Relationship of Apolipoprotein E and Age at Onset to Parkinson Disease Neuropathology

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Abstract

Previous studies investigating the association between apolipoprotein E (APOE) genotypes and Parkinson disease (PD) have yielded conflicting results, and only a few have addressed APOE as a possible determinant of PD pathology. Therefore, we aimed to evaluate the relationship between APOE and PD as well as APOE and PD pathology. We studied 108 pathologically verified patients with PD and 108 controls pair-matched for age and gender. Allele frequencies of APOE differed between patients with PD and controls (p = 0.02). The frequency of e4 allele increased (p = 0.01), whereas that of e3 allele decreased with advancing PD pathology (p = 0.002). Only age of PD onset was an independent predictor for the rate of progression of PD pathology in which late-onset patients appeared to reach end point PD pathology more rapidly than early-onset patients (p = 0.001). In conclusion, our findings suggest that APOE may express its effect on the risk of PD by modifying the occurrence of PD pathology, but age of PD onset seems to be the principal determinant of the progression rate of PD pathology.

Key Words: Age of onset, Apolipoprotein E (APOE), Dementia, Disease progression, Neuropathologic staging, Parkinson disease, Risk factors.

INTRODUCTION

Parkinson disease (PD) is the most common movement disorder and the second leading cause of neurodegeneration after Alzheimer disease (AD) among the elderly. Clinically, patients with PD display diverse motor dysfunctions and often show autonomic and cognitive deficits (1, 2). Although the clinical phenotype of PD largely depends on a lesion of the substantia nigra, PD-related neuropathology also occurs in extranigral regions of the brain (3) and outside the brain (4).

Briefly, PD is characterized by pathologic findings showing a progressive dopaminergic neurodegeneration in the pars compacta of the substantia nigra and the presence of intraneuronal inclusions such as Lewy bodies (LBs), Lewy neurites (LNs), and pale bodies in the remaining nigral neurons as well as in other vulnerable neurons of the central and peripheral nervous system (5, 6). Misfolded and aggregated α-synuclein molecules constitute the major components of these inclusion bodies (7).

From a neuropathologic point of view, the severity of PD-related lesions varies greatly both from case to case and within the brain in individual cases (3). In addition, pathologies related to AD (i.e., formation of neurofibrillary tangles [NFTs] and deposition of β-amyloid [Aβ]) are common in older persons and frequently co-occur with PD-associated lesions (5, 8–10). The present pathologic diagnostic criteria for PD, however, subscribe to a unifying concept of the pathologic variability of PD that is exclusively limited to the assessment of specific somatomotor dysfunctions (8, 11, 12). As such, these criteria fail to account for the full range of pathologic changes inherent in the complex nature of the neuropathology in PD.

Several lines of evidence indicate that, in the course of PD, the lesions increase steadily in their topographic distribution pattern (3, 13). Thus, instead of using nigrostriatal degeneration to define PD, an alternative view would acknowledge the considerable interindividual variability in PD, which would result in a broader definition of the disease. To this end, a recently proposed neuropathologic staging protocol for sporadic PD takes into account the topographic distribution pattern and increasing severity of the lesions in selectively vulnerable regions of the brain (3).

The reasons for the interindividual variability of neuropathologic lesions in PD and the biologic basis for their rate of progression are poorly understood. Identification of different disease-causing genetic alterations in several genomic segments with either an autosomal-dominant or -recessive mode of inheritance (14, 15), as well as the existence of a number of gene polymorphisms that alter the risk for disease development (16, 17), indicate that PD is a genetically complex disorder. A large number of genes and polymorphisms have been assessed as candidate risk determinants for
PD (17). In particular, many case–control studies have addressed the putative role of apolipoprotein E (APOE) in PD susceptibility (17, 18). The ε4 allele of APOE is a widely recognized genetic risk factor for AD, whereas the ε2 allele is thought to confer protection against the disease (19).

Although the results have not been consistent, most studies did not detect a significant difference in APOE allele frequencies between patients with PD and control subjects (20–38). A recent meta-analysis of 2,157 patients with PD, however, revealed that APOE is indeed positively associated with sporadic PD (18). Furthermore, another most recent study suggested that APOE is probably responsible for the chromosome 19 linkage peak for PD (39). On the other hand, only a few studies have explicitly focused on assessing APOE as a possible determinant of PD-related pathology (32, 40–43). Thus, the possibility that APOE may modulate disease rather than play a causative role has not been addressed in depth.

Therefore, the present study is designed to examine the detailed neuropathologic features of PD cases differing in the occurrence of PD neuropathology and to identify the impact of APOE on disease progression. The definition of wider neuropathologic phenotypes of PD may augment the ability to identify genetic contributions to disease progression.

**MATERIALS AND METHODS**

**Case Material**

One hundred 8 consecutive cases of PD (46 women and 62 men; age range, 55–89 years; mean age ± standard deviation [SD], 75.1 ± 6.9 years) from the neurologic unit of the MST Hospital Group in Enschede, The Netherlands, were included in this study. PD diagnosis had been made during life and was confirmed at autopsy. Cases were included in the study after review of the final postmortem reports, clinical records, and brain sections. All autopsies have been performed between 1993 and 2003 and met the criteria for the clinical diagnosis of PD (11, 44). Clinical data collected included gender, age at death, age of PD onset, and duration of disease. To exclude AD cases with extrapyramidal signs, cognitive decline was not a criterion for exclusion if the symptoms occurred one year after the onset of parkinsonism (45). Indeed, none of the 108 PD cases experienced cognitive impairment at PD onset and none had developed dementia within the first year of PD. Thus, none fulfilled the clinical criteria for AD or dementia with Lewy bodies. The Mini-Mental State Examination (MMSE) was used to evaluate cognitive function (46). Scores used to assess the cognitive status in each case were those obtained within 12 to 18 months before death.

Because of conflicting data based on case–control studies with healthy control subjects, we investigated an additional control population (n = 108, 46 women and 62 men; age range, 55–89 years; mean age ± SD, 75.1 ± 6.9 years). Each of the 108 PD cases was pair-matched with one control on the basis of exact similarity with respect to age and gender. In few PD cases, an age deviation of ±2 years was tolerated on the part of the corresponding controls. All controls were obtained at routine autopsy from the Department of Pathology in Enschede, The Netherlands. These controls represented consecutive randomly selected autopsy series in which all cases were autopsied between 1999 and 2003. All controls underwent neuropathologic examination and none exhibited PD-related pathology. Additionally, the clinical data of all controls were collected retrospectively from the medical records and none experienced neurologic disease. Consent for autopsy was obtained for all controls as well as all PD cases and the study approved by local ethics review committees.

**Neuropathologic Analysis**

At autopsy, all PD cases fulfilled published criteria for neuropathologic diagnosis of PD (12). None of the cases had other disorders associated with the presence of major cerebral infarctions, multiple territorial infarctions,Binswanger disease, or evidence of major subcortical multiple small-vessel disease. In addition, tissue sections from all cases were evaluated according to a recently published protocol for staging of PD-related pathology (3). To determine the degree of concomitant AD-related lesions in each case, a procedure permitting differentiation of stages I–VI in the development of NFT (Braak staging) (47) and phases 1–4 in the evolution of AB deposition (48) was used. In addition, AD pathology was rated according to the guidelines of The Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) (49).

PD-related lesions were visualized with immunoreactions for α-synuclein as previously described (3). Immunostained sections were partially counterstained with hematoxylin to permit recognition of immunopositive material in particular types of nerve cells and confirm the location of the immunopositive material within specific brain regions. Areas examined included the brainstem (dorsal glossopharyngeus–vagus complex, locus ceruleus, and substantia nigra), frontoparietal and temporal neocortex, anterior and posterior hippocampus with the entorhinal and transentorhinal cortex, and visual cortex.

**Apolipoprotein E Genotyping**

For APOE genotyping, the genomic DNA was extracted from formaldehyde-fixed and paraffin-embedded brains. APOE genotypes were determined blind to all clinical and pathologic information of the cases as described previously (50).

**Statistical Methods**

Statistical analysis was performed using Pearson’s χ² test or Fisher exact test for comparisons of categorical data, and using one-way analysis of variance (ANOVA) or independent-samples t-test for comparison of mean values for normally distributed continuous outcomes among groups. The Kruskal-Wallis H-test and Mann-Whitney U test were used for non-normally distributed or ordered variables, whereas the Spearman’s rho statistics were used for measuring rank-order correlation between 2 discrete scores. To avoid a false-positive result owing to deviation from the Hardy-Weinberg equilibrium (HWE) in controls, the HWE test was performed using the standard observed-expected χ² goodness-of-fit test with df = 3 for 3 alleles.

An increase or decrease in the frequencies of APOE alleles with progression of PD stages was assessed using the Cochran-Armitage trend test. For comparison of the age at PD
RESULTS

The association of APOE with the severity of PD-related pathology (regional distribution of the pathology) could be studied in a cohort of 108 patients with PD and 108 pair-matched controls. Comparisons of demographic, clinical, pathologic, and genetic features of all PD cases and controls are summarized in Table 1. Here, the underlying pathology in all PD cases ranged between PD stages 3 and 6. Given the varying degrees of PD-related pathology in this series as assessed by the newly introduced PD staging system (3), cases also were analyzed by subgroups according to their PD pathology: 1) PD cases with moderate pathology (PD stages 3–4) and 2) those with severe or endpoint pathology (PD stages 5–6).

As shown in Table 2, 2 of the 108 PD cases were devoid of AD-related lesions. NFT pathology was not prominent in PD cases, with 80% of all cases exhibiting no or mild NFT pathology ranging from NFT stages 0–II (median, Braak stage II). Thirty-one cases lacked Aβ deposits, whereas most showed abundant Aβ deposits (median, Aβ phase 2). Furthermore, Table 3 shows that only 2 PD cases fulfilled the neuropathologic criteria for high likelihood AD (CERAD C, Braak stages V–VI), and only 10 PD cases met the criteria for intermediate likelihood AD (CERAD B–C, Braak stages III–IV). Most PD cases (n = 96) revealed low likelihood AD (CERAD 0–A, Braak stages 0–III [n = 85] and CERAD B–C, Braak stages 0–II [n = 11]).

According to their last MMSE scores before death, all patients with PD could be stratified as nondemented (MMSE scores 30–25; n = 17) or demented (MMSE scores < 25; n = 84). MMSE scores were unavailable for 7 cases. The distribution of the APOE polymorphism in PD individuals and controls appears in Table 1. The overall allele frequencies for all PD individuals were 10.2% for ε2, 67.2% for ε3, and 22.7% for ε4. By comparison, the allele frequencies for controls were 6.9% for ε2, 77.8% for ε3, and 15.3% for ε4. The distribution of APOE genotypes in controls was comparable to that in other studies of APOE genotype frequencies in the general population.

### TABLE 1. Characteristics of All Controls and Patients with Parkinson Disease (PD)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>Stage 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size, n</td>
<td>108</td>
<td>16</td>
<td>45</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>Men/women</td>
<td>62/46</td>
<td>12/4</td>
<td>28/17</td>
<td>21/19</td>
<td>1/6</td>
</tr>
<tr>
<td>Mean age at death</td>
<td>75.1 ± 6.9</td>
<td>73.4 ± 7.4</td>
<td>75.5 ± 7.1</td>
<td>75.6 ± 6.5</td>
<td>73.7 ± 6.8</td>
</tr>
<tr>
<td>Mean age at onset</td>
<td>—</td>
<td>66 ± 11</td>
<td>65.9 ± 10</td>
<td>65.3 ± 9.7</td>
<td>67.2 ± 7</td>
</tr>
<tr>
<td>Disease duration (range)</td>
<td>—</td>
<td>1–26</td>
<td>1–36</td>
<td>1–38</td>
<td>4–10</td>
</tr>
<tr>
<td>Disease duration (median)</td>
<td>—</td>
<td>6</td>
<td>9.5</td>
<td>10</td>
<td>7.5</td>
</tr>
<tr>
<td>Mean MMSE score*</td>
<td>—</td>
<td>21.6 ± 4.5</td>
<td>18.1 ± 6.3</td>
<td>14.1 ± 5.2</td>
<td>13.2 ± 3.6</td>
</tr>
<tr>
<td>Median Braak stage†</td>
<td>—</td>
<td>I</td>
<td>II</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Median Aβ phase‡</td>
<td>—</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Median CERAD§</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Frequency of APOE genotypes</td>
<td>—</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>ε2/ε2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>70</td>
<td>13</td>
<td>25</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>21</td>
<td>1</td>
<td>11</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Frequency of APOE alleles¶</td>
<td>—</td>
<td>15 (0.069)</td>
<td>2 (0.063)</td>
<td>8 (0.089)</td>
<td>11 (0.0138)</td>
</tr>
<tr>
<td>ε2</td>
<td>168 (0.778)</td>
<td>27 (0.844)</td>
<td>63 (0.7)</td>
<td>48 (0.6)</td>
<td>7 (0.5)</td>
</tr>
<tr>
<td>ε3</td>
<td>33 (0.153)</td>
<td>3 (0.094)</td>
<td>19 (0.211)</td>
<td>21 (0.263)</td>
<td>6 (0.429)</td>
</tr>
</tbody>
</table>

* Mean values are expressed as mean ± standard deviation; ages and duration of disease (range and median) are given in years; parenthetical values represent actual frequencies of genotypes and alleles.
† Correlation between PD stages and MMSE score: r = −0.49; p < 0.0001.
‡ Correlation between PD stages and Braak stages: r = 0.43; p < 0.0001.
§ Correlation between PD stages and CERAD: r = 0.47; p < 0.0001.
¶ Allele distribution, PD individuals versus controls: χ² = 8.02; p = 0.02.
Aβ, β-amyloid; APOE, apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer’s disease; MMSE, Mini-Mental State Examination.
**TABLE 2. Aβ Phases and Braak Stages in All Individuals With Parkinson Disease**

<table>
<thead>
<tr>
<th>Aβ Phases (0–4)</th>
<th>0 (n = 31)</th>
<th>1 (n = 15)</th>
<th>2 (n = 24)</th>
<th>3 (n = 27)</th>
<th>4 (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braak Stages (0–VI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (n = 2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I (n = 39)</td>
<td>15</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>II (n = 45)</td>
<td>13</td>
<td>4</td>
<td>13</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>III (n = 19)</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>IV (n = 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>V (n = 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>VI (n = 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Aβ, β-amyloid.

**TABLE 3. CERAD and Braak Stages in All Individuals With Parkinson Disease**

<table>
<thead>
<tr>
<th>CERAD (0–C)</th>
<th>0 (n = 75)</th>
<th>A (n = 10)</th>
<th>B (n = 14)</th>
<th>C (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braak Stages (0–VI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (n = 2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I (n = 39)</td>
<td>37</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>II (n = 45)</td>
<td>31</td>
<td>4</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>III (n = 19)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>IV (n = 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>V (n = 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>VI (n = 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

CERAD, Consortium to Establish a Registry for Alzheimer Disease.

The distribution of APOE alleles (Table 1) was significantly altered between PD individuals and controls ($\chi^2 = 8.02$, df = 2, $p = 0.02$). In the total patient cohort, the Cochran-Armitage trend test revealed a significant reciprocal change in the e3 and e4 alleles of APOE across PD stages (see also Table 1 and Fig. 1). Although the frequency of the e4 allele showed a significant progressive increase from PD stage 3 (9.4%) to PD stage 6 (42.9%) ($p = 0.01$), the e3 allele frequency decreased from PD stage 3 (84.4%) to PD stage 6 (50%) ($p = 0.002$). In addition, there was a trend toward an increasing frequency of the e2 allele with advancing PD stages, although not significant ($p = 0.2$). In the statistical analyses, no distinction was made between individuals heterozygous and homozygous for e2 and e4 alleles of APOE because of the small number of homozygotes in the present sample ($n = 6$ for e2 allele and $n = 10$ for e4 allele).

By contrast, no relationship existed between APOE polymorphism and age at death ($p = 0.8$), MMSE scores ($p = 0.4$), Aβ phases ($p = 0.9$), CERAD ($p = 0.8$), or Braak stages ($p = 0.7$). However, although not significant ($p = 0.6$), disease duration was longer in carriers of the e4 allele than in their counterparts across all PD stages. As shown in Figure 2, Kaplan-Meier survival analysis revealed no influence of APOE polymorphism (neither in the analysis of carriers of the e4 allele versus noncarriers nor in the analysis of carriers of the e2 and e4 alleles versus homozygotes for the e3 allele) on the age at PD onset (log rank = 0.2, df = 1, $p = 0.7$). The Cox proportional hazard model, however, revealed that APOE genotype and gender were not significant predictors of the rate of disease progression. Rather, only age of PD onset was an independent predictor for rate of disease progression, whereby late-onset patients experienced more rapid progression to the neuropathologic end point than early-onset patients. This creates prognostic gradients within groups of PD cases; for example, a person with an onset of 60 years would reach PD stages 5 to 6 within a median of 15 years, whereas an older person of age 70 years would reach these stages within 10 years (Fig. 2).
studies have been conducted involving more than 2,500 PD (17). Since the appearance of that review, however, other reported the lack of association of APOE polymorphism with a meta-analysis performed in 2000 of 21 independent studies inconsistencies. limited statistical power, may have contributed to these study design, coupled with the fact that most studies had very able literature indicates that differences in ethnic back- showing no association (20–29, 31–34, 36–38). The avail- showing significant associations (30, 52–62) and others previous studies generated conflicting results with some polymorphism on the risk for PD has been uncertain because APOE polymorphism may influence PD by modifying the PD- PD was apparent. Taken together, it can be speculated that increase in the frequency of the e allele of APOE with advancing neuropathologic stages of individuals with PD as opposed to controls. Furthermore, an increase in the frequency of the e allele and a decrease of the e3 allele of APOE with advancing neuropathologic stages of PD was apparent. Taken together, it can be speculated that APOE polymorphism may influence PD by modifying the PD-related pathologic burden in the brain.

Despite many population studies, the impact of APOE polymorphism on the risk for PD has been uncertain because previous studies generated conflicting results with some showing significant associations (30, 52–62) and others showing no association (20–29, 31–34, 36–38). The available literature indicates that differences in ethnic background, PD end points (demented or nondemented), and study design, coupled with the fact that most studies had very limited statistical power, may have contributed to these inconsistencies.

To understand the cumulative evidence on this topic, a meta-analysis performed in 2000 of 21 independent studies reported the lack of association of APOE polymorphism with PD (17). Since the appearance of that review, however, other studies have been conducted involving more than 2,500 additional patients with PD. The most recent meta-analysis (18) of more than 22 association studies of PD supports the notion that the e2 allele is significantly related to an increased risk for PD, whereas the e4 allele lacks such an effect. The results of the present study are partially in accord with this most recent meta-analysis regarding the e2 allele.

Although meta-analyses can at least partially alleviate the problem of inadequate statistical power, they cannot control the problems of publication and reporting bias. In this context, there are some potential limitations of the meta-analyses performed in 2000 and 2004 regarding PD and APOE (17, 18). First, neither study took into account the effects of cognitive status, ethnic background, and neuropathologic status. Second, it seems that the effect of the e2 allele of APOE in PD was not considered in the initial meta-analysis, and this could have led to discrepancies in the conclusions reached by both meta-analyses (17, 18). Thus, results from meta-analyses of published data cannot definitely exclude a possible role for the e4 allele of APOE in PD. The crucial discrepancies in previous studies, including those from both meta-analyses, dominate the literature, and this should caution us to reserve judgment about debates regarding the association of PD with APOE. In this context, it should be mentioned that another recent report suggested that APOE is probably responsible for the chromosome 19 linkage peak for PD (39).

On the other hand, several potential limitations of most association studies of APOE and PD should be noted. First, from a total of 31 genetic association studies of APOE with PD reported to date, 8 involved fewer than 50 PD cases (20, 22, 26, 33, 36, 55, 58, 62). These studies are too small and apparently lacked the statistical power to reliably confirm or refute the putative effects of APOE on PD. Second, 22 from 31 studies included clinically diagnosed PD without pathologic diagnostic confirmation (21, 22, 27–31, 34–38, 52, 54–56, 59–64). These studies may blunt any existing genetic association with PD because clinicopathologic studies have shown significant false-positive and false-negative rates for diagnosing PD (44, 53). Third, 13 of 31 studies did not provide information about the cognitive status of their PD cases, whereas another 9 studies included only nondemented PD cases (20, 21, 27, 34–36, 38, 61, 62). Here, it should be pointed out that previous studies have reported less conflicting results regarding the association between APOE and PD plus dementia: most studies reported a positive association (43, 52, 54, 55, 58, 59); fewer reported the lack of an association (28, 29, 31, 33). Our results are in line with those from studies that show an association between APOE and PD plus dementia because 80% of our patients were demented. In fact, to the best of our knowledge, the present study involves the highest number of demented patients with PD for association of APOE with PD; the average number of clinically and pathologically verified PD cases in previous studies was only 11 (33, 43, 58). There were no significant differences in the frequencies of APOE genotypes and alleles between patients with PD with and without dementia in our series, but demented individuals were older than their nondemented counterparts. Nonetheless, we cannot draw firm conclusions about whether APOE polymorphism is associated with PD without dementia because the number of patients in this group was too small.

FIGURE 2. Kaplan-Meier curves comparing ages at onset of Parkinson disease (PD) according to apolipoprotein E e allele status (dashed line represents carriers and continuous line represents noncarriers of e4 allele). The logrank test was not significant (p = 0.7).

DISCUSSION

The present study aimed to assess the impact of APOE genotype on the neuropathologic lesions in PD with and without dementia. In addition, the prevalence of APOE alleles between PD individuals and controls was compared. Our results reveal that APOE polymorphism was associated with PD in which the alleles e2 and e4 were overrepresented in individuals with PD as opposed to controls. Furthermore, an increase in the frequency of the e4 allele and a decrease of the e3 allele of APOE with advancing neuropathologic stages of PD was apparent. Taken together, it can be speculated that APOE polymorphism may influence PD by modifying the PD-related pathologic burden in the brain.

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The neuropathology in PD varies considerably in severity. To facilitate allocation of patients to different PD severity groups, we used a staging system for sporadic PD proposed by Braak et al (3). Patients were classified as having moderate or severe PD with respect to their PD stages. Here, our results indicate that APOE genotype accounts for PD pathology in which the frequency of ε4 allele increased and that of ε3 allele decreased with higher neuropathologic PD stages. Additionally, although not significant, there was a trend toward increased frequency of ε2 allele of APOE with increasing PD stages. The lack of significance could be explained by a small number of ε2 allele carriers in the PD cases examined here. These findings lend support to the pathophysiological relevance of APOE for the PD-related pathologic process. In this context, most of the earlier studies that measured the association between PD-related pathology and APOE have consistently showed a positive association (40–43). However, some methodological features of our study should be mentioned in comparison with previous ones. We used a neuropathologic staging system for PD that assumes that the severity of the underlying neuropathologic process as a whole is largely defined by the topographic distribution of the key lesions in specific brain regions. This hierarchically based procedure enabled us to explore the impact of APOE on the severity of the PD-related brain lesions. By contrast, previous studies used a quantitative neuropathologic approach measuring the density of LBs in given brain regions. They applied arbitrary numeric cutoffs for PD-related lesions to define the severity of PD for each individual.

Caution, however, is needed to avoid misreading the results gained from lesional density analyses of samples. For instance, PD is characterized by depletion of neurons and the presence of intraneuronal LBs and Lewy Ns (LNs) in a proportion of surviving neurons at nigral and extranigral brain sites. According to this definition of PD, neurons are required for the production of LBs while at the same time implying that brain regions with marked neuronal loss may lack the lesions. This could introduce a degree of inaccuracy in individual classifications with respect to lesional density because patients with severe PD may display extensive neuronal loss and less lesional density than patients with moderate PD. For this reason, assessment of the anatomic distribution of PD-related pathology according to the proposed PD staging system for sporadic PD may be preferable to define the severity of PD-related pathology because the staging system takes into account the extent of the brain regions and various neuronal systems involved.

We reasoned that if APOE indeed modifies the development of the underlying PD pathology, there might be differences in severity of disease—and the rate at which the disease progresses—between groups with and without the ε4 allele. The rate of disease progression can be estimated when age, disease onset, and duration of disease are considered in a multivariate model. Hence, when using the multivariate hazard proportional ratio, the age at onset turned out to be the major determinant for the rapid disease progression of PD and not APOE.

In this context, it is known that progression rates of PD vary between subjects and previous clinically based studies have consistently reported that patients with PD with late-onset show a more rapid rate of disease deterioration than early-onset ones (65–68). Our findings, which show the importance of age of PD onset for subsequent deterioration of the neuropathologic picture in PD, corroborate these studies. This indicates that aging may have an effect on the rate of PD progression. Thus, age of PD onset may prove to be of important prognostic value in clinical practice. This, in turn, has important implications as the number of patients with PD increases with age.

That is not to say, however, that the ε4 allele has no effect on the neuropathologic course of PD. Instead, the effect of APOE on the rate of PD progression could not be detected when duration of disease is considered, thereby indicating that disease duration might be a factor that potentially interferes with the association between APOE and PD pathology. In this connection, it should be mentioned that, although not significant, disease duration was longer in carriers of the ε4 allele than in their counterparts across all PD stages. This implies that duration of disease indeed is a potential confounder in which the ε4 allele carriers might survive longer than their counterparts after disease onset, thus allowing PD cases with ε4 allele to reach higher PD stages. Taken together, we think that the APOE ε4 allele is associated with the severity or progression of PD pathology in that it modifies the development of the PD-related pathology without necessarily affecting the rate at which the pathology progresses. Alternatively, if it does, this effect is likely to be modest. In other words, APOE by itself is not a reliable indicator for the presence or rate of PD progression and can only be interpreted in the context of its association with PD pathologic phenotype. Finally, because it is difficult to speculate about the underlying biologic mechanisms by which APOE influences the development of PD-related pathology, we suggest that an adequate explanation of these neural underpinnings can only be provided when knowledge of the basic biology of this complex disease improves.

Several studies have assessed the association of APOE polymorphism with age of onset. Some of them reported an association of APOE with an earlier age of PD onset (30, 57, 64), whereas others produced the opposite results (23, 28, 34, 59). Our findings do not show a significant influence of APOE genotypes on the age of disease onset. These inconsistencies may be a result of case characteristics in which previous studies with a positive association consisted primarily of cases with a family history of PD (57, 64). Because the individuals in the present series had no family history of the PD, a possible effect of APOE on the age of onset of familial PD cannot definitely be ruled out.

Most of our patients with PD did not have significant AD-related pathology and 10% showed moderate AD-related pathology without sufficient lesional burdens to warrant a postmortem diagnosis of AD. Remarkably, MMSE scores of patients were significantly correlated with stages of the AD-related pathology and PD pathology. Conversely, although PD stages were associated with Aβ and NFT stages as previously reported (69) and, as observed already, with APOE polymorphism; nevertheless, the extent of the AD-related lesions in our PD series was not influenced by APOE genotypes. This last finding confirms some earlier reports (20, 22, 33) but conflicts with another (43). In all PD cases investigated here,
the frequency of the ε4 allele of APOE remained elevated (22.5%) even when cases with advanced concomitant AD pathology were excluded. It is worth noting, however, that although it failed to reach significance, there was a trend toward an increase in the frequency of the ε4 allele of APOE with advancing medians of AD-related lesions, thereby pointing to a possible influence of APOE on AD-related lesions in PD cases. Here, the lack of any association between APOE and the presence of AD pathology may reflect the mild occurrence of these lesions in the PD cases studied. A possible effect of APOE polymorphism on the development of AD pathology in individuals with PD, therefore, cannot be eliminated definitively because most persons with PD in this series had negligible or no AD pathology in which a floor effect no longer can discriminate between group differences.

In summary, the study provides evidence that APOE is associated with PD and PD-related pathology, thereby indicating that APOE genotypes may influence PD risk, at least in part, through modification of the cardinal neuropathologic PD phenotype. Moreover, our findings reveal that age of disease onset, but not APOE, is a principal determinant for a more rapid progression on the part of PD-related pathology. The strength of our association study lies in the careful characterization of the clinical and pathologic phenotype of PD cases, the inclusion of a relatively large number of patients of PD, and the close individual matching of PD cases and controls for demographic features.

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REFERENCES


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