Apolipoprotein E ε4 Allele, Temporal Lobe Atrophy, and White Matter Lesions in Late-Life Dementias

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**Objective:** To examine the relationship between the apolipoprotein E (APOE) ε4 genotype, medial temporal lobe atrophy, and white matter hyperintensities on magnetic resonance imaging in late-life dementias.

**Design:** Structural magnetic resonance imaging study using T2-weighted and proton density–weighted axial scans and T1-weighted coronal scans.

**Setting:** Community-dwelling population of elderly patients prospectively chosen from a clinical case register of consecutive referrals to old age psychiatry services.

**Subjects:** Twenty-five subjects with Alzheimer disease (by criteria of the National Institute of Neurological and Communication Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association; mean age, 77.8 years), 22 subjects with dementia with Lewy bodies (consensus criteria; mean age, 77.2 years), and 24 subjects with vascular dementia (by criteria of the National Institute of Neurological and Communication Disorders and Stroke and the Alzheimer International pour la Recherche et l’Enseignement en Neurosciences; mean age, 76.9 years) were selected. Subjects were well matched for age, sex, duration of illness, and cognitive function.

**Main Outcome Measures:** The APOE genotype was determined using the polymerase chain reaction method, and medial temporal lobe atrophy and white matter hyperintensities (periventricular and deep white matter) were visually rated using standardized scales.

**Results:** In all subjects with dementia, no significant associations were noted between APOE ε4 status and medial temporal lobe atrophy (mean score: 0 ε4 = 4.5, 1 ε4 = 4.5, and 2 ε4 = 4.3; P = .90), periventricular hyperintensities (0 ε4 = 3.3, 1 ε4 = 3.1, and 2 ε4 = 2.9; P = .83), and white matter hyperintensities (0 ε4 = 5.3, 1 ε4 = 4.9, and 2 ε4 = 4.9; P = .79).

**Conclusions:** The APOE ε4 allele does not determine medial temporal lobe atrophy or white matter lesions, as measured by magnetic resonance imaging in patients with Alzheimer disease, vascular dementia, or dementia with Lewy bodies. Although APOE ε4 may modify the risk for acquiring dementia, this finding provides further evidence that APOE ε4 does not influence pathological processes thereafter.

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The presence of the apolipoprotein E (APOE) ε4 allele has been identified as a major risk factor for both sporadic and familial late-onset Alzheimer disease (AD). Located on chromosome 19 at q13.2, the APOE gene codes for 3 major isoforms: ε2, ε3, and ε4. An increased frequency of the APOE ε4 allele has been observed in subjects with AD and those with other types of dementia, including dementia with Lewy bodies (DLB) and vascular dementia (VaD). The pathological pathway through which the apolipoprotein E exerts influence has yet to be determined. In particular, it is not known whether the effects of the apolipoprotein E are specific to AD or expressed through a common pathway, independent of the diagnosis.

Recently, several studies have reported a link between the presence of APOE ε4 and specific morphologic changes identified on neuroimaging. Lehtovirta and colleagues, Tanaka et al, and Juottonen et al studied subjects with AD and found increased atrophy in medial temporal lobe structures on magnetic resonance imaging (MRI) in those possessing an APOE ε4 allele. The relationship between medial temporal lobe atrophy (MTA) on MRI and the APOE ε4 status in subjects with DLB and VaD has not yet been examined.

Imaging studies have also reported a high prevalence of white matter changes.
SUBJECTS AND METHODS

RECRUITMENT AND DIAGNOSIS OF SUBJECTS

Seventy-one subjects older than 60 years who fulfilled the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, criteria for dementia were recruited from a community-dwelling population of patients with an informant in regular contact. Subjects were prospectively chosen from a clinical case register of consecutive referrals to Old Age Psychiatry Services, Newcastle General Hospital, Newcastle upon Tyne, England. The recruitment of subjects with DLB was supplemented by referrals to a specialist dementia clinic. The research was approved by the local ethics committee, and all subjects, as well as the nearest relative for patients, gave informed consent after the nature of the procedures had been fully explained.

Standardized clinical diagnostic criteria were used to characterize the type of dementia. The diagnoses of AD, VaD, and DLB were made in accordance with criteria of the National Institute of Neurological and Communication Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association,20 the National Institute of Neurological Disorders and Stroke and the Association International pour la Recherche et l’Enseignement en Neurosciences,21 and the Consortium on DLB International Workshop,22 respectively, by consensus among 3 experienced raters (J.O’B., C.B., and I.McK.). The diagnosis was made in a manner blinded to MRI findings and APOE status. Applying these criteria, 25 subjects had AD, 24 had VaD, and 22 had DLB. Cognitive function was measured using the Mini-Mental State Examination23 within 3 months of obtaining an MRI scan.

MRI SCANNING PROCEDURE

All scans were performed on a 1.0-T MRI scanner (Siemens Magnetom Impact; Siemens Analytical X-ray Instruments, Erlangen, Germany). To assess MTA, T1-weighted coronal images of the whole temporal lobe were obtained with a slice thickness of 5 mm and no inter slic e gap. These images were acquired by reformatt ing a 3-dimensional data set perpendicular to the long axis of the hippocampus (magnetization prepared rapid-acquisition gradient echo: repetition time, 11.4 milliseconds; echo time, 4.4 milliseconds; inversion time, 400 milliseconds; time delay, 50 milliseconds; matrix, 256 x 256; and slice thickness, 1 mm).

To assess white matter lesions, whole-brain axial images of 5-mm thickness (0.5-mm gap) were acquired using proton density-weighted and T2-weighted turbo or fast-spin echo sequences (rapid acquisition with relaxation enhancement: repetition time, 2800 milliseconds; echo time, 14/85 milliseconds; matrix, 256 x 256; field of view, 230 mm, giving a pixel size of 0.92 x 0.92 mm; and acquisition time, 4 minutes 13 seconds).

MTA AND WHITE MATTER RATING

All scans were rated with the diagnosis and the APOE genotype blinded. A standardized scale24 was used to rate left and right MTA from copies of T1-weighted coronal images. This scale rates MTA as 0 (indicating absent) to 4 (indicating severe) according to the width of the surrounding cerebrospinal fluid spaces (choroidal fissure and temporal horns) and the height of the hippocampal formation, which includes the hippocampus proper, the subiculum, and the parahippocampal and dentate gyri. The scans were scored using the following criteria: 0 = no change; 1 = mild increase in the width of the choroidal fissure, normal temporal horn width, and the height of hippocampal

Table 1. Characteristics of Subjects With Alzheimer Disease (AD), Vascular Dementia (VaD), and Dementia With Lewy Bodies (DLB)*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients</th>
<th>Age, y</th>
<th>Age at Onset, y</th>
<th>Duration of Illness, mo</th>
<th>Sex, M/F</th>
<th>MMSE Score</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>25</td>
<td>77.8 (4.4)</td>
<td>74.2 (4.6)</td>
<td>40.7 (19.9)</td>
<td>9.16</td>
<td>15.4 (5.3)</td>
<td>APOE e2 0.06  APOE e3 0.52  APOE e4 0.42</td>
</tr>
<tr>
<td>VaD</td>
<td>24</td>
<td>76.9 (6.7)</td>
<td>73.5 (6.6)</td>
<td>41.0 (24.4)</td>
<td>14.10</td>
<td>18.0 (3.7)</td>
<td>APOE e2 0.0  APOE e3 0.75  APOE e4 0.25</td>
</tr>
<tr>
<td>DLB</td>
<td>22</td>
<td>77.2 (6.3)</td>
<td>74.0 (6.1)</td>
<td>37.6 (19.4)</td>
<td>14.8</td>
<td>14.5 (7.6)</td>
<td>APOE e2 0.02  APOE e3 0.68  APOE e4 0.30</td>
</tr>
</tbody>
</table>

*Values are given as mean (SD) except as noted. MMSE indicates Mini-Mental State Examination. Differences among the dementia groups in characteristics were not significant.

in subjects with AD13,14 and VaD.15,16 These lesions have been linked to vascular causes and diseases, and in postmortem studies, APOE e4 has been strongly associated with cerebral amyloid angiopathy,17,18 suggesting the possibility of an association between APOE e4 status, microvascular ischemia, and white matter lesions.

The primary aim of this study was to examine the relationship between the APOE e4 status, MTA, and white matter lesions on MRI and to investigate whether any association was specific to AD or present in other dementias when associated with APOE e4. Our hypotheses were that APOE e4 would be associated with increased preva-
formation; 2 = moderate increase in the width of the choroid fissure, mild increase in the width of the temporal horn, and mild decrease in the height of the hippocampal formation; 3 = severe increase in the width of the choroid fissure, moderate increase in the width of the temporal horn, and moderate decrease in the height of the hippocampal formation; and 4 = severe increase in the width of the choroid fissure and temporal horn and severe decrease in the height of the hippocampal formation. Scans were rated by consensus among 3 experienced raters (J.O'B., A.G., and P.S.). For the purposes of analysis, left and right MTA scores were summed to give a combined score, ranging from 0 to 8.

White matter lesions were rated using a semiquantitative scale from copies of proton density- and T2-weighted axial images by an experienced rater (P.S.). The scale provided a measurement of periventricular hyperintensities and deep white matter hyperintensities. Periventricular hyperintensities (maximum score = 6) represented the combined score for bands and frontal and occipital caps according to their size (0 = absent; 1 = ≤5 mm in width; and 2 = >5 mm and <10 mm in width). White matter hyperintensities were rated in 4 regions: frontal, temporal, parietal, and occipital (0-6 in each area, maximum total score = 24) according to the size and number of lesions (0 = absent; 1 = <3 mm, ≤5 lesions; 2 = <3 mm, ≥6 lesions; 3 = 4-10 mm, ≤5 lesions; 4 = 4-10 mm, ≥6 lesions; 5 = ≥11 mm, ≥1 lesions; and 6 = confluent).

DETERMINATION OF APOE GENOTYPE

The APOE genotypes were analyzed using the standard polymerase chain reaction method. Genomic DNA was isolated from white blood using a proprietary extraction method according to the manufacturer’s instructions (QIAamp Blood Kit; QIAGEN, Crawley, England). Amplification of the APOE gene containing the allelic sites was performed by a modification of the method of Wenham et al\textsuperscript{2} and Hixson and Vernier.\textsuperscript{22} The following polymerase chain reaction primers were used in the amplification: forward: 5’-TCCAAGGAGCTGGAGGCGGGA-3’, and reverse: 5’-ACAGAATTGCCCGGGCCCTGCTGA-3’. The reactions were performed in a final 50-mL volume of standard buffer containing 15 pmol of each primer; Taq polymerase (Pharmacia Biotech Ltd, St Albans, England), 1.0 U; 10% dimethyl sulfoxide; 200 mmol/L of each deoxynucleotide; and DNA, 200 ng. Reaction conditions were an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of annealing at 65°C for 30 seconds, an extension at 70°C for 90 seconds, and denaturation at 94°C for 30 seconds. The resultant amplification products were then digested overnight at 37°C with CfoI enzyme (Boehringer, Lewes, England), 2.5 U, in the buffer supplied. Following digestion, the polymerase chain reaction products were electrophoresed through a composite 3% NuSieve and 1% standard agarose (Flowgen, Lichfield, England) gel, and the bands were visualized using ethidium bromide fluorescence.

STATISTICAL ANALYSIS

A commercial statistical software package (Statistical Package for the Social Sciences for Windows, release 7.5; SPSS Institute, Inc, Chicago, Ill) was used for data analysis. Differences between groups on continuous variables were assessed using analysis of variance with the post hoc Scheffé test. For nonparametric data, the Kruskal-Wallis test, Mann-Whitney test, or Pearson χ² statistic was used as appropriate, with the Fisher exact probability test calculated for 2 × 2 tables when the expected cell frequency was less than 5. All statistical tests were 2-tailed and were regarded as significant at P<.05.

<table>
<thead>
<tr>
<th>Imaging Variables, Mean Score</th>
<th>MTA</th>
<th>PVH</th>
<th>WAH</th>
</tr>
</thead>
</table>
| Mean rating scores for medial temporal lobe atrophy (MTA), periventricular hyperintensities (PVH), and white matter hyperintensities (WMH) for all subjects with dementia (n = 71) according to APOE ε4 status. Lightly shaded bars indicate absence of ε4 allele; gray bars, presence of 1 × ε4 allele; and black bars, presence of 2 × ε4 alleles.

ε4 = 3.1, and 2 ε4 = 2.9; P = .83), and white matter hyperintensities (0 ε4 = 3.3, 1 ε4 = 4.9, and 2 ε4 = 4.9; P = .79).

When each dementia group was analyzed separately according to the presence or absence of the APOE ε4 allele, no significant differences were noted between MRI ratings and the presence of ε4 in subjects with AD, VaD, or DLB, as shown in Table 2.
No association was found between the presence of the APOE e4 allele and MTA and white matter lesions on MRI in subjects with late-onset AD, VaD, or DLB. The frequency of APOE e4 in the different dementia groups was consistent with that in previous studies, and in common with other findings, subjects with APOE e4 were more likely to have a family history of dementia in line with other reports. Overall, subjects were well matched for age, age at onset of illness, duration of illness, and the severity of cognitive impairment.

**MEDIAL TEMPORAL LOBE ATROPHY**

Our hypothesis of an association between the presence of APOE e4 and MTA and white matter lesions on MRI was not supported by the findings of this study. Furthermore, subjects homozygous for the APOE e4 allele had similar MTA ratings as those with 1 allele, and therefore, we did not observe an e4 allele dosage effect, as reported by Lehtovirta and colleagues.

Several factors may explain the discrepancy between the findings of this study and those reporting an association between APOE e4 and atrophy of medial temporal lobe structures. Lehtovirta et al. and Juottonen et al. used volumetric measurements to assess atrophy of the hippocampus and the entorhinal cortex, respectively. By comparison, our ratings of MTA may have lacked the sensitivity to detect small changes in volume. Alternatively, subjects in our study were older, and the association between the possession of the APOE e4 allele and subsequent disease may weaken with advancing age, in parallel with the diminishing effect of APOE e4 on the risk of dementia developing. Although subjects in a study by Tanaka et al. were of an equivalent age or even older, they were fewer (n = 34 vs 71), and the authors used a different scanning protocol (acquiring thicker slices from a low-resolution scanner) and method to rate atrophy.

The results of this study are consistent with the findings of Jack et al. Examining subjects of a similar age, they found that the APOE e4 genotype and hippocampal atrophy were independently linked with AD. They suggested that the lack of an association between APOE e4 and hippocampal atrophy could be explained by the differential association between hippocampal atrophy and the neurofibrillary tangle burden on the one hand and a putative link between APOE e4 and plaque burden on the other.

**WHITE MATTER LESIONS**

Our hypothesis of an association between the possession of APOE e4 and white matter lesions was, again, not supported by our findings. Skoog et al. likewise, found no direct link between APOE e4 and white matter lesions on computed tomography of subjects aged 85 years with AD or VaD. Other studies have also failed to find an association between APOE e4 and white matter lesions on MRI in normal elderly subjects and those with Dutch hereditary cerebral angiopathy. Overall, this finding indicates that the APOE e4 allele is unlikely to play a direct role in the pathogenesis of white matter lesions in late-onset dementias, although given the variety of disorders underlying the development of white matter lesions, confirmatory clinicopathological studies are necessary. Interestingly, the data are consistent with reports postulating that the APOE e4 allele and cerebrovascular disease, including white matter lesions, are independent but synergistic risk factors for the development of dementia.

**CLINICAL VS PATHOLOGICAL STUDIES**

A possible criticism of this and other antemortem studies would be the reliance on standardized clinical, rather than pathological, diagnoses. This might account for some of the homogeneity among the diagnostic groups, given that a proportion of patients with clinical diagnoses of late-onset dementias turn out to have overlapping features on pathological examination.

The role of APOE in the pathogenesis of dementias remains to be determined, and so far, postmortem studies have produced conflicting results. Although several studies have failed to find a specific association between APOE e4 status and the type or amount of disease, others have reported isoform-specific associations with impaired neu plasticity, neurofibrillary tangle formation, amyloid deposition, and cholinergic dysfunction. In contrast, a consistent pattern is emerging from antemortem studies indicating that a range of clinical changes, including the severity and pattern of cognitive impairment, noncognitive symptoms, and neurologic features, are not influenced by APOE e4 genotype. Although controversial, taken together, these clinical studies suggest that APOE e4 can modify the risk of acquiring dementia but not subsequent pathological processes.

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### Table 2. Scores of Imaging Variables by Diagnostic Group and APOE e4 Status

<table>
<thead>
<tr>
<th>MRI Variable</th>
<th>APOE e4 Status</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DLB (n = 22)</td>
</tr>
<tr>
<td>Medial temporal lobe atrophy</td>
<td>Absent</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>4.3</td>
</tr>
<tr>
<td>Periventricular hyperintensity</td>
<td>Absent</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>3.1</td>
</tr>
<tr>
<td>White matter hyperintensity</td>
<td>Absent</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*Values are given as mean scores. MRI indicates magnetic resonance imaging; DLB, dementia with Lewy bodies; AD, Alzheimer disease; and VaD, vascular dementia. Differences between APOE e4 status and imaging variables for dementia groups were not significant.*
The APOE ε4 allele was not associated with either MTA or white matter lesions in subjects with late-onset AD, DLB, or VaD. Furthermore, in this cross-sectional study, we found no evidence of a specific association between the type of dementia, the APOE ε4 genotype, and MRI ratings. Longitudinal studies may provide further insights into the pathogenic role of APOE ε4, but the results of this study are consistent with other studies, suggesting that APOE ε4 exerts an early influence, modifying the risk of acquiring dementia and age at onset, but appears not to have significant pathological effects thereafter.

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