Presence of an APOE4 Allele Results in Significantly Earlier Onset of Parkinson’s Disease and a Higher Risk With Dementia

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Abstract: The ε4 allele of the apolipoprotein E gene (APOE4) has been consistently associated with a greater risk of Alzheimer’s disease (AD) as well as an earlier onset of AD. It is possible that APOE4 may also play a role in the etiology of other neurodegenerative disorders, such as Parkinson’s disease (PD). APOE genotype, age of onset, disease duration, smoking history, and dementia status were collected for families with PD, yielding 324 Caucasian families with complete information. Logistic regression employing one individual per family and including age of onset and disease duration as covariates demonstrated a significantly increased risk of dementia for those individuals having inherited at least one ε4 allele (OR = 3.37; P = 0.002). Survival analyses also demonstrated a significantly earlier age of onset for those subjects with at least one ε4 allele (59.7 years) as compared with those homozygous for the more common ε3 allele (62.4 years; P = 0.009). Thus, consistent with previous studies, we find evidence that the presence of an ε4 allele results in significantly earlier onset of PD and a greater likelihood of dementia. It appears the similarities between PD and AD may be due to an overlap in the diseases’ genetic etiology. © 2005 Movement Disorder Society

Key words: apolipoprotein E; age of onset; Parkinson’s disease; dementia

During the past decade, a number of genes have been identified that, when mutated, result in either autosomal dominant or autosomal recessive Parkinson’s disease (PD).1 However, in the vast majority of cases of idiopathic PD, the disease etiology is more complex, with both genetic and environmental factors contributing to disease risk. One of the first identified susceptibility genes contributing to the risk for a complex neurodegenerative disease was apolipoprotein E (APOE), which is an important risk factor for Alzheimer’s disease (AD).2 The ε4 allele has consistently been shown to lead to both an increased risk for AD as well as an earlier age of onset of disease.2 The ε2 allele has a protective effect, decreasing the risk of AD and delaying its onset.3 PD and AD share similarities in their disease etiologies: both neurodegenerative diseases are associated with advancing age, exhibit protein aggregation as part of their pathology, and appear to be exacerbated by oxidative stress.4 The clinical symptoms of AD and PD may also overlap in some patients. There is growing evidence that cognitive impairment and dementia are more common than originally thought among PD patients. Although estimates of the frequency of dementia among PD patients vary widely among studies, a recent review of a number of studies estimated the prevalence of dementia in PD to be 30%.5 In a large longitudinal study of AD patients, at least one motor sign was detected in 44% of patients during at least one of their study visits.6 Because of these similarities, it has been postulated that
there may be common pathways affected in both diseases. To test this hypothesis, several groups have examined the role of APOE, a known risk factor for AD, in patients with PD.

Evidence for the role of APOE in PD has been inconsistent. Similar to the relationship seen in AD, some studies have shown that PD or PD with dementia are associated with the ε4 allele, while other studies report an association with the ε2 allele. Still others report a lack of association of APOE with both phenotypes. Similarly, there has been inconsistent data regarding the role of APOE in onset of PD. In this study, we have examined the role of the APOE ε2 and ε4 alleles on the age of onset of PD and the risk for dementia in a sample of familial PD patients.

PATIENTS AND METHODS

Study Design and Data Collection

A total of 366 families (n = 783 individuals with DNA), consisting of at least one pair of living siblings diagnosed with PD, were recruited through 59 Parkinson Study Group (PSG) sites located throughout North America. All study participants completed a uniform clinical evaluation (UPDRS) and a diagnostic checklist with inclusion criteria consisting of clinical features highly associated with autopsy-confirmed PD and exclusion criteria highly associated with other non-PD pathological diagnoses. Sixteen families of Hispanic descent and five families of Asian, Pacific Islander, or African American descent were removed from the sample to control for potential heterogeneity due to ethnicity. Age of disease onset, disease duration, education, smoking status, and results from the Mini Mental State Exam (MMSE) were available for 324 Caucasian families.

Uhlmann and Larson found that education-specific thresholds optimize the performance of the MMSE as a screening test for dementia. In this study, we employ their lower limits of normal: 21 for middle school, 23 for high school, and 24 for college/graduate school attainment. The average age of onset of all the PD subjects included in these analyses was 60.9, with a range of 18 to 87 years. Age of onset information was obtained from three sources: the subject’s medical records, the family history questionnaire, and the case report form. The three measures of age of onset of PD showed excellent agreement (reliability, R = 0.94), strengthening confidence in the reliability of the family-reported age of clinical onset for PD. The age of onset used in the analysis came primarily from the family history questionnaire and was supplemented with data collected on the case report form when needed. Disease duration was calculated as the number of months from age of onset to the date of examination. An individual was classified as a smoker if they had smoked more than five packs of cigarettes in their lifetime.

Peripheral blood was obtained from all individuals after appropriate written informed consent approved by each individual institution’s institutional review board was completed. Total genomic DNA was prepared as previously described. APOE genotyping was carried out using Taqman assays developed for each of the two polymorphisms that comprise the three different alleles. APOE genotypes were obtained blind to all clinical information.

For age of onset analyses, patients were categorized into the following genotypic groups: ε4 carriers (ε3ε4 and ε4ε4), ε3 homozygotes (ε3ε3), and ε2 carriers (ε2ε3 and ε2ε2). Carrier groups were used in lieu of individual genotypes due to the small number of homozygotes for the rarer ε2 and ε4 alleles. Individuals with the ε2ε4 genotype (n = 17) were removed from all analyses so that the effect of the rarer alleles could be determined independently.

Data Analysis

Two hypotheses were tested as part of the data analyses. First, odds ratios were calculated using logistic regression to test the hypothesis that particular APOE genotypic groups increased the risk for PD with dementia. Second, Kaplan–Meier survival analysis was used to test the hypothesis that the age of PD onset differed between the APOE genotypic groups. All analyses were carried out using SAS software (release 6.12; SAS Institute, Cary, NC).

Logistic regression was used to assess whether the presence of an ε4 allele or an ε2 allele increased the risk of dementia as compared to the most common ε3ε3 genotype after controlling for possible confounders. In this logistic model, presence of dementia (as determined by MMSE using an education-specific cutoff) was the dependent variable and two binary variables, indicating presence of an ε4 allele or an ε2 allele, were included as independent variables. The remaining imputed state (ε3ε3) was used as the reference. Age of onset, disease duration, gender, and smoking history were included as covariates in the initial model, and those found to be significant were kept in the final reduced model. To ensure statistical validity, a single individual was sampled at random from each of the families. This was repeated 1,000 times, and the median bootstrapped t statistic was determined for each variable in the equation, and the corresponding P value and odds ratio are reported.

To determine the effect of APOE genotype on age of disease onset, Kaplan–Meier survival analysis was used to plot age of onset distribution curves for each of the
three genotypic groups and to test the difference between the curves using log-rank statistics. The SAS LIFETEST procedure was used to detect the effects of education, gender, and smoking on survival, and those covariates found to be nonsignificant were dropped from the model. Similar to the approach used for the logistic regression, a single individual from each of the families was sampled at random, and 1,000 test statistics were generated. Common resampling techniques (bootstrapping) were employed to obtain a representative value. The statistic from the resampling distribution and its corresponding P value are reported.

**RESULTS**

No deviation from Hardy–Weinberg equilibrium was seen in the distribution of APOE genotypes of a representative independent sample ($\chi^2 = 1.53; \text{df} = 3; P = 0.68$), indicating that if any genotyping error was present, then the amount was likely negligible. Among the familial PD subjects, 14.2% had an MMSE score less than their education-specific cutoff, thereby meeting our criteria for dementia. When a single individual was sampled from each family, on average 15.4% met our criteria for dementia (Table 1). When a logistic regression model was fitted to predict dementia status, age of onset and disease duration were significant ($P < 0.001$ for both) while gender and smoking were not ($P > 0.30$ for both). After accounting for the effects of the significant covariates, there was a significant increase in the risk for dementia given the presence of an ε4 allele (OR = 3.38; $P = 0.002$; Table 2). The presence of an ε2 allele did not have a significant protective or deleterious effect on the risk for dementia.

Subsequently, we tested the hypothesis that APOE genotype is associated with the age of disease onset. All of the covariates (education, gender, smoking history) were nonsignificant ($P > 0.40$), but the age of PD onset was significantly earlier in those patients who carried at least one ε4 allele (59.7 years of age) compared with those patients homozygous for the common ε3 allele (62.4 years of age; log-rank statistic = 6.79; $P = 0.009$; Table 3). When the χ² statistic was bootstrapped 5,000 times, the statistic remained robust (95% CI = 6.62–6.96). The ε2 carriers did not have significantly earlier age of onset (mean age of onset = 61.5; median bootstrapped $\chi^2 = 0.70$; 95% CI = 0.64–0.75) as compared to the ε3 homozygotes.

**DISCUSSION**

Similar to previous studies, we have found a significant association between age of onset of PD and APOE genotypes. Age of onset was significantly earlier in those individuals with at least one ε4 allele when compared with those homozygous for the common ε3 allele. The average reduction in age of onset was 2.7 years, which is similar to the reduction seen in other studies. Zareparsi and colleagues reported a reduction of 3.4 years in their preliminary sample and 3.5 years in an expanded sample, and Li and colleagues reported a reduction of roughly 3 years when comparing ε4 carriers with non-ε4 carriers. Several studies have not seen this trend with age of onset. However, as noted by Zareparsi and colleagues, this could be due to the smaller size and/or differing ethnicities of these samples. The studies showing a significant association with the ε4 allele represent three of the four largest samples that have addressed the role of APOE genotype on age of PD onset. The reduc-

**TABLE 1.** Average APOE genotype cell counts for 50,000 replicates, choosing one individual per family

<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>Demented</th>
<th>Not demented</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε4ε4</td>
<td>1.0 (2.0%)</td>
<td>5.5 (2.0%)</td>
</tr>
<tr>
<td>ε3ε4</td>
<td>18.0 (36.0%)</td>
<td>56.7 (20.7%)</td>
</tr>
<tr>
<td>ε3ε3</td>
<td>24.0 (48.0%)</td>
<td>166.4 (60.7%)</td>
</tr>
<tr>
<td>ε2ε3</td>
<td>7.0 (14.0%)</td>
<td>43.9 (16.0%)</td>
</tr>
<tr>
<td>ε2ε2</td>
<td>0.0 (0.0%)</td>
<td>1.5 (0.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>50.0 (100.0%)</td>
<td>274.0 (100.0%)</td>
</tr>
</tbody>
</table>

*On average, 9.5 individuals would have had the genotype ε2ε4 (all were not demented and had an average age of onset of 63.7); however, these individuals were removed from both sets of analyses in order to account for the effect of ε2 and ε4 carriers separately.

**TABLE 2.** Logistic regression model for predicting dementia based on MMSE with an education-specific cutoff

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Beta (odds ratio)</th>
<th>T statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε4 carriers</td>
<td>3.38</td>
<td>10.14</td>
<td>0.0015</td>
</tr>
<tr>
<td>ε2 carriers</td>
<td>1.11</td>
<td>0.45</td>
<td>0.0544</td>
</tr>
<tr>
<td>Age of onset (yr)</td>
<td>1.12</td>
<td>21.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>1.18</td>
<td>26.97</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

“Carrier” indicates the presence of at least one ε4 or ε2 allele (ε2ε4 genotypes were removed). The ε3ε3 genotype is imputed from the dichotomous carrier variables. The statistics reported are the median values from 1,000 bootstrapped replicates.

**TABLE 3.** Mean age of onset and comparison of survival curves for the presence of each allele compared to the common genotype, ε3ε3

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Age of onset (mean)</th>
<th>Median χ² (P)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε4 carriers</td>
<td>81.9</td>
<td>59.7</td>
<td>6.79 (0.009)</td>
</tr>
<tr>
<td>ε3ε3 homozygotes</td>
<td>194.6</td>
<td>62.4</td>
<td></td>
</tr>
<tr>
<td>ε2 carriers</td>
<td>52.5</td>
<td>61.5</td>
<td>0.68 (0.41)</td>
</tr>
</tbody>
</table>

“Carrier” indicates the presence of at least one ε4 or ε2 allele (ε2ε4 genotypes were removed).

*Log-rank statistic from Kaplan–Meier analysis.
tion in age of onset due to the presence of an ε4 allele is modest (2.7 years); however, any significant predictor of age of onset has potential clinical relevance for future pharmacological interventions designed to delay or prevent PD onset. Furthermore, by accounting for the variation due to APOE, the power of a study to identify additional susceptibility genes for PD is increased.

One limitation of this study is the use of self-reported information in determining age of disease onset. The reliability between the age of onset collected from medical records, family history questionnaires, and case report forms was high \( (R = 0.94); \) however, these sources are not independent. In many individuals, the onset of nonmotor manifestations may precede the onset of motor symptoms by many years. Therefore, it is likely that for most individuals the self-reported age of onset used in our analyses is a few years later than the actual disease onset. It is also possible that those with more education might notice clinical PD symptoms earlier than those with less education, and it is possible that greater education might have a protective effect on dementia, similar to that observed in Alzheimer’s disease. However, we did not detect a significant association between education and age of onset. Unfortunately, the relationship between education and age of onset cannot be tested further in our sample, since the age-of-onset information was not obtained through rigorous longitudinal assessments.

The association of APOE genotype with the risk of PD with dementia was significant as well. The increase in the risk for PD with dementia among the ε4 carriers as compared with the common ε3 genotype was highly significant after adjusting for age of onset and duration of the disease \( (OR = 3.38; P = 0.002). \) This is consistent with several studies showing that the ε4 allele is associated with Lewy body dementia and AD with PD, and PD with dementia. In our own study, the relationship between the ε4 allele and dementia was less significant if we failed to adjust for the effects of age of onset and disease duration \( (P = 0.02), \) thus illustrating the importance of controlling for these factors.

There are several explanations for the observed association between dementia and the ε4 allele. For example, it is possible that the individuals having PD with dementia in fact have both PD and AD, and the association we have observed between the ε4 allele and dementia is simply the known association between ε4 and AD. Unfortunately, we do not have a sufficient number of autopsies completed in individuals with PD and dementia to test this hypothesis. On the other hand, it is also possible that PD and AD represent a continuous spectrum of the same disease and that risk factors for one phenotype influence the other as well. The significant association with an earlier age of PD onset (note that dementia was associated with later age of onset in this sample) supports this hypothesis.

Another limitation of this study is its cross-sectional assessment of cognitive impairment. Some subjects demonstrate some cognitive impairment, but it is not sufficient to be classified as dementia using our study criteria. It is possible that these individuals will progress and in the future will meet our criteria for PD with dementia. To consider this possibility, we have used age of onset and disease duration as covariates in our analyses examining the relationship between APOE and dementia.

Another limitation of our study is the exclusive focus on familial PD. We cannot determine whether the ε4 allele also increases the risk of dementia and decreases the age of PD onset among individuals without a family history of PD. Finally, we have used the MMSE score to define dementia. While this is a convenient and rapidly administered instrument, there are more detailed cognitive batteries that could be performed, which would have greater sensitivity and specificity in evaluating dementia and cognitive impairment.

The genotyping of our sample of PD families bolsters the evidence that variation in the APOE gene is associated with PD age of onset and with the presence of dementia as a comorbidity of PD. Presence of an ε4 allele results in earlier onset of PD symptoms and a higher risk of dementia. Taken together, these data further suggest that PD and AD etiology may be due to common pathways.

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APPENDIX

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