Association between apolipoprotein E e4 allele and arteriosclerosis, cerebral amyloid angiopathy, and cerebral white matter damage in Alzheimer’s disease

J Tian, J Shi, K Bailey, C L Lendon, S M Pickering-Brown, D M A Mann

Objective: To investigate the association between white matter damage, as evidenced by myelin loss (ML), the extent of cerebral amyloid angiopathy (CAA), or arteriosclerosis (Art), and apolipoprotein E (ApoE) e4 allele in Alzheimer’s disease (AD), in order to understand the causes of damage to white matter in AD and its contribution to the pathogenesis of the disorder.

Materials and methods: Brain tissues were obtained from 94 patients with AD confirmed by autopsy. ApoE genotyping was performed by PCR on DNA extracted from frontal cortex or cerebellum. CAA and Art were assessed on Weigert’s haematoxylin and eosin stained sections in frontal, temporal, parietal, and occipital cortices; the extent of ML was scored on Luxol fast blue stained sections of these regions.

Results: The ApoE e4 allele frequency in the 61 patients with ML was not significantly different from that in the 33 patients without ML, nor did this differ in the 84 patients with Art from that in the 10 patients without Art. There were no significant differences in the proportions of patients with genotypes containing 0, 1, or 2 ApoE e4 alleles in the presence or absence of ML or Art. The mean ML, Art, or CAA scores within each region, and the total scores summed across all four brain regions, did not differ between patients with 0, 1, or 2 ApoE e4 alleles. However, the mean ML severity score in the occipital cortex was significantly greater than that in the frontal or temporal cortices in patients with 1 or 2 ApoE e4 alleles. The severity of CAA in the occipital cortex was significantly higher than that in other areas of cortex in patients with 0 or 2 ApoE e4 alleles. The mean Art score in the occipital cortex was greater than that in the temporal cortex in patients with two ApoE e4 alleles and was higher than that in the frontal cortex in patients with one ApoE e4 allele.

Conclusions: The likelihood of patients with AD suffering from CAA, Art, or ML is not influenced by ApoE e4 allele, nor is the overall burden of these pathological changes in the brain. However, the distribution of ML, CAA, and Art within the brain is at least partly influenced by genotype and dosage of ApoE e4 allele, with the occipital cortex being more severely affected by all of these pathological changes in e4 allele bearers, particularly when two ApoE e4 alleles are present.

Damage to the white matter of the cerebral cortex, in the form of signal hyperintensities seen on magnetic resonance imaging (MRI), is common in patients with Alzheimer’s disease (AD). The deep cerebral white matter is particularly vulnerable to ischaemia, because medullary arteries with few anastomoses, and arteries from the leptomeningeal border zones to the deep white matter, must pass through this region. However, the actual cause of white matter damage and loss in AD remains controversial. Although this could result from Wallerian degeneration resulting from neuronal and axonal loss associated with neurofilibrillary degeneration, vascular abnormalities are considered to be the main culprits.

One of the major vascular pathologies in AD involves the deposition of amyloid β protein (Aβ) within the walls of blood vessels supplying and penetrating the grey matter of the cerebral cortex and cerebellum, producing the condition known as cerebral amyloid angiopathy (CAA). The prevalence of CAA within the brain in patients with AD varies from about 81 to 96% cases. CAA within the leptomeningeal blood vessels is thought to cause dysfunction of the autoregulation of cerebral blood flow and may therefore predispose to ischaemia in areas distal to the lesions. However, arteriosclerosis (Art) of the arteries of the deep white matter is common in elderly subjects, including those with AD, and it is therefore also possible that white matter damage and loss in AD results from a chronic cerebral hypoperfusion caused by fibrohyalinosis of the medullary arteries.

Although the apolipoprotein E (ApoE) e4 allele may be associated with a higher burden of Aβ (especially Aβ1–40 but see Chalmers et al7) NFT,18 and phosphorylated tau19 in the brain parenchyma, the influence of the ApoE genotype on the extent of vascular pathology and white matter changes in AD is controversial. In one study,20 patients with the ApoE e3/e4 and e4/e4 genotypes had more extensive white matter lesions than patients with the e3/e3 genotype. Skoog et al21 reported that risk of dementia in very old people is associated with white matter loss and the ApoE e4 allele (but not each alone), suggesting an interaction between these two factors in the causation of dementia. However, in other reports,22–24 no associations with the presence or degree of white matter loss have been found. The extent of CAA has been linked in some studies to possession of the ApoE e4 allele, but see Chalmers et al7 especially in patients with the e4/e4 genotype,7 but other workers25 26 have not found such an association. Variations in findings may in part be due to the small sample sizes often

Abbreviations: Aβ, amyloid β protein; AD, Alzheimer’s disease; Apo, apolipoprotein; Art, arteriosclerosis; BA, Brodmann area; CAA, cerebral amyloid angiopathy; LFB, Luxol fast blue; ML, myelin loss; MRI, magnetic resonance imaging; WHE, Weigert’s haematoxylin and eosin.
employed, often with few patients homozygous for the ApoE e4 allele, and the variety of assessment methods used.

In this study we have investigated whether the ApoE e4 allele may constitute a risk factor for cerebral white matter damage in AD in its own right, or through its interactions with either CAA or Art. For this, we investigated the presence and distribution of white matter damage (in terms of myelin loss; ML), CAA, and Art in four brain regions of a large cohort of 94 patients with AD, and correlated these with possession of one or two copies of the ApoE e4 allele.

MATERIALS AND METHODS
All brain tissues used in this study had been obtained from patients in whom the diagnosis of AD had been confirmed at autopsy according to CERAD neuropathological criteria. Patients with diffuse Lewy bodies, large embolic infarcts, or primary vascular dementia in addition to AD lesions were excluded from the study. Ninety four patients (50 females: mean (SD) age at onset, 65.4 (10.3) years; age at death, 74.3 (10.1) years; duration of illness, 7.9 (2.9) years; 44 males: age at onset, 64.1 (10.4) years; age at death, 73.1 (9.4) years; duration of illness, 8.1 (3.3) years) with clinical and neuropathological evidence of AD were investigated. There were no significant differences in age at onset, age at death, or duration of illness between males and females.

Formalin fixed blocks of frontal (Brodmann area (BA) 8/9), temporal (BA 21/22), occipital (BA 17/18), and parietal (BA 39/40) cortex were similarly and routinely embedded in paraffin wax, and consecutive sections, cut at a thickness of 6 μm, were mounted onto 3-aminopropyltriethoxysilane coated slides. Sections were stained by Weigert’s haematoxylin and eosin (WHE) method to assess the severity of CAA and Art, and by Luxol fast blue (LFB) to assess the extent of ML as an index of white matter damage. The severity of CAA, the degree of Art, and the extent of ML were assessed semiquantitatively in each of the four cortical areas, as described elsewhere, and scores were summed across regions to provide a combined severity score for each patient. Our previous studies have shown that the severity of CAA can be detected as efficiently in WHE stained sections as in sections immunostained for Ab. LFB, however, is not a progressive stain, requiring differentiation in lithium carbonate for optimal histological demonstration of myelin. This could potentially induce some batch to batch variation in stained product. However, in this present study, LFB staining was quality controlled by including a known normal “control” case in each batch of sections from the AD cases in which the degree of myelin staining could be checked. Hence, it was possible to compare the degree of myelin staining in each of the AD cases on an equivalent basis. Moreover, even in those AD cases where ML was severe, there was residual well stained myelin in undamaged parts of the white matter in one or more of the sections from each case that could further act as an “internal standard” to check on the reproducibility of LFB staining compared with that in the control case.

ApoE genotyping was determined from DNA extracted from frozen samples of frontal cortex or cerebellum using PCR.

One way non-parametric analysis of variance (Kruskal-Wallis, using SPSS software) was applied to categorical variables when comparing differences in pathology ratings across brain regions for bearers of 0, 1, or 2 ApoE e4 alleles, or between bearers of 0, 1, or 2 ApoE e4 alleles for each brain region. Mann-Whitney U test was used post hoc to identify significant differences between groups/regions. χ² test was used to identify differences in frequency of affected cases within genotype groups. Two tailed p values ≤0.05 were considered statistically significant throughout all analyses.

RESULTS
Myelin loss
Myelin loss was found in 61 patients (65%). The ApoE e4 allele frequency in these 61 cases with ML (50/122 alleles, 0.41) was not significantly different from that in the 33 cases without ML (23/66 alleles, 0.35), nor were there any significant differences in the percentages of patients with each ApoE genotype, or between the percentage of patients with 0, 1, or 2 e4 alleles, in the presence or absence of ML (fig 1A). The mean ML score within each region in the 61 patients with ML (table 1), and the total score summed across all four brain regions (fig 2A) did not differ according to ApoE genotype, or between patients with 0, 1, or 2 e4 alleles. However, there were significant differences in the severity of ML scores between the four brain regions according to possession of ApoE e4 allele (table 1). Thus, the mean ML score in the occipital cortex in patients with 1 or 2 ApoE e4 alleles was higher (p<0.05–0.01) than that in frontal and temporal cortex (and parietal cortex when 2 e4 alleles were present) whereas there were no such regional differences in patients with no e4 alleles (table 1).

CAA
All 94 patients showed CAA to a greater or lesser extent, although not all brain areas were always or equally affected in every patient. The presence of CAA was therefore independent of ApoE genotype. The mean CAA score within each region and the mean total CAA score summed across all four brain regions (fig 2B) did not differ between patients with 0, 1, or 2 ApoE e4 alleles. However, the severity of CAA in the occipital cortex in cases with 0 or 2 ApoE e4 alleles was significantly higher (p<0.05–0.01) than that in the other regions of cortex, but there were no such regional differences in patients with 1 ApoE e4 allele (table 1).

Arteriosclerosis
Arteriosclerosis was detected to some degree in 84 patients (89%). The ApoE e4 allele frequency in the 84 cases with Art (67/168 alleles, 0.40) was not significantly different from that in the 10 cases without Art (9/20 alleles, 0.45), nor were there any significant differences in the percentages of patients with 0, 1, or 2 e4 ApoE alleles in the presence or absence of Art (fig 1B). The mean Art score within each region (table 1) and the mean total Art score summed across all four brain regions
preferentially affected by all three of the pathological ApoE e4 allele. Hence, the occipital cortex seems to be
may be at least partly influenced by genotype and dosage of
distribution of ML, CAA and Art within the brain with AD
changes in the brain so influenced. However, the actual
genotype, nor is the overall burden of these pathological
Present data suggest that the likelihood of patients with AD
cases is given in parenthesis.

**DISCUSSION**

The influence of ApoE genotype on the extent of white
matter damage and loss in AD is unclear. In MRI studies, the
possession of the ApoE e4 allele has been linked in some
studies to white matter lesions but in other MRI based
studies no correlation between white matter scores and ApoE
genotype was found. 22–26 28–30 25–28 29–32 33 34 35 Autopsy based studies are few, and
often based on small cohorts, but these too suggest no
association between the ApoE e4 allele and white matter
loss. 34 35 36 37 Present data, based on 94 autopsy assessed AD
patients, indicate that while the ApoE e4 genotype is not
associated with the likelihood of ML occurring in the brain
in AD, it may influence the location and severity of ML, with
the occipital cortex being more frequently and more severely
affected, compared with other brain regions, in patients with
ApoE e3/e4 and e4/e4 genotypes. Further studies are needed
to clarify the association between loss and damage to white
matter and the ApoE genotype.

The prevalence of CAA within the brain in patients with
AD has been estimated to vary from about 81 to 98% of
cases. 38–40 41–43 As previously reported, as most patients with AD
in this present study were overall moderately or severely
affected by CAA, with the occipital cortex being affected more
than the frontal, temporal, and parietal cortices. Possession of
the ApoE e4 allele in AD has been claimed to correlate with
the extent of CAA, especially in patients bearing ApoE
C4 genotype. However, not all investigators have confirmed
such an association. 24 25 In the present study we were unable to show any effect of possession of ApoE e4 allele on
either the likelihood of CAA being present in the brain, or the
overall severity of CAA. It is possible that different ways of
histologically demonstrating CAA and grading the degree of
severity between the various studies may have led to such
discrepancies of finding. Although in the present report, CAA
was assessed on WHE stained sections, we have found a
similar lack of effect of ApoE e4 allele in a subset of the 94
patients examined here using β-amyloid immunostaining
(J Tian, unpublished data). Most previous studies on CAA in
AD have not studied the effects of ApoE genotype on the
distribution and severity of CAA in the brain. Our
present data suggest that the distribution of CAA in AD may
be, at least partly, influenced by genotype and dosage of
ApoE e4 allele, with the occipital cortex being most
frequently and most severely damaged by CAA in those
patients carrying ApoE e4/e4 genotype. This predilection
for occipital cortex to be affected severely in cases with ApoE
e4/e4 genotype was also noted by Zarow et al. 24–26 27
Hence, the distribution and severity of CAA within the brain in
a patient with AD may be “driven” by the ApoE e4 allele,
although variations in overall severity of CAA between
patients with AD cannot be explained by the ApoE genotype
alone.

(See Fig 2C) did not differ between patients with 0, 1, or 2 ApoE e4
alleles. The mean Art score in the occipital cortex was
higher (p<0.05) than that in the temporal cortex in
patients with 2 ApoE e4 alleles. In patients with one
ApoE e4 allele, mean Art score in the occipital cortex was
higher (p<0.05) than that in the frontal cortex.

### Table 1

<table>
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<th>e4 alleles</th>
<th>Number of cases</th>
<th>Frontal</th>
<th>Temporal</th>
<th>Parietal</th>
<th>Occipital</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>21</td>
<td>0.9 (1.0)</td>
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<td>0.4 (0.7)**</td>
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<td>1.2 (0.9)</td>
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<td>2</td>
<td>10</td>
<td>0.3 (0.7)**</td>
<td>0.6 (1.1)</td>
<td>0.6 (0.7)*</td>
<td>1.4 (0.8)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>None</td>
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<td>1.6 (0.7)*</td>
<td>1.7 (0.9)</td>
<td>2.3 (0.8)</td>
</tr>
<tr>
<td>1</td>
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<td>1.6 (0.8)</td>
<td>1.7 (0.9)</td>
<td>2.0 (0.9)</td>
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<td>1.8 (0.6)**</td>
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</tr>
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<td>2.7 (2.7)</td>
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* * * p<0.05 and ** p<0.01 compared to occipital cortex.

**Figure 2** Mean (+SD) severity scores for ML (A), CAA (B) and Art (C) according to the presence of 0, 1, or 2 ApoE e4 (e4) alleles. Number of cases is given in parenthesis.
In the present study the distribution (but not the overall severity) of Art was also related to the ApoE e4 allele, with the occipital cortex again being affected more than any other brain region, especially in patients with the ApoE e4/e4 genotype. Therefore, as with CAA, the distribution of Art in the brain with AD may likewise be partly influenced by the ApoE e4 allele, which may act as a shared risk factor, explaining (in part) the association between the extent of CAA and Art in the brain in AD, although it is likely that other genetic or non-genetic factors may also be responsible.

It is interesting that the occipital lobe has more severe pathology for all three measures, ML, CAA, and Art, but only in ApoE e4 allele bearers. Does this mean that there is something intrinsically different about the occipital lobe, or the posterior cerebral circulation, compared with the rest of the brain, which makes this part of the brain especially vulnerable to vascular pathology? If so, the ApoE e4 protein may exacerbate such inherent vulnerability, perhaps through impaired tissue maintenance and response to damage.

Authors’ affiliations
J Tian, J Shi, K Bailey, D M A Mann, Clinical Neuroscience Research Group, University of Manchester, Hope Hospital, Salford, Manchester M6 8HD, UK
J Tian, J Shi, Department of Care of the Elderly, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 100700, China
Department of Psychiatry, University of Birmingham, Birmingham B15 2QZ, UK
S M Pickering-Brown, Department of Old Age Psychiatry, Institute of Psychiatry, London SE5 8AF, UK

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REFERENCES

2 Pantoj I, Garcia JH, Gutierrez JA. Cerebral white matter is highly vulnerable to ischaemia. Stroke 1996;27:1641–6