Apolipoprotein Eε4 Modifies Alzheimer’s Disease Onset in an E280A PS1 Kindred

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We previously have identified a large kindred from Colombia in which Alzheimer’s disease (AD) is caused by the E280A presenilin 1 (PS1) mutation. The objective of this study was to examine whether environmental and genetic factors are responsible for variation in the phenotypic expression of the E280A PS1 mutation. We genotyped coding and promoter polymorphisms of the APOE gene in carriers of the E280A PS1 mutation. Kaplan–Meier product-limit and Cox proportional hazard models were used in the statistical analyses. DNA was available from 114 carriers of the E280A PS1 mutation, including 52 subjects with AD. APOE ε4 allele carriers were more likely to develop AD at an earlier age than subjects without the ε4 allele (hazard ratio, 2.07; 95% confidence interval, 1.07–3.99; p = 0.030). Subjects with low education were more likely to develop AD later than those with higher education (hazard ratio, 0.476; 95% confidence interval, 0.26–0.87). Low educational level was associated with rural residence (p < 0.001). Promoter APOE variants did not influence either the onset or the duration of the disease. This study is the first to our knowledge to demonstrate that genetic and environmental factors influence age of onset in a kindred with a familial AD mutation.


Research over the past two decades has established that familial early-onset Alzheimer’s disease (FAD) is a genetically heterogeneous disorder that can be caused by mutations in three genes: β-amyloid protein precursor gene (APP) on chromosome 21, presenilin 1 (PS1) gene on chromosome 14, and presenilin 2 (PS2) gene on chromosome 1.1–3 In addition, many studies have identified the ε4 allele of the apolipoprotein E gene (APOE ε4) as a dose-dependent risk factor for AD. Presence of APOE ε4 alleles is correlated with earlier age of onset of AD.4–7 Linkage to additional susceptibility loci for familial LOAD has been reported, but the responsible genes have not yet been identified.8,9

Because the APOE ε4 allele is the only known genetic risk factor for sporadic AD, the hypothesis that it may also modify age of onset within specific kindreds with FAD has been investigated. An association between the presence of the APOE ε4 allele and age of onset in FAD has been suggested in small kindreds carrying APP mutations, but not those carrying PS mutations.10–13 Nonetheless, the wide range of age of onset associated with some PS mutations14 suggests that variants in APOE or in other genes could act as modifiers of the phenotypic expression of FAD.

APOE polymorphisms in the promoter region also have been associated with an increased risk of developing AD in some studies.15,16 Two of these polymorphisms (−491A/T and −427 T/C) play a role in the level of expression of APOE in vitro.16–19 However, no studies to date have examined the role of APOE promoter polymorphisms as modifiers of phenotypic expression in specific AD families.

In 1987, a Colombian family with early-onset autosomal dominant AD was described.20 The most frequent presentation in this family is memory loss, followed by behavior and personality changes, and progressive language impairment. In the final stages, gait disturbances, seizures, and myoclonus are frequent.21 Screening of the PS1 gene showed a single base pair change from A to C (gAa→gCa) at codon 280, which results in a Glu to Ala substitution associated with the disease.10 In subsequent years, 24 additional families with the E280A PS1 mutation have been identified.22 Most members of the E280A kin-
dred live in Medellin and in the surrounding mountainous region in Colombia. Analysis of markers surrounding the PS1 gene support the existence of a founder effect, and, after examination of baptismal records, notary registries, and clinical records, a person who was originally from Northern Spain during the 1500s was identified as a common ancestor of 13 of these families. This large family with similar environmental exposures and homogeneous disease cause is a unique resource for the study of modifier genes. The purpose of this study is to examine whether age of onset and duration of FAD caused by the E280A PS1 mutation is associated with coding and/or promoter APOE variants.

In a previous study with a sample of 28 individuals carrying the E280A PS1 mutation from the same kindred, we failed to detect an effect of APOE genotype on age of onset. In the years since that investigation, however, the number of subjects available for study has increased substantially, thereby increasing the power to detect a difference between the APOE groups. For this reason, reexamination of the impact of APOE genotype on FAD expression is warranted.

Subjects and Methods
All participants gave informed consent and the Washington University School of Medicine Ethics Committee, and the Human Subjects Committee of the University of Antioquia (Colombia) approved the study. Members of the E280A Colombian kindred who agreed to participate visited the Neurology Department and Neuroscience Group of the University Hospital San Vicente de Paul, Medellin, Colombia, for an initial assessment and were invited to return annually for subsequent assessments. Some members were examined at their home. Blood samples and clinical and demographic information, including age, sex, years of education, and whether the subject lived in a rural or urban area, was collected during the initial visit. Subjects who resided in the Greater Medellin area were considered to live in an urban environment, and all others were classified as living in a rural environment. During all visits, a brief interview and a neuropsychological battery were administered to kindred members. Neuropsychological assessment was conducted using the Spanish versions of the Consortium to Establish a Registry for Alzheimer’s Disease and Clinical Dementia Rating assessment instruments. A neurological examination was also administered to subjects who reported memory loss. Diagnosis of AD was based on the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria for “probable AD” or “definite AD.” The criterion of “onset between ages 40 and 90 years” was not considered for the diagnosis of AD. All physicians and psychologists involved were blind to the E280A PS1 mutation status of the participants. The presence of the E280A PS1 mutation in a subject affected with AD was necessary for phenotype assignment to minimize the possible confounding effects of any other causes of dementia in this family.

For subjects who met diagnostic criteria for AD at the time of the first visit, age of onset was assigned historically as the age at which memory impairment began to interfere with daily activities as reported by at least two collateral sources, usually first-degree relatives. For 10 subjects who developed AD during follow-up, age of onset was defined as the age at which the patient presented with memory impairment that interfered notably with daily activities. Duration of disease was defined as the number of years from onset of disease to death.

Analysis of APOE Polymorphisms
Genomic DNA was isolated from blood samples using QiAmp DNA blood mini kits from Qiagen (Valencia, CA). Detection of the E280A PS1 mutation was performed as previously described. Genotyping of the APOE ε polymorphism was performed using polymerase chain reaction amplification of a 244bp fragment followed by digestion with HhaI as described in Hixson and Vernier. Genotyping of the APOE promoter polymorphisms (−219G/T, −491A/T, and −427 T/C) was performed as described in Wang and colleagues.

Statistical Analyses
Survival analyses were used to assess differences in age of onset and duration of disease as a function of gender, educational level, rural versus urban environment, APOE ε, and the −491A/T and −219 G/T APOE promoter polymorphisms. Survival techniques are used to analyze the average time to an event of interest when that event may not have occurred for all subjects during the observation period. Inclusion of data from asymptomatic E280A PS1 carriers can prevent underestimating the average time to AD onset or death. The dependent measure for these subjects is the age at last assessment, rather than the age at which the event is experienced. Survival curves of time to AD onset and duration of disease were estimated using the Kaplan–Meier product-limit method (SAS/STAT PROC LIFETEST; SAS, version 8.02.02 for SunOS, SAS Institute, Cary, NC, 1999). Cox proportional hazard models (SAS/STAT PROC PHREG; SAS, version 8.02.02 for SunOS, SAS Institute, Cary, NC, 1999) were used to test the effect of APOE genotype together with educational level and with rural versus urban environment on the survival functions of AD onset. In the survival analyses using age of onset as the dependent variable, participants who had not received a diagnosis of AD as of their most recent clinic visit were censored on the date of that visit. Only kindred members with diagnoses of AD were used in the survival analyses assessing duration of disease. For subjects with diagnoses of AD who had died, duration of disease was defined as the number of years from onset of the disease to death. Kindred members with diagnoses of AD who were still living as of their last assessment were censored on the date of that assessment. A value less than or equal to 0.05 was considered statistically significant in all analyses.

Assessment of linkage disequilibrium and the estimated frequencies of haplotypes among the different APOE polymorphisms was determined using the FASTEHPLUS pro-
gram. The 2LD-two-locus linkage disequilibrium (LD) calculator was used for the two-locus linkage disequilibrium analysis (both are available at: http://www.iop.kcl.ac.uk/IoP/Departments/PsychMed/GEpiBS/software.shtml)

Results

Subjects

DNA is available from 246 members of the Colombian kindred. Screening of the E280A PS1 mutation showed that 114 of these subjects were heterozygous for the mutation. No homozygous carriers of the E280A mutation were identified. One patient with a diagnosis of probable AD with onset at age 83 years was excluded from the study because he did not carry the E280A mutation. A total of 109 subjects carrying the E280A mutation made up the final sample (Table) because 5 E280A carriers with memory impairment insufficient for a diagnosis of probable AD were excluded from the analyses. Women composed 66% of the sample (N = 72). Fifty-seven subjects remained asymptomatic throughout the study period, and 52 received a diagnosis of probable AD at or before their last visit. Postmortem evaluation of 11 brains confirmed the diagnosis of AD. Age of onset ranged from 35 to 62 years, with a mean of 45.2 years (standard deviation [SD], 5.7), and 75% of AD patients had an age of onset between 39 and 51 years (Fig 1a). For patients without AD, age at last assessment ranged from 24 to 65 years with a mean of 40.5 years (SD, 8.5; see Fig 1b).

Table 1. APOE Genotype Distribution within the E280A PS1 Carriers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Not Affected (N = 57)</th>
<th>Affected (N = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>39</td>
<td>68.4</td>
</tr>
<tr>
<td>Male</td>
<td>18</td>
<td>31.6</td>
</tr>
<tr>
<td>Years of education</td>
<td>Mean = 5.1, SD = 3.8</td>
<td>Mean = 4.0, SD = 3.0</td>
</tr>
<tr>
<td>APOE ε4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>7</td>
<td>12.3</td>
</tr>
<tr>
<td>ε2/ε4</td>
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<tr>
<td>ε3/ε3</td>
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<td>1.8</td>
</tr>
<tr>
<td>−219 G/T</td>
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</tr>
<tr>
<td>GG</td>
<td>16</td>
<td>28.1</td>
</tr>
<tr>
<td>TG</td>
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<tr>
<td>−427 T/C</td>
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<td></td>
</tr>
<tr>
<td>CT</td>
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</tr>
<tr>
<td>TT</td>
<td>52</td>
<td>91.2</td>
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<tr>
<td>−491 A/T</td>
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</tr>
<tr>
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</tr>
<tr>
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</table>

APOE = apolipoprotein E; PS1 = E280A presenilin 1; SD = standard deviation.

Demographic Variables

AGE OF ONSET. There was no significant difference in the survival curves for time to AD onset for men and women (p = 0.694). The mean educational level of subjects was low (4.6 years, SD, 3.5 years). To investigate whether educational level was associated with age of AD onset within this kindred, we stratified subjects into low (0–3 years, N = 52) and high (>3 years, N = 48) education groups. Kaplan–Meier analyses indicated that subjects in the low education group were more likely to develop AD at a later age than were subjects in the high education group (log-rank test; p = 0.017, Fig 2a), and that subjects residing in a rural area were more likely to develop AD at a later age than...
were those who lived in an urban setting (log-rank test; \( p = 0.007 \), see Fig 2b). Educational level was related to residential area (\( \chi^2 \) test; \( p < 0.001 \)), such that 68.6% of subjects in the low education group lived in a rural area, compared with 35.4% of those in the high education group.

DURATION OF DISEASE. Among the 24 deceased AD patients, duration of disease ranged from 5 to 23 years, with a mean of 11.1 years (SD, 4.7). The log-rank test indicated that gender had a marginal effect (\( p = 0.093 \)) on duration of disease, such that women were more likely to have a longer duration of disease than men. Duration of AD did not vary significantly by education group (\( p = 0.620 \)) or residential environment (\( p = 0.777 \)).

APOE \( \varepsilon \) POLYMORPHISM. Only one (0.9%) of the 109 carriers of the E280A mutation was homozygous for the APOE \( \varepsilon 4 \) allele. Twenty-eight (25.7%) were heterozygous (N = 25 for \( \varepsilon 4/\varepsilon 3 \) and N = 3 for \( \varepsilon 4/\varepsilon 2 \)), and 80 (73.4%) had no \( \varepsilon 4 \) alleles (N = 72 for \( \varepsilon 3/\varepsilon 3 \) and N = 8 for \( \varepsilon 3/\varepsilon 2 \)). In the survival analyses, subjects were dichotomized into two groups based on presence or absence of the \( \varepsilon 4 \) allele.

AGE OF ONSET. The log-rank test showed a statistically significant difference (\( p = 0.045 \)) among the two survival functions of time to AD onset in the two groups (see Fig 2c). Kindred members with the \( \varepsilon 4 \) allele were more likely to develop AD at an earlier age than were members without the allele. Cox proportional hazards models were used to examine the effect of the \( \varepsilon 4 \) allele together with the effect of education and the effect of residential area on age of onset.

A regression model using APOE \( \varepsilon 4 \) status and education group as independent variables confirmed that there was a statistically significant difference in the survival functions for the APOE \( \varepsilon 4 \) groups when educational level was controlled (HR, 2.07; 95% confidence interval [CI], 1.07–3.99; \( p = 0.030 \)). Cox regression analysis also confirmed that level of education is independently related to age of AD onset (HR, 0.476; 95% CI, 0.26–0.87; \( p = 0.016 \)). A regression model testing APOE \( \varepsilon 4 \) status, education group, and the interaction term indicated that APOE \( \varepsilon 4 \) status and education did not have an interactive effect (\( p = 0.278 \)) on age of onset.

Given the aforementioned association between educational level and residential area, it is not surprising that when residential area was substituted for education in the Cox proportionate hazards model, both residential environment (HR, 0.447; 95% CI, 0.24–0.82; \( p = .009 \)) and APOE \( \varepsilon 4 \) status (HR, 2.00; 95% CI, 1.04–3.86; \( p = 0.038 \)) were independently related to age of onset. Similarly, residential area and APOE \( \varepsilon 4 \)
status did not have an interactive effect \((p = 0.191)\) on age on onset. The finding of an inverse relationship between age of onset and the presence of the \(APOE \varepsilon4\) allele led us to investigate a possible protective effect of the \(APOE \varepsilon2\) allele. In these analyses, the survival curve of time to AD onset of subjects with \(\varepsilon3/\varepsilon2\) and \(\varepsilon4/\varepsilon2\) genotypes \((N = 11)\) was compared with the survival function of subjects with \(\varepsilon4/\varepsilon3\) and \(\varepsilon4/\varepsilon4\) genotypes \((N = 26)\) and with all subjects without the \(APOE \varepsilon2\) allele \((N = 98)\) using Kaplan–Meier product-limit method. Although the survival curves suggest a modest protective influence of the \(APOE \varepsilon2\) allele \(\text{(see Fig 2c)}\), the difference between the groups did not reach statistical significance \((p = 0.106 \text{ and } p = 0.15, \text{ respectively})\).

**Duration of Disease.** Duration of AD did not vary significantly by \(APOE \varepsilon4\) status \((\text{log-rank test, } p = 0.713)\). Because only three subjects with \(\varepsilon3/\varepsilon2\) or \(\varepsilon4/\varepsilon2\) genotype had developed AD by the time of their last assessment, we were unable to analyze the relationship between the \(APOE \varepsilon2\) allele and duration of disease.

**\(APOE\) Promoter Polymorphisms**

**Age of Onset.** To determine the effect of specific variants of the \(-491\ A/T\) and \(-219\ G/T\) \(APOE\) promoter polymorphisms on age of onset, we conducted survival analyses comparing every genotype against the other two. No significant differences between the genotypes were found \((all \ p \ values \geq 0.30)\). We were unable to test the effect of specific variants of the \(-427\ \text{T/C}\) polymorphism, because only four subjects had the CT genotype and the remainder had the TT genotype.

**Duration of Disease.** None of the survival tests using duration of AD as a dependent variable were statistically significant \((all \ p \ values \geq 0.120)\).

**Linkage Disequilibrium and Haplotype Analysis of \(APOE\) Polymorphisms**

To investigate the degree of independence among the \(APOE\) polymorphisms and their association with clinical traits and demographic variables, we performed an Estimating haplotype-frequencies \((\text{EH})\)-based haplotype analysis. In the four- and the two-locus analyses for the \(APOE\) promoter and \(APOE\ \varepsilon\) polymorphisms, the estimated haplotype frequencies under the hypothesis of association showed a significant representation of specific haplotypes, but most of the analyses showed incomplete disequilibrium \((\text{disequilibrium coefficient [D'] between } -0.5 \text{ and } +0.5)\). An exception was the two-locus analysis for \(APOE -427\text{T/C}\) and of \(APOE \varepsilon\) polymorphism where, as a consequence of the low frequency \((0.0265)\) of the C allele, \(D'\) was close to 1 \((0.906)\).

**Discussion**

This article is the first to our knowledge to report a modulating effect the \(APOE \varepsilon4\) allele and environmental factors in a large family with an FAD mutation. Presence of the \(APOE \varepsilon4\) allele is associated with earlier expression of clinical symptoms of AD in E280A PS1 carriers. Subjects with the \(APOE \varepsilon4\) allele develop AD at approximately twice the rate of subjects without the \(APOE \varepsilon4\) allele. Furthermore, the presence of the \(APOE \varepsilon2\) allele may be associated with later onset of the disease, although the difference did not reach statistical significance. The possible protective effect of the \(APOE \varepsilon2\) allele warrants reexamination in a larger sample. Thus, \(APOE\) and \(PSI\) genes display an epistatic effect resulting in variable age of onset of disease.

Previous work suggested that the \(APOE \varepsilon4\) allele influences age of onset in FAD in some kindreds carrying APP mutations, but the effect has not been found in families carrying PS1 mutations. However, the largest of these studies analyzed subjects from unrelated pedigrees with different PS1 mutations. Variation in the age of onset associated with \(PS\) mutations thus may have obscured the epistasis associated with \(APOE \varepsilon\) alleles.

A study using brain tissue from patients in the E280A kindred strongly suggests that mutant PS1 protein alters the proteolytic processing of the \(\beta\)-APP at the C terminus of A\(\beta\) to favor deposition of A\(\beta\) 42. Studies in transgenic animals have conclusively demonstrated that \(APOE \varepsilon\) alleles influence risk for AD by modifying A\(\beta\) deposition. Based on these studies, the effect of the \(APOE \varepsilon4\) allele on modifying the age of onset in E280A carriers most likely influences fibril formation and/or clearance of the increased A\(\beta\) 42, thus accelerating A\(\beta\) 42 deposition. Given this pathological mechanism, it was surprising that onset of AD would be affected in APP but not PS mutations families. These new results therefore are more consistent with our understanding of AD pathogenesis, that is, both APP and PS mutations increase A\(\beta\) 42 levels and A\(\beta\) deposition. Because \(APOE \varepsilon\) alleles also influence A\(\beta\) deposition by increasing fibrillogenesis and/or decreasing clearance, the effect of \(APOE\) plus APP/PS mutations would be expected to be additive. Nevertheless, the theoretical possibility that \(APOE\) genotype affects only certain PS1 mutations, such as E280A and no others, remains.

Our study failed to detect any associations between \(APOE\) promoter polymorphisms and age of onset or duration of disease in the FAD Colombian kindred.
The analysis of the multiple-locus estimate haplotype frequencies of the polymorphisms along the APOE gene showed partial linkage disequilibrium among the polymorphisms, suggesting that the modulator effect of APOE ε polymorphism on age of onset is not dependent on specific allelic variants of the APOE promoter region. Case–control studies with these polymorphisms have produced mixed results; furthermore, none of the studies have reported an effect of these polymorphisms on age of onset rather than risk for disease. However, a meta-analysis has reported that the effect of the −219T APOE allele is strongest in subjects with a later age of onset.

Sixty-six percent of sampled subjects were women. In general, subjects who volunteer to participate in research are more likely to be female than male. In addition, a survey of 51 members of the E280A PS1 kindred (F. Lopera, unpublished data) indicates that 53% (N = 16) of the 30 women surveyed work as homemakers, whereas almost all men work outside the home. Women in the Medellin area may be better able than men to arrange their schedules so that they can participate in research.

Although subject gender was unrelated to age of onset, two other demographic characteristics, level of education and residential environment, were associated with this variable. Previous studies examining late-onset AD have reported that low educational level is a risk factor for AD. However, our finding that AD onset is earlier among kindred members with a “high” educational level relative to those with less education, is not unprecedented. Antonelli and colleagues found that a late diagnosis of dementia was associated with less formal education and a lower occupational role. The researchers suggested that dementia had a heavier social and economic impact in the group with higher educational attainment resulting in an earlier diagnosis. Note that most of the subjects in this kindred have a low absolute level of education when compared with participants in other studies (mean, 4.6 years; SD, 3.5 years). Our sample showed a strong correlation between low education and rural environment, suggesting that low education often is linked to rural residence in the Medellin area. Antonelli and colleagues also found that rural residence was associated with a late diagnosis of dementia. It is possible that factors associated with education and residential environment (such as diet, pollution, availability of health care), rather than those variables themselves, are responsible for the observed associations with age of onset. The relationship between education, residential environment, and onset of AD shown in this investigation highlights the crucial importance of further characterizing the cultural and demographic variables that can modulate the detection of memory impairment.

In conclusion, the large size of the E280A FAD kindred enabled us to take a novel approach to identifying modifying genetic factors of FAD, providing strong support for the role of APOE ε alleles in modifying age of onset. Furthermore, we demonstrate that despite the presence of a deterministic mutation age of onset may be modified by environment.

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References