Influence of Apolipoprotein E Genotype on Senile Dementia of the Alzheimer and Lewy Body Types

Significance for Etiological Theories of Alzheimer’s Disease

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Alzheimer’s disease (AD) is associated with an increased frequency of the apolipoprotein E type e4 allele. To address both the disease and the allele specificity of this association, we have examined the apolipoprotein E allele distribution in 255 elderly persons including those with autopsy-confirmed AD, senile dementia of the Lewy body type (SDLT), vascular dementia, Parkinson’s disease (PD) or Huntington’s disease and in non-demented controls either with or without coronary complications. The e4 allele frequency was increased in SDLT (0.365) and AD (0.328) as compared with controls (0.147), PD (0.098), or Huntington’s chorea (0.171). Coronary disease and vascular dementia were associated with marginally higher e4 allele frequencies than in controls. In PD, amyloid β-protein accumulated to a greater extent in those cases possessing an e4 allele than in those without. Those PD cases with dementia were not distinguished from either controls or PD cases without dementia, whether tested biochemically or by apolipoprotein E genotype. It is the comparison of the results in AD and SDLT that yielded the most significant findings. There was a 1.8-fold excess of amyloid β-protein in AD as compared with controls, and the levels in SDLT were intermediate between those in AD and controls. In contrast, AD was discriminated from both controls and SDLT by the substantial accumulation of paired helical filament tau and phosphorylated tau (both increased more than 20-fold as compared with controls). SDLT was nevertheless characterized by an increased e4 allele frequency in the absence of significant tau pathology (at least 10-fold less than that in AD). These findings indicate that tau processing is more specifically associated with AD than is amyloid β-protein accumulation and that presence of the e4 allele is not an etiological factor that accounts for tau pathology. (Am J Pathol 1994, 145:1472–1484)

Apolipoprotein E (apoE) is a sialoglycoprotein that is secreted as a protein with a relative molecular mass of 34,200.1,2 There are three major isoforms of apoE (E2, E3, and E4) that are the products of three alleles (e2, e3, and e4) encoded for by a single gene (APOE) located on the long arm of chromosome 19. These isoforms give rise to six different phenotypes, the most common of which is E3/E3.3 The three isoforms differ by the interchange of Cys and Arg residues at positions 112 and 158 of the mature protein. ApoE2

Supported in part by the Medical Research Council (UK). CRH, MR, and CMW were supported by The Leopold Müller Estate.

Accepted for publication August 22, 1994.

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has Cys residues in both of these positions, apoE3 has Cys-112 and Arg-158, and apoE4 has Arg in both positions.

ApoE is involved in the mobilization and redistribution of cholesterol during neuronal growth and after injury. Astrocytes and microglia are the predominant cell types responsible for producing apoE in brain, and this protein is also involved in lipid transport in cerebrospinal fluid. Neurons do not produce apoE but express the apoE-binding receptor, low-density lipoprotein receptor-related protein, by which it can be internalized. When complexed with lipoproteins, apoE can stimulate neurite outgrowth by the delivery of cholesterol. Upon addition of free apoE to apoE-enriched lipoprotein complexes, the apoE decreases neurite branching and promotes neurite extension away from the cell body.

Several recent lines of investigation have connected apoE with the pathogenesis of Alzheimer's disease (AD). (1) The levels of apoE4 mRNA are increased in AD brains relative to controls. (2) ApoE protein has been associated immunohistochemically with both plaques and neurofibrillary tangles, predominantly of the extracellular type, in AD. (3) ApoE binds avidly to synthetic amyloid β-protein (Aβ) and to soluble Aβ in cerebrospinal fluid. Oxygen-mediated binding of Aβ to the lipoprotein-binding domain of apoE4 creates a sodium dodecyl sulfate (SDS)-resistant complex, with apoE4 binding more avidly than apoE3. The hypothesis that apoE acts as a pathological chaperone that modulates and/or promotes aggregation of Aβ has been proposed, and it has been speculated that apoE can bind and transport hydrophobic peptides, such as soluble Aβ in cerebrospinal fluid, in a manner that would prevent fibril formation.

The presence of the ε4 allele is associated with late onset familial AD and sporadic AD, suggesting that the ε4 allele may be a possible risk factor or susceptibility gene for AD. Although only 2 to 3% of people are homozygous for the ε4 allele, the penetrance of AD in homozygotes approaches 90%, and heterozygotes are at substantially increased risk as compared with controls and develop the disease earlier in life. Nonetheless, the observation that AD can occur in persons without an ε4 allele is consistent with the notion that AD is a heterogeneous disorder in which several factors, both genetic and environmental, exert an influence on the course and progression of the disease. The ε4 allele frequencies in patients with other amyloid-forming diseases (Creutzfeldt-Jakob disease, Down's syndrome, and familial amyloid polyneuropathy) do not differ from control groups, despite the observation that apoE has been associated immunohistochemically with the prion plaques in kuru.

There are several outstanding issues concerning the association between apoE and AD. First, it is unclear whether it is the presence of the ε4 allele or the absence of one of the other two alleles that is the important risk-determining factor for AD. Second, it is possible that the association with ε4 might be due to linkage disequilibrium between APOE, AD and a further locus nearby. Third, APOE genotype influences a number of disorders and some of these may influence survival rate such as longevity, coronary heart disease, and other forms of dementia that have not been examined. For example, an increase in the ε4 allele frequency has been reported in multifocal dementia in Japan. Finally, it is not understood how different apoE isoforms might influence the pathogenesis of AD.

Dementia associated with cortical Lewy bodies has emerged as the second most common form of degenerative dementia in the elderly after AD, exceeding the prevalence of vascular dementia. The Lewy body is an intraneuronal inclusion body composed of amorphous material and 10- to 20-nm filaments consisting of cytoskeletal neurofilament proteins and ubiquitin. Senile dementia of the Lewy body type (SDLT) was identified recently by its unusual neuropathological profile among psychiatric patients presenting with dementia. They were subsequently found to have a clinical presentation that is distinct from both AD and PD. SDLT is characterized by the presence of a moderate number of neocortical Lewy bodies and sparse or absent neurofibrillary lesions. Moreover, SDLT and PD both lack the paired helical filaments (PHFs) that are characteristically abundant in AD. These two forms of dementia, therefore, offer an ideal opportunity to assess the influence that particular APOE genotypes might have on PHF formation and to understand further the molecular basis for the differences that identify AD and the Lewy body diseases (SDLT and PD) from each other and from normal aging.

To determine the disease specificity and the influence that the APOE genotype has in different neurodegenerative disorders, we have assessed the APOE genotype in elderly persons having autopsy-confirmed diagnoses of AD, SDLT, PD, or Huntington's chorea. We have compared their genotypic distribution with nondemented controls and assessed the influence that the presence or absence of the ε4 allele has on the processing of tau protein and the deposition Aβ that occur in AD and Lewy body diseases.
Materials and Methods

Patients and APOE Genotyping

Frozen brain tissue from autopsy-confirmed cases was obtained from the Cambridge Brain Bank Laboratory, Newcastle General Hospital, and the Antwerp Brain Bank. AD was diagnosed according to McKhann et al.28 SDLT was identified initially by a neuropathological profile distinct from AD.23 These patients presented with dementia and did not have parkinsonian features. They are associated with a pattern of clinical features distinct from that found in AD.23,26 PD patients presented with classic parkinsonian movement disorder (tremor, rigidity, and akinesia). Control cases consisted of age-matched patients capable of living an independent existence with no recorded neurological or psychiatric abnormalities or neuropathological evidence of AD or PD. Of the 58 control cases, 40 were preselected (before genotyping) on the basis of the presence or exclusion of coronary complications. The latter included atherosclerosis, myocardial infarction, ischemia, electrocardiographic changes, or angina. The details for 255 cases are summarized in Table 1.

Brain tissue (approximately 50 mg wet weight) was treated with 0.3 ml proteinase K (0.5 mg/ml) in Tris-HCl (10 mmol/L, pH 8.3), containing 50 mmol/L KCl, 0.01% gelatin, 0.45% Nonidet P-40, and 0.45% Tween 20, at 60 C for 1 hour with occasional agitation. The reaction was terminated by transferring the tubes to a thermal block at 95 C for 10 minutes. The mixture was centrifuged at 13,000 x g for 2 minutes and the supernatant used as the source of genomic DNA. The fourth exon of the APOE gene was amplified by the polymerase chain reaction (PCR) by using biotylated primers, and the APOE genotype determined by using a reverse DNA hybridization test as recommended by the manufacturer (INNO-LiPA Apo E kit; Innogenetics N.V., Ghent, Belgium).

Immunochromical Analysis

Brain tissue was homogenized and separated into fractions containing normal tau protein, sarkosyl-insoluble hyperphosphorylated tau protein, protease-resistant PHF-tau, and SDS-insoluble Aβ. Tau proteins and Aβ were measured by competitive immunoassays, using MAb 7.51 for normal tau,29 MAb AT8 for phosphorylated tau,30 MAB 423 for C-terminally truncated, protease-resistant PHF-tau,31 and BR301 for Aβ in an SDS-insoluble fraction.27 The fractionations, antibodies, and immunoassays have been described in detail previously.27,29,32

Statistical Analysis

Allele frequencies were determined by the gene counting method. Comparison of the distribution of genotype and allele frequencies and determination as to whether genotypes were in Hardy-Weinberg equilibria were tested by using Pearson’s χ² test or Fisher’s exact test when necessary. Odds ratios (ORs) for AD or SDLT associated with the presence of a particular APOE allele were calculated with exact 95% confidence intervals (CIs), using the iterative procedure described by Fleiss.33 The weighted averages for allele frequencies were calculated from 15 reports and ORs for the association of particular alleles with AD were determined by using data from those studies for which all data were available.

Analysis of variance was used to make intergroup comparisons of biochemical data for control, AD, SDLT, and PD groups. Comparisons were made for data obtained from both cingulate and occipital cortices. For data in which significant F ratios were obtained, the Scheffé multiple comparison test was used to determine which groups were significantly different from each other at a significance level of P < 0.05. Student’s t-test was used to compare quantitative biochemical differences between subgroups with or without the e4 allele. Levene’s test for inequality of variance was used when necessary. Data were analyzed by using SPSS for Microsoft Windows, version 6.0 (SPSS Inc., Chicago, IL).

Results

APOE Genotype

The reverse DNA hybridization test used in this study provided a fast (approximately 6 hours) and simple

<table>
<thead>
<tr>
<th>Table 1. Case Details</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>No. of cases</td>
</tr>
<tr>
<td>Sex (male/female)</td>
</tr>
<tr>
<td>Age at death</td>
</tr>
<tr>
<td>Range</td>
</tr>
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</table>
method of determining APOE genotype with small amounts of frozen brain tissue. Exon 4 of the APOE gene was amplified by PCR and detected directly by its binding to a set of four oligonucleotide probes specific for each of the four base changes. Each phenotype results in a different pattern of positive probes. A representative set of results shows the pattern obtained for each genotype (Figure 1). This test was applied to 255 elderly cases for which autopsy diagnoses were available. With the exception of Huntington’s chorea, all groups were age matched, with a mean age at death between 77 and 79 years (Table 1); six patients with Huntington’s chorea died in their sixth decade. The distribution of the six genotypes and the frequencies of the three alleles are given in Table 2. For all of the different populations examined, the APOE genotype frequencies did not depart significantly from the Hardy-Weinberg equilibrium ($P > 0.60$).

The most common phenotype in controls was the E3/E3 homozygote (Table 2). The AD and SDLT groups were both characterized by an allele distribution that was distinct from controls and other neurological diseases. In AD and SDLT, the e4 allele frequencies were 0.328 and 0.365, respectively, as compared with 0.147 in age-matched controls. The most common phenotype in AD and SDLT was E3/E4. The only cases that were homozygous for the e4 allele had the diagnosis of either AD (6 of 67) or SDLT (2 of 26). The ORs for one or two e4 alleles being associated with AD and SDLT were 3.27 (95% CI, 1.41 to 7.14) and 4.56 (95% CI, 1.53 to 13.84), respectively.

When the distribution of individual alleles was examined, the AD and SDLT groups were distinct from each other (Table 3). AD was associated with an elevated e4 allele frequency (OR = 3.27) that was compensated for by decreases in the frequencies of both e2 (OR = 0.34; 95% CI, 0.08 to 1.29) and e3 (OR = 0.26; 95% CI, 0.04 to 1.43) alleles. In contrast, SDLT was associated with high e4 (OR = 4.56) and low e3 (OR = 0.43; 95% CI, 0.04 to 4.6) allele frequencies, but there was no significant change in the e2 allele frequency (OR = 0.71; 95% CI, 0.14 to 3.28).

For PD, Huntington’s chorea, and vascular dementia, there were no significant differences from the controls. The frequency of the e4 allele was increased, although not to a significant extent, both in vascular dementia (0.25) as compared with all controls and in those controls with coronary conditions as compared with controls without coronary disease (0.20 and 0.10, respectively; Table 2).

An increased e2 allele frequency combined with a decreased e4 frequency in centenarians as compared with those younger than 100 years has been reported.18 When the control group in the present study was divided into those over 85 years at death (22 alleles) and those aged 85 years and under (94 alleles), the only significant difference in the allele frequencies between the two groups was for an increased e2 allele frequency in the older group (0.227 compared with 0.073; $\chi^2 = 6.0, P = 0.014$).

**Immunochromic Analysis**

Previously, we have discriminated AD not only from controls but also from SDLT on the basis of the quantification of PHF-tau and phosphorylated tau. In this study, the biochemical changes in tau protein processing and in Aβ deposition were examined in control, AD, SDLT, and PD groups (Table 4). These patients were selected for biochemical analysis before APOE genotyping. The distribution of genotypes among these groups did not differ from that described above for the larger samples.

When all cases for each group were taken into account, normal tau protein was depleted by almost 50% in all three disease categories as compared with controls. In addition, AD was characterized by the accumulation of both phosphorylated tau (increased 86-fold in AD as compared with controls) and protease-resistant PHF-tau (23-fold). Although both forms are elevated as compared with controls, these proportional increases need not necessarily reflect the proportion of the total PHF-tau pool that is phosphorylated in AD. The later can represent less than 15% of the total PHF-tau pool in AD (C. M. Wischik et al, in preparation). The level of protease-resistant PHF-tau in SDLT was less than 10% of that found in AD, and it did not differ significantly from controls or PD (Table 4). Likewise, there was no significant ac-
Table 2. Distribution of APOE Genotypes and the APOE Allele Frequencies for Different Populations

<table>
<thead>
<tr>
<th>Category (no. of cases)</th>
<th>APOE genotype</th>
<th>APOE allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (58)*</td>
<td>33</td>
<td>7</td>
</tr>
<tr>
<td>Coronary control (20)</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Noncoronary control (20)</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Alzheimer's disease (67)</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Parkinson's disease (51)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Huntington's disease (41)</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>Vascular dementia (12)</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

* Coronary and noncoronary control cases listed separately are also included in the total control sample of 58 cases.

Table 3. Comparison of APOE Allele Frequencies in Patients with Different Neurodegenerative Disorders with Nondemented Controls

<table>
<thead>
<tr>
<th>Diagnosis (no. of alleles)</th>
<th>Six genotypes (5 df)</th>
<th>e2, e3, e4 alleles (2 df)</th>
<th>e2 allele (1 df)</th>
<th>e3 allele (1 df)</th>
<th>e4 allele (1 df)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X²</td>
<td>P</td>
<td>X²</td>
<td>P</td>
<td>X²</td>
</tr>
<tr>
<td>Alzheimer's disease (134)</td>
<td>14.4</td>
<td>0.013</td>
<td>13.35</td>
<td>0.001</td>
<td>2.75</td>
</tr>
<tr>
<td>SDLT (52)</td>
<td>14.2</td>
<td>0.014</td>
<td>10.24</td>
<td>0.006</td>
<td>0.11</td>
</tr>
</tbody>
</table>

* Pearson’s X² test (with continuity correction) was used to compare genotype and allele distributions in AD and SDLT with control patients (116 alleles). No significant differences from controls were found for any of these distributions in Parkinson’s disease (102 alleles), Huntington’s chorea (82), or vascular dementia (24).

Table 4. Effect of the Presence or Absence of the e4 Allele on Biochemical Parameters

<table>
<thead>
<tr>
<th>Biochemical parameter (units)</th>
<th>Controls</th>
<th>AD</th>
<th>SDLT</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>e4 allele present (n = 5)</td>
<td>e4 allele absent (n = 6)</td>
<td>e4 allele present (n = 9)</td>
<td>e4 allele absent (n = 3)</td>
<td>e4 allele present (n = 9)</td>
</tr>
<tr>
<td>Normal tau (U/g)</td>
<td>11.56 ± 2.06</td>
<td>8.39 ± 1.03</td>
<td>5.33 ± 1.1</td>
<td>7.98 ± 2.74</td>
</tr>
<tr>
<td>Phosphorylated tau (ng/g)</td>
<td>3.48 ± 3.30</td>
<td>1.0 ± 0.78</td>
<td>161 ± 34</td>
<td>247 ± 148</td>
</tr>
<tr>
<td>PHF tau (U/g)</td>
<td>0.94 ± 0.23</td>
<td>1.0 ± 0.31</td>
<td>22.5 ± 4.6</td>
<td>19.3 ± 9.5</td>
</tr>
<tr>
<td>Aβ (ng/g)</td>
<td>295 ± 63</td>
<td>225 ± 59</td>
<td>488 ± 39</td>
<td>467 ± 69</td>
</tr>
</tbody>
</table>

* The effect of the presence of an e4 allele was tested for each disease category and for each of four biochemical parameters (Student’s t-test). The only significant difference between cases with and without an e4 allele was observed for Aβ in PD (P = 0.008).

1 The differences between the disease and control groups at a significance level of P < 0.05 (Scheffé procedure) were as follows: 1) cases with the e4 allele: normal tau, control with AD and PD; phosphorylated tau and PHF-tau, AD with control, SDLT, and PD; Aβ, AD with control, SDLT, and PD and 2) cases without the e4 allele: normal tau, none, phosphorylated tau and PHF-tau, AD with control, SDLT, and PD; Aβ, AD with control and PD, SDLT with PD. For all other combinations, the differences were not significant at P < 0.05.

2 n, number of cases.

3 The mean determination (± SEM) is given for each group based upon determinations from both cingulate and occipital cortices for each case.

The accumulation of phosphorylated tau in SDLT, PD, or controls. Phosphorylated tau was measured by using an antibody (MAb Aβ8) that is dependent upon phosphorylation of tau at Ser-202, just one of a number of phosphorylation sites in tau protein.30 In contrast, Aβ accumulation did not show such striking differences between the AD, SDLT, and control groups. Detergent-insoluble Aβ in SDLT amounted to 69% of the level found in AD, whereas the amount found in controls was 77% of that in SDLT (Table 4). These results demonstrate that, whereas AD and SDLT are characterized by increased Aβ, in excess of that found in normal aging, there is a far greater specificity for abnormal tau accumulation in AD.

The association of the e4 allele with changes in these biochemical parameters was examined for each of the four groups (Table 4). From this analysis, the only significant difference observed was a
four-fold increase in SDS-insoluble Aβ in those PD cases with an ε4 allele as compared with those without \( (P = 0.008) \). The PD group consisted of seven patients that presented with dementia and five that did not. The association of the ε4 allele with Aβ accumulation was not reflected by the presence of high levels of Aβ in those PD cases with dementia (not shown). Paradoxically, in SDLT there was a tendency toward the opposite being true; the levels of Aβ were marginally higher in those SDLT cases without an ε4 allele (Table 4).

No significant changes were observed for any of the four categories of patients with respect to the levels of either PHF-tau or phosphorylated tau in cases with and without the ε4 allele (Table 4). When individual cases were compared, a slight overlap was found between AD and SDLT in terms of abnormal tau accumulation (Figure 2). The extent of this overlap is compatible with the 95% agreement (19 of 21 cases) between clinical diagnosis of SDLT on the one hand and its pathological diagnosis on the other.26 There were inadequate numbers of cases with an ε2 allele or that were homozygous for the ε4 allele to allow for an extensive analysis of the influence that might be exerted by these genotypes.

Elevated levels of normal tau protein, with a tendency toward significance, were observed in those control and SDLT cases in which an ε4 allele was present (Table 4). In contrast, normal tau protein levels were lower in those AD cases possessing an ε4 allele than those without, although these differences did not reach statistical significance. There was a tendency for the levels of normal tau protein in AD to decrease in an allele dose-dependent manner (Figure 3A). Normal tau levels in AD (mean ± SE) were 7.32 ± 2.97, 5.94 ± 1.33, and 3.2 ± 1.34 for cases with 0, 1, and 2 ε4 alleles, respectively. In contrast, there was no such relationship in SDLT (3.95 ± 1.61, 6.11 ± 1.30, and 7.1 ± 0.70 for 0, 1, and 2 ε4 alleles, respectively; Figure 3B).

**Discussion**

**APOE Genotyping**

Before the APOE gene sequence was known, isoelectric focusing of apoE isoforms was used to determine phenotypes. Several genotyping methods have now been reported on the basis of the PCR amplification of genomic DNA.34–37 The latter methods are more accurate and prevent the relatively high proportion

![Figure 2](image-url)  
**Figure 2.** Biochemical comparison of AD and SDLT cases with and without an ε4 allele. Normal tau (A), PHF-tau (B), phosphorylated tau (C), and Aβ (D) were measured in cingulate and occipital cortex. (△), ApoE4-positive AD cases; (▲), ApoE4-negative AD cases; (○), ApoE4-positive SDLT cases; (●), ApoE4-negative SDLT cases.

![Figure 3](image-url)  
**Figure 3.** Normal tau protein levels in AD (A) and SDLT (B) patients on the basis of the number of ε4 alleles present. (△), no ε4 allele; (○), heterozygous for ε4; (▲), homozygous for ε4.
(from 2 to 15%) of erroneous results that can be obtained by traditional phenotyping. The hybridization assay used to determine the genotype in this study has been validated by typing blood samples of known genotype and in which partial sequence analysis was used for confirmation (J. Louwagie et al., in preparation). Here we demonstrate that the method is suitable also for use with small quantities of brain tissue. It is a rapid method that obviates the need for restriction endonuclease digestion and electrophoretic separation of PCR products. In addition, only one step is required for the hybridization of the different DNA products to all four probes.

**APOE Genotype and AD**

Presence of the ε4 allele has been associated with both sporadic and familial cases of late onset AD but not with certain kindreds with early onset familial AD. Several independent studies have confirmed these findings, but in a recent study, the ε4 allele was not associated with Swedish patients having sporadic AD. When the weighted average from 15 independent studies was determined, the following allele frequencies in AD were calculated: ε2, 0.044; ε3, 0.587; ε4, 0.380 for a total of 2896 alleles (Table 5). Data from 10 of these investigations were combined with the data from the present study to determine the likelihood of each allele being associated with AD (907 AD cases and 1115 controls). The ORs were: ε2, 0.41 (95% CI, 0.31 to 0.55); ε3, 0.55 (0.41 to 0.73); ε4, 4.52 (3.72 to 5.50). Thus, the estimated risk of having AD is almost five-fold higher in those patients having at least one ε4 allele, an association that is compensated for by decreases in both the ε2 and ε3 frequencies. The protective effect of the ε2 allele against AD has been documented recently by other investigators. The ε4 allele frequency (0.328) in the present sample of 67 autopsy-confirmed AD cases was not significantly lower than the weighted average value of 0.380 from the 15 studies described above (Table 5) and higher than values from individual studies for sporadic AD (0.24, 0.22, and 0.26) and early onset familial AD (0.19). Furthermore, it has been noted that the frequency in AD varies between different ethnic groups.

Despite the clear association of the ε4 allele with some cases of AD, homozygosity for this allele does not always result in dementia. Indeed, 12 of 26 Swedish people between the ages of 65 and 98 years (non-demented controls) were homozygous for the ε4 allele. Large sample sizes are required to determine whether particular subtypes of familial AD are associated closely with a particular genotype. An extensive analysis of familial AD found that the ε4 allele was associated with late onset AD (ε4 = 0.51) but not with AD in Volga Germans or in early onset, non-Volga Germans. In contrast, sporadic AD cases in the United Kingdom with early onset have been reported with an increased ε4 frequency (0.29) and a significant association between the ε4 allele and early onset AD in The Netherlands was found in those patients with a positive family history of the disease. It was suggested that apoE4 modifies the expression of an AD phenotype that is determined by other genetic and/or environmental factors underlying the familial aggregation of AD.

**Comparison between AD and SDLT**

Although an increased ε4 frequency was reported recently for cortical Lewy body disease, 12 of the 18 cases examined in the latter study had only a clinical diagnosis and no biochemical data was available. In this study, we have examined 26 patients with senile dementia that had neuropathologically diagnosed SDLT. These patients had minimal or absent Alzheimer-type pathology, and a biochemical study of 13 of these indicated that they were not characterized by PHF pathology. In this study, we observed that the ε4 allele frequency (0.365) was increased in cases of SDLT to an extent similar to that observed in AD (0.328). In SDLT, the proportion of E3/E4 heterozygotes, compared with E4/E4 homozygotes, was greater than that observed for AD. Of the 8 patients who were homozygous for the ε4 allele in this study, 6 had a diagnosis of AD and the other 2,

<table>
<thead>
<tr>
<th>APOE allele</th>
<th>Control (n = 116)*</th>
<th>Control (n = 5008)†</th>
<th>AD (n = 134)*</th>
<th>AD (n = 2896)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2</td>
<td>0.086</td>
<td>0.077 (0.024–0.145)</td>
<td>0.030</td>
<td>0.044 (0.013–0.08)</td>
</tr>
<tr>
<td>ε3</td>
<td>0.767</td>
<td>0.789 (0.722–0.896)</td>
<td>0.642</td>
<td>0.588 (0.440–0.75)</td>
</tr>
<tr>
<td>ε4</td>
<td>0.147</td>
<td>0.134 (0.05–0.190)</td>
<td>0.328</td>
<td>0.380 (0.236–0.52)</td>
</tr>
</tbody>
</table>

* This study, (n, number of alleles).
† Weighted frequency (with ranges) from 15 studies.
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SDLT. As with AD, several SDLT cases did not possess a single ε4 allele. Whereas increased prevalence of the ε4 allele in AD patients was associated with decreases in both the ε2 and ε3 allele frequencies, SDLT was not associated with a concomitant decrease in ε2 frequency. In SDLT, the increase in ε4 was compensated for by a decrease only in the ε3 frequency. The significance of these subtle differences is unclear at present. There was a tendency toward an ε4 allele dose-dependent depletion of normal tau in AD but not SDLT. Whether this could be accounted for by a different protective capacity for the ε2 allele in the two disorders remains to be tested.

It seems unlikely that the increased ε4 allele frequency in SDLT is due to the inclusion of AD cases. In this study, we have examined a population of pure SDLT that was confirmed by the absence of significant tau pathology. In these cases, there was no evidence that the ε4 allele was associated with those few SDLT patients in which there was overlap with AD (Figure 3A). These results are inconsistent with those described recently.52 In the latter investigation, the ε4 allele frequency was increased in AD but not in pure Lewy body disease, as compared with controls. The frequency was intermediate for those cases described as the Lewy body variant of AD.24 It is possible that the minimal Alzheimer-type changes in tau pathology that occur in some SDLT cases may arise from the chance coincidence of a specific neurobiological condition and the widely prevalent presence of plaques.

There are both similarities and differences between AD and SDLT. The first similarity is that they are both associated with a significant increase in the frequency of the ε4 allele as compared with controls. Second, they have elevated levels of Aβ deposition. Finally, normal tau protein is depleted to a similar extent in both AD and SDLT as compared with controls. In contrast, the two groups are separated not only by their clinical presentation and extent of neurofibrillary pathology23 but also by a 10-fold increase in protease-resistant PHF-tau accumulation in AD as compared with SDLT. Furthermore, the levels of phosphorylated tau in SDLT are less than 1% of those found in AD.

APOE Genotype in Other Diseases

The extent of the association of the ε4 allele with types of dementia other than AD remains to be established. Increased ε4 allele frequencies have not been observed in patients with Creutzfeldt-Jakob disease, Down’s syndrome, or familial amyloid polynucleopathy.16,56 In Down’s syndrome, an increase in the ε2 allele frequency was reported.54 Frontal lobe dementia has been associated with ε4 allele frequencies of 0.28 and 0.16,57,58

Other conditions that are influenced by the APOE genotype might indirectly affect the development of AD. Aging and vascular changes are both factors that are influenced by APOE genotype. Longevity is associated with the ε2 allele.16,19 an allele that also predisposes patients toward hyperlipoproteinemia.19 In the present study, the ε2 frequency was higher in those nondemented persons who survived beyond 85 years. Longevity is likely to be a complex product of both genotypic and environmental influences.

There is evidence for factors other than the APOE genotype being involved in the pathogenesis of AD. Mutations in the amyloid precursor protein gene account for early onset AD in approximately 20 families;59 germline mutations of mitochondrial DNA have been associated with AD plus PD,60 and trisomy 21 is associated with Alzheimer-type dementia in many elderly Down’s syndrome individuals. Although it is not known whether the mitochondrial DNA mutations are associated with particular APOE genotypes, Down’s syndrome individuals13,56 are not distinguishable from controls in terms of their APOE genotype. Early onset familial AD and AD linked with an as yet unidentified locus on chromosome 14 are not associated with an increased ε4 allele frequency.16,61 It is unclear whether apoE has a role in patients with amyloid β-protein precursor mutations.62,63

The APOE allele frequencies for control patients in this study were similar to both those obtained in studies with larger populations of healthy controls19,64,65 and to the weighted average for controls derived from 15 studies, which were: ε2, 0.077; ε3, 0.789; and ε4, 0.134 for a total of 5008 alleles (Table 5).6,10,14,20,39–49 Certain ethnic populations, however, differ considerably from the average. For example, the Finnish (0.23), Sudanese (0.29), and Aborigines (0.39) have high ε4 frequencies in the general population.19,64,66 whereas the Chinese and Japanese have low frequencies (0.074).19 These populations with high and low frequencies for ε4 have correspondingly high and low prevalences for coronary artery disease.

In this study, both coronary artery disease (in nondemented controls) and vascular dementia were associated with small though nonsignificant increases in the ε4 frequency. The extent of this increase was comparable with the significant differences observed in studies with larger sample sizes19,67–71 although contradictory findings have been reported.19 Patients with an ε4 allele are exposed to higher atherogenic lipid concentrations throughout life and this may lead to an increased risk of atherosclerosis.19,65
The variability between the association of the ε4 allele and different forms of dementia indicates that, whereas the presence of this allele may be a risk factor in certain individuals, predictive testing for AD remains unlikely to be of value at present. A number of factors may affect the genotypic frequencies that have been reported. First, some studies include both familial and sporadic late onset cases from families with a high incidence of dementia. Such sampling and selection criteria need not necessarily be representative of the general population. As mentioned above, ethnic backgrounds of populations may differ in their APOE genotype frequency. Finally, non-AD cases such as multi-infarct dementia,25 coronary artery disease,19,67–71 and SDLT may have increased ε4 frequencies. Nevertheless, it will be important to determine the genotype of different patients to investigate how a particular APOE genotype might influence the progression of dementia in well defined types of dementia. The rapid test used in this study could be applied in the determination of APOE genotype in prospectively assessed elderly patients and controls.

Neither PD nor Huntington’s chorea were distinguishable from controls by their APOE genotype. Furthermore, those PD cases that had dementia were not distinguished from those without. PD that is characterized by Lewy bodies in the brain stem and occasionally throughout the neocortex72 was not influenced by APOE genotype despite sharing some clinical overlap with SDLT.23 Down’s syndrome exemplifies a further distinct pathogenetic process. Despite the development of Alzheimer-type pathology in most elderly patients with Down’s syndrome, the latter is not associated with an increased ε4 allele frequency.16,54,56

AD is a disease that is characterized by PHF accumulation and its pathogenesis is associated with the presence of ε4 in the absence of ε2 and ε3 alleles. Of the neurodegenerative disorders other than AD, only SDLT is associated with the presence of ε4. In SDLT, the ε2 allele does not appear to be protective. Inasmuch as there is less than one tenth the amount of insoluble abnormal tau protein in SDLT than found in AD, it appears that several factors are likely to influence the development of these distinct yet overlapping dementia syndromes.

APOE Genotype and Neuropathology

Two studies have reported that plaque densities are elevated in those AD cases homozygous for the ε4 allele.6,73 In contrast, we failed to find any evidence for biochemical changes associated with the presence of an ε4 allele in either AD or SDLT (Table 4). There was a significant decrease in the amount of Aβ in cingulate and occipital cortex in PD cases not carrying an ε4 allele as compared with controls and PD cases heterozygous for ε4. It is possible that specific brain regions may be affected preferentially. For example, whereas apoE immunoreactivity is strong within cerebellar and cortical diffuse plaques, it is absent or very weak in diffuse plaques found in striatum/thalamus.74 Although apoE has been shown to bind to plaques and tangles,6,8,10 the pathological significance of such binding to extracellular protein deposits has not been established. It has been proposed that apoE acts as a molecular chaperone for pathologically processed proteins.9 Oxidized apoE binds with high avidity to Aβ and the apoE4 isoform binds more avidly to synthetic Aβ than does apoE3 in vitro.12,73 Furthermore, these two isoforms have differential effects on neurite outgrowth that may reflect their interaction with certain cellular proteins.75 Inasmuch as apoE is an abundant brain protein that is secreted by astrocytes that invade areas of degeneration, its binding to Aβ per se may not affect the progression of the disease directly. There is no evidence that apoE alters the neurotoxicity of soluble Aβ. In fact, rabbit apoE (a protein that exhibits 80% sequence identity to human apoE3) eliminated the neurotoxicity of Aβ for hippocampal cultures.76 The possibility that apoE is involved in mediating clearance of extracellular Aβ has been proposed on the basis of the finding that apoE and low-density lipoprotein receptor-related protein, one of the receptors for apoE complexes, are colocalized in senile plaques in AD.6

It has been suggested that apoE3 (or possibly apoE2) may be protective against PHF formation.17 This hypothesis is not substantiated by our findings, inasmuch as no differences in the levels of abnormal tau proteins were observed between AD patients with or without an ε4 allele. Similarly, from an analysis of the APOE genotype in Chamorros, Waring et al 77 found no evidence in support of apoE3 being protective against tangle formation. To determine whether or not apoE2 is protective will require the analysis of a larger sample of cases possessing the ε2 allele. Those AD patients with an ε4 allele had a tendency toward having lower levels of normal tau than those AD patients with an ε4 allele. This raises the possibility that apoE4 might indirectly affect cellular metabolism in pyramidal cells in AD without affecting the rate of abnormal tau accumulation.
Implications for Pathogenesis of AD

Although homozygosity or heterozygosity for the ε4 allele confers an increased risk factor for both AD and SDLT and a decreased age of onset in AD, the presence of this allele does not appear to be either essential or necessary for the development of either type of dementia in all cases. Despite features in common between the two diseases, AD and SDLT remain distinct at the clinical, neuropathological, and biochemical levels. AD is a neurodegenerative disease characterized by the accumulation of abundant neocortical PHFs, and SDLT is a disease associated with the presence of neocortical Lewy bodies in the relative absence of PHFs. In both SDLT and AD, there is a greater load of Aβ in the medial temporal lobe, as compared with controls, and an increased amount of SDS-insoluble Aβ. Furthermore, the plaques in SDLT, in general, lack tau-immunoreactive neurites, a feature that is substantiated by the absence of PHF-tau in these patients. This makes it unlikely that the association with the ε4 allele can account for those pathological changes that are most closely correlated with the clinical signs of dementia in AD, ie, neuritic plaques and neurofibrillary tangles.

Etiological theories for AD have included debate over the relevant importance and primacy between Aβ and neurofibrillary pathology. Aβ is regarded by many investigators as the specific etiological agent in AD. In this study and others, Aβ deposition is increased only 1.8-fold in AD as compared with controls and the level in SDLT is intermediate. In contrast, AD is associated with substantial accumulation of abnormal tau protein (increased more than 20-fold in AD as compared with controls). Thus it emerges that the changes in the abnormal processing of tau protein, rather than Aβ deposition, are more specifically associated with the clinical, pathological, and immunohistochemical factors that distinguish AD from both SDLT and normal aging.

References

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