Apolipoprotein E ε4 is a Determinant for Alzheimer-Type Pathologic Features in Tauopathies, Synucleinopathies, and Frontotemporal Degeneration

Keith A. Josephs, MST, MD; Yoshio Tsuboi, MD; Natalie Cookson, BSc; Hilary Watt, MSc; Dennis W. Dickson, MD

Objectives: To determine if apolipoprotein E ε4 influences the frequency of Alzheimer-type pathologic features in tauopathies, synucleinopathies, and frontotemporal degeneration and to determine if the frequency of Alzheimer-type pathologic features in synucleinopathies is similar to the frequency of such features in tauopathies and frontotemporal degeneration.

Methods: A total of 285 patients with pathologically proven neurodegenerative disorders, including diffuse and transitional Lewy body disease, frontotemporal degeneration, progressive supranuclear palsy, corticobasal degeneration, and multiple system atrophy, with a mean age of 75.1 ± 9.3 years, were suitable for genetic and pathological analysis. Disorders were grouped as tauopathies (progressive supranuclear palsy and corticobasal degeneration), synucleinopathies (Lewy body disease and multiple system atrophy), and frontotemporal degeneration. Braak neurofibrillary tangle staging and quantitative scores of senile plaques were used to determine the degree of concomitant Alzheimer-type pathologic features in each case, and apolipoprotein E genotype was determined from DNA isolated from frozen brain tissue. The relationship of apolipoprotein E ε4 to Alzheimer-type pathologic features was determined.

Results: Across all neurodegenerative disorders, apolipoprotein E ε4 and older age independently predicted the co-occurrence of Alzheimer-type pathologic features (P < .001), whereas female sex had a lesser effect (P = .03). When divided into the 3 subgroups (tauopathies, synucleinopathies, and frontotemporal degeneration), apolipoprotein E ε4 had a similar effect, whereas older age and female sex were less predictive. There was a significant difference between the frequency of Alzheimer-type pathologic features in synucleinopathies and the frequency of such features in tauopathies and frontotemporal degeneration (P < .001 for both). The frequency of apolipoprotein E ε4 allele was not significantly different among the 3 groups.

Conclusions: Apolipoprotein E ε4, independent of older age and sex, contributes to the co-occurrence of Alzheimer-type pathologic features in tauopathies, synucleinopathies, and frontotemporal degeneration, but this does not explain why Alzheimer-type pathologic features are significantly more likely to coexist with synucleinopathies than with either tauopathies or frontotemporal degeneration.

Arch Neurol. 2004;61:1579-1584

Apolipoprotein E ε4 (APOE ε4) is a known risk factor for the development of Alzheimer disease (AD) with increased amyloid burden but with less consistent effect on tau pathology. In one series of patients with late-onset probable AD who underwent autopsies, the chance of having autopsy-confirmed AD, given the presence of an APOE ε4 allele, was 75%. Because of the influence of APOE ε4 in AD, many researchers have wondered whether the APOE ε4 allele may also increase the risk for the development of other neurodegenerative disorders. Although there is no evidence that APOE ε4 influences the pathologic features of progressive supranuclear palsy (PSP), multiple system atrophy, or frontotemporal degeneration, studies on Lewy body disease (LBD) have been controversial. (In this study, the term LBD includes braintem-LBD, transitional-LBD, and diffuse-LBD.) The controversy with LBD stems from the fact that when LBD and Alzheimer-type pathologic features (senile plaques [SP] and neurofibrillary tangles [NFT]) coexist, some researches have postulated 2 separate diseases, but others have postulated a variant of LBD, the Lewy body variant.

Regardless, we and other authors have demonstrated an increased frequency of the APOE ε4 allele in cases of mixed LBD and Alzheimer-type pathologic features. Also, more recently, Mann et al demonstrated a similar finding in cases of frontotemporal degeneration and Alzheimer-type pathologic features.
Because the most recent classification of neurodegenerative disorders includes 3 main categories (tauopathies, synucleinopathies, and frontotemporal degeneration), we set out to determine if having the ApoE ε4 genotype is a determinant for coexisting Alzheimer-type pathologic features across all tauopathies, synucleinopathies, and frontotemporal degeneration. We also set out to determine if there would be a difference between each of the 3 groups in terms of the coexistence of Alzheimer-type pathologic features.

METHODS

CASES

The autopsy data bank at the Mayo Clinic in Jacksonville, Fla, was used to find all autopsy-confirmed cases of tauopathies, synucleinopathies, and frontotemporal degeneration between 1998 and 2002. Tauopathies were PSP (n = 137) and corticobasal degeneration (n = 18). Synucleinopathies were diffuse-LBD/transitional-LBD (n = 93) and multiple system atrophy (n = 12). Frontotemporal degeneration cases were Pick disease, frontotemporal dementia with parkinsonism linked to chromosome 17, frontotemporal lobar degeneration with or without motor neuron disease but with motor neuron disease–type inclusions, and dementia lacking distinct histopathological features (n = 23). Cases were selected upon availability of both fixed tissue for histopathologic studies and frozen tissue for genetic studies.

NEUROPATHOLOGIC METHODS

Neuropathologic diagnoses were made using a standardized dissection, sampling and staining protocol that also included counts of SP and NFT in multiple brain regions using thioflavin-S fluorescent microscopy to assess Alzheimer-type pathologic features according to our laboratory’s routine procedures. Alzheimer-type pathologic features that were assessed in all cases included lesion counts of SP and NFT in multimodal association areas of middle frontal, superior temporal, and inferior parietal cortices, as well as in primary motor and visual cortices using thioflavin-S fluorescent microscopy. The maximal density of SP and NFT in each area was recorded; the averages of each lesion type in the 3 association areas and the 2 primary areas were calculated for each case and used for further analysis. The distribution and severity of Alzheimer-type neurofibrillary degeneration were also estimated with Braak NFT staging.

DIAGNOSTIC CATEGORIES OF ALZHEIMER-TYPE PATHOLOGIC FEATURES

All cases met consensus pathologic criteria for PSP,18 corticobasal degeneration,19 transitional-LBD/diffuse-LBD,20 frontotemporal degeneration,21 and multiple system atrophy.22 Alzheimer-type pathologic features occurring in each of the 3 groups (tauopathies, synucleinopathies, and frontotemporal degeneration) were subdivided into AD or pathologic aging. Alzheimer disease was diagnosed if there were numerous cortical SP (sufficient to meet Khachaturian criteria for AD, but composed mostly of diffuse, nonneuritic amyloid deposits, and with a Braak stage of III or lower.23 Cases with few or no SP and a Braak stage of III or lower were considered to be pure (pure disease). Alzheimer-type pathologic features imply either AD or pathologic aging.

APOE GENOTYPE

Apolipoprotein E testing was performed on all 285 cases. Genomic DNA was extracted from fresh frozen brain using the QIAamp DNA mini kit (Qiagen, Bothell, Wash). Amplification was carried out using the single-day apolipoprotein ε method used by Crook et al,26 with the following modifications. The DNA was amplified using a Hybrid Touchdown thermal cycler (Hybaid, Cambridge, England). Conditions were an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 60°C to 50°C touchdown annealing for 30 seconds at 0.5°C/cycle, and 72°C for 45 seconds, with a final extension at 72°C for 10 minutes. The primer sequences used for this amplification were forward 5'-TAAGCTTGACCGGTGTCCAAGGA-3' and reverse 5'-ACAGAATTCCGCCC-GGCTGGTACAC-3'. After amplification, 5 units of Cfo I (Promega, Madison, Wis) enzyme and its buffer were added directly to 20 µL of polymerase chain reaction product and were incubated at 37°C for 5 hours. The digest was run on a 4% agarose gel with 1 × Tris-borate-EDTA buffer giving main fragment sizes of 91, 83, 72, and 48 base pairs.

STATISTICAL ANALYSIS

The differences in the frequencies of the ApoE ε4 allele between diagnostic categories of pure disease and Alzheimer-type pathologic features within each group and the difference in the frequency of cases with Alzheimer-type pathologic features between groups were tested by using a 2-by-2 contingency analysis of the χ² test. For small numbers, Fisher exact test was used. Multiple logistic regression analysis was used to evaluate associations between ApoE ε4 allele frequency, age, sex, disease, and odds of having concomitant AD pathologic features. Data were analyzed by using Stata statistical software (Stata 6.0; Stata Corp, College Station, Tex), with a significance level set at P < .05.

RESULTS

Comparisons of the demographics and the pathologic and genetic features of all 3 groups are summarized in Table 1. The mean ages were 75.1 years for all 285 patients included in this study, 74 years for patients with tauopathies, 77.5 years for patients with synucleinopathies, and 71.2 years for patients with frontotemporal degeneration. Of all 285 patients, 49% were women. The mean ± SD Braak NFT stage was 3.0 ± 1.7, and the average SP count was 21.7. As expected based on the diagnostic criteria algorithm, the Braak NFT stage and SP count were higher in patients with AD than in patients with pathologic aging and patients with pure disease; they were higher for all patients and for patients with tauopathies, synucleinopathies, and frontotemporal degeneration. Of all the patients, 35% had at least 1 ApoE ε4 allele.

Of the 285 cases, 156 (55%) were considered pure, whereas 129 (45%) had concomitant Alzheimer-type pathologic features, which are defined as consisting of all cases with pathologic aging (n = 43, 15%) plus all cases...
with concomitant AD (n=86, 30%). The majority (79%) of patients with a diagnosis of synucleinopathies had a concomitant diagnosis of Alzheimer-type pathologic features compared with only 24% and 35% of patients diagnosed as having either tauopathies or frontotemporal degeneration, respectively (P<.001 for association between concomitant Alzheimer-type pathologic features and disease diagnosed). Of all patients with concomitant AD, 65% were women, compared with 40% of patients with pure disease. This trend of more women among patients with concomitant AD is statistically significant (P=.001) and remained across all 3 groups: tauopathies (71% of patients with AD were women vs 42% of patients with pure disease), synucleinopathies (63% vs 35%), and frontotemporal degeneration (100% vs 40%). Similarly, patients with concomitant AD tended to be older at death (P<.001). The average age at death was 72 years in patients with pure disease, 77.1 years in patients with pathological aging, and 79.5 years in patients with concomitant AD. Of the patients with pure disease, 15% had at least 1 APOE ε4 allele, compared with 47% of patients with pathological aging and 65% of patients with AD (P<.001 for differences in presence of ε4 allele(s) by status of AD pathologic features). When each group was analyzed separately, similar results were noted as follows. The presence of at least 1 ε4 allele was significantly higher in tauopathies/Alzheimer-type pathologic features (43%) than in tauopathies/pure disease (15%; P=.001), higher in synucleinopathies/Alzheimer-type pathologic features (64%) than in synucleinopathies/pure disease (13%; P<.001), and higher in frontotemporal degeneration/Alzheimer-type pathologic features (75%) than in frontotemporal degeneration/pure disease (20%; P=.02).

Table 2 shows the results for associations between risk factors and concurrent Alzheimer-type pathologic features, shown as odds ratios (OR) for concurrent Alzheimer-type pathologic features (and then specifically for pathological aging and AD) compared with pure disease. Unadjusted OR are shown, as well as OR adjusted for other predictor variables, derived from multiple logistic regression analysis. These show that the associations found are largely independent of each other (ie, adjusted OR are similar in size to unadjusted OR). The presence of at least 1 APOE ε4 allele (OR, 8.41; P<.001), and age at death (OR, 2.41 for a 10-year increase; P<.001) independently predicted the co-occurrence of Alzheimer-type pathologic features (P<.001), whereas female sex was less predictive (OR, 2.13; P=.03). When OR for being

### Table 1. Demographics of All Cases, Tauopathies, Synucleinopathies, and Frontotemporal Degeneration

<table>
<thead>
<tr>
<th></th>
<th>No. of Patients</th>
<th>Age at Death, y Mean ± SD</th>
<th>Women, %</th>
<th>Braak Neurofibrillary Tangle Stage, Mean ± SD</th>
<th>Average Senile Plaque Count</th>
<th>Apolipoprotein E ε4/+, %*</th>
<th>Apolipoprotein E ε4/−, %**</th>
<th>Apolipoprotein E ε4+, %‡</th>
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<tr>
<td><strong>All cases</strong></td>
<td>285</td>
<td>75.1 ± 9.3</td>
<td>49</td>
<td>3.0 ± 1.7</td>
<td>21.7</td>
<td>30</td>
<td>5</td>
<td>35</td>
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<tr>
<td><strong>Pure disease cases‡</strong></td>
<td>156</td>
<td>72.0 ± 9.6</td>
<td>40</td>
<td>1.9 ± 1.1</td>
<td>3.1</td>
<td>15</td>
<td>0</td>
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<td><strong>Pathologic aging</strong></td>
<td>43</td>
<td>77.1 ± 9.8</td>
<td>51</td>
<td>2.5 ± 0.7</td>
<td>38.5</td>
<td>40</td>
<td>7</td>
<td>47</td>
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<td><strong>Alzheimer disease</strong></td>
<td>86</td>
<td>79.5 ± 7.3</td>
<td>65</td>
<td>5.1 ± 0.8</td>
<td>46.9</td>
<td>51</td>
<td>14</td>
<td>65</td>
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<td><strong>Alzheimer-type pathologic features</strong></td>
<td>129</td>
<td>78.7 ± 7.5</td>
<td>60</td>
<td>4.2 ± 1.4</td>
<td>44.1</td>
<td>47</td>
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<td>74.0 ± 8.3</td>
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<td>2.3 ± 1.2</td>
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<tr>
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<td>73.1 ± 8.5</td>
<td>42</td>
<td>2.1 ± 1.1</td>
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<td><strong>Pathologic aging</strong></td>
<td>23</td>
<td>76.9 ± 7.2</td>
<td>61</td>
<td>2.5 ± 0.7</td>
<td>36.9</td>
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<td>76.4 ± 7.9</td>
<td>71</td>
<td>4.4 ± 0.5</td>
<td>41.4</td>
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<td>50</td>
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<td><strong>Alzheimer-type pathologic features</strong></td>
<td>37</td>
<td>76.7 ± 7.3</td>
<td>65</td>
<td>3.2 ± 1.1</td>
<td>38.6</td>
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<td><strong>All cases</strong></td>
<td>107</td>
<td>77.5 ± 9.2</td>
<td>53</td>
<td>4.2 ± 0.7</td>
<td>37.7</td>
<td>42</td>
<td>11</td>
<td>53</td>
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<tr>
<td><strong>Pure disease cases‡</strong></td>
<td>23</td>
<td>69.5 ± 11.0</td>
<td>35</td>
<td>2.0 ± 1.0</td>
<td>2.9</td>
<td>13</td>
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<td>14</td>
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<td>36</td>
<td>2.4 ± 0.9</td>
<td>42.6</td>
<td>36</td>
<td>14</td>
<td>50</td>
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<td><strong>Alzheimer disease</strong></td>
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<td>80.2 ± 7.2</td>
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<td>5.2 ± 0.7</td>
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<td>53</td>
<td>14</td>
<td>67</td>
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<td><strong>Alzheimer-type pathologic features</strong></td>
<td>83</td>
<td>79.5 ± 7.4</td>
<td>58</td>
<td>4.8 ± 1.3</td>
<td>47.2</td>
<td>50</td>
<td>14</td>
<td>64</td>
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<td>14.1</td>
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<tr>
<td><strong>Pure disease cases‡</strong></td>
<td>15</td>
<td>66.8 ± 14.0</td>
<td>40</td>
<td>1.1 ± 1.2</td>
<td>1.9</td>
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<tr>
<td><strong>Pathologic aging</strong></td>
<td>6</td>
<td>79.5 ± 10.4</td>
<td>50</td>
<td>2.5 ± 0.8</td>
<td>35.1</td>
<td>67</td>
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<td><strong>Alzheimer disease</strong></td>
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<td>77.0 ± 4.2</td>
<td>100</td>
<td>4.0 ± 0.0</td>
<td>43.0</td>
<td>100</td>
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<td><strong>Alzheimer-type pathologic features</strong></td>
<td>8</td>
<td>78.9 ± 9.0</td>
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<td>37.1</td>
<td>75</td>
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*+/− indicates the presence of 1 apolipoprotein E ε4 allele.
†+/+ indicates the presence of 2 apolipoprotein E ε4 alleles.
‡+ indicates the chance of having either 1 apolipoprotein E ε4 allele or 2 apolipoprotein E ε4 alleles.
§Cases with few or no senile plaques and a Braak stage of III or lower were considered to be pure.
affected by pathologic aging and AD were considered separately, stronger OR were found with AD than with pathologic aging, which is as expected because pathologic aging is somewhere between pure disease and AD. For instance, the OR of having pathologic aging (compared with pure disease) among those with an ApoE ε4 allele is 5.63, compared with an OR of 12.8 for those with AD (compared with pure disease). An association between Alzheimer-type pathologic features and synucleinopathies was significantly different compared with Alzheimer-type pathologic features and tauopathies (OR, 9.99; P <.001) and Alzheimer-type pathologic features and frontotemporal degeneration (OR, 7.37; P <.01). There is no evidence that strengths of association between age, sex, and ApoE ε4 status are any different between different diseases (assessed by testing for interactions between disease status and each predictor variable in turn).

In this series of 285 cases of neurodegenerative disorders, including frontotemporal degeneration, corticobasal degeneration, PSP, LBD, and multiple system atrophy, the frequency of having at least 1 ApoE ε4 allele was 35%. This finding is similar to what we and others have reported in smaller pathologically confirmed series of non-AD dementias (40%) and PSP (29%). When all of our cases were divided into pure cases vs cases with Alzheimer-type pathologic features, the frequency of ApoE ε4 was found to be significantly different, 15% and 59%, respectively (P <.001). The 15% observed in our pure cases is similar to what we observed in pure PSP cases (23%) and similar to what others have reported in autopsy-proven controls, whereas 59% is within the range of what we and others have reported in autopsy-proven AD cases. This significant difference demonstrates that the ApoE ε4 allele is a determinant for Alzheimer-type pathologic features across all neurodegenerative disorders. We also found that this significance remained true for diseases when classified as tauopa-thies, synucleinopathies, and frontotemporal degeneration, again demonstrating that ApoE ε4 does influence coexisting Alzheimer-type pathologic features in synucleinopathies, tauopathies, and frontotemporal degeneration. This finding is similar to what we reported in a smaller series of patients with PSP only, where we also demonstrated significant correlations between the ApoE ε4 carrier status and coexisting Alzheimer-type pathologic features. In keeping with the above finding was the fact that the frequency of the ApoE ε4 allele/alleles increased as the Alzheimer-type pathologic features evolved from absent (pure) cases to cases with pathologic aging and finally to cases with full-blown AD for all neurodegenerative disorders as well as for each of the 3 groups, synucleinopathies, tauopathies, and frontotemporal degeneration (Figure 1). We also demonstrated that in addition to ApoE ε4, older age and female sex are also independent risk factors for the development of Alzheimer-type pathologic features.

One of the most interesting findings was a significant difference in the frequency of Alzheimer-type pathologic features in the synucleinopathies compared with the tauopathies and frontotemporal degeneration. Of the 107 synucleinopathies, 84 (79%) had coexisting Alzheimer-type pathologic features compared with only 24% of the tauopathies and 38% of the frontotemporal degeneration cases (Figure 2). This difference suggests that Alzheimer-type pathologic features are more likely to coexist with synucleinopathies than with tauopathies or frontotemporal degeneration. This difference was not explained by a difference in age or sex between patients with synucleinopathies and the other 2 groups. We had considered the possibility that this difference between the synucleinopathies group and the other 2 groups was due to ApoE ε4. However, when we separated the cases with Alzheimer-type pathologic features from each group and studied the effect of ApoE ε4, we did not find a significance difference (Figure 3). The absence of a correlation suggests that other factors, genetic and/or environmental, exist that account for this significant difference.
At the molecular level, this is an important finding that implies an increased tendency for the α-synuclein protein and the Aβ protein to coexist. We postulate that other upstream genes or other genetic loci may help to explain this increased association.

This study demonstrates that ApoE ε4, older age, and female sex are determinants for Alzheimer-type pathologic features in all neurodegenerative disorders, synucleinopathies, tauopathies, or frontotemporal degeneration. However, Alzheimer-type pathologic features are more likely to coexist with synucleinopathies than with tauopathies or frontotemporal degeneration, a finding that cannot be explained by an association with ApoE ε4.

Accepted for Publication: March 22, 2004.
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Author Contributions: Study concept and design: Josephs and Dickson. Acquisition of data: Josephs, Tsuboi, Cookson, and Dickson. Analysis and interpretation of data: Watt and Dickson. Drafting of the manuscript: Josephs, Cookson, Watt, and Dickson. Critical revision of the manuscript for important intellectual content: Josephs and Tsuboi. Statistical expertise: Tsuboi, Watt, and Dickson. Administrative, technical, and material support: Josephs, Tsuboi, Cookson, and Dickson. Study supervision: Tsuboi and Dickson.

Funding/Support: This study was supported by grants AG16574, AG17216, AG14449, AG03949, NS40256, National Institutes of Health, Bethesda, Md; Mayo Foundation, Rochester, Minn; State of Florida Alzheimer Disease Initiative, Tallahassee, Fla; and The Society for Progressive Supranuclear Palsy, Baltimore, Md.

Previous Presentation: This study was presented at the 8th International Conference on Alzheimer’s Disease and Related Disorders, Stockholm, Sweden, July 20-25, 2002.

REFERENCES


