Cerebral amyloid angiopathy in traumatic brain injury: association with apolipoprotein E genotype

P D Leclercq, L S Murray, C Smith, D I Graham, J A R Nicoll, S M Gentleman

Objective: In view of the association of the apolipoprotein E (APOE) ε4 allele with poor outcome after traumatic brain injury we determined the frequency of cerebral amyloid angiopathy (CAA) and the extent of haemorrhagic pathology in relation to APOE genotype in an autopsy series of 88 head injured cases.

Methods: Tissue sections from the frontal and temporal lobes were immunostained for amyloid peptide (Aβ) and stained for Congo red to identify vascular amyloid pathology. A semiquantitative assessment of contusions, the total contusion index, was used to estimate the severity of the haemorrhagic pathology. APOE genotypes were determined by polymerase chain reaction of genomic DNA extracted from paraffin embedded tissue sections.

Results: CAA was present in 7/40 (18%) ε4 carriers compared with 1/48 (2%) non-ε4 carriers (p = 0.021, 95% confidence interval [CI] for difference in proportions with APOE 3% to 29%) with 6/40 (4 with CAA) ε4 carriers being homozygotes. Thus the risk of having CAA for ε4 carriers was 8.4 times that for the non-ε4 carriers. However, there was no clear tendency for patients with CAA to have more severe or more numerous contusions (median contusion index 19 (CAA) v 14.5, p = 0.23, 95% CI for difference in medians 5 to 14).

Conclusions: Presence of CAA in head injured cases was significantly associated with possession of an APOE ε4 allele but not with the severity of contusions.

METHODS

Case material

Formalin-fixed paraffin embedded brain tissue and associated clinical information for 88 cases of traumatic head injury were obtained from the archive of the Department of Neuropathology, Institute of Neurological Sciences, Glasgow. All work carried out with this tissue was approved by the ethics committee of the Southern General Hospital. For each case, paraffin sections were selected from both the frontal and medial temporal lobes. The immunohistochemistry was assessed blind to clinical details, pathological features, and APOE genotypes.

Determination of APOE genotypes

The APOE genotypes of the cases were already available from a previous study and had been determined by amplifying genomic DNA extracted from formalin-fixed tissue using a published method.

Immunohistochemistry

Paraffin sections were pretreated in 80% formic acid solution for eight minutes and incubated overnight at 4 °C with an antiserum raised against residues 18–22 of the human form of Aβ (clone 1E8, 1:2000 dilution; Glaxo SmithKline, UK). Aβ immunoreactivity was detected using the avidin biotin peroxidase method (ABC Elite kit, Vectors Laboratories, UK) and counterstained with Meyer’s haematoxylin.

Abbreviations: APOE, apolipoprotein E; CAA, cerebral amyloid angiopathy; Aβ, amyloid β-protein
method of Congo red was also used to stain amyloid and birefringence was visualised under polarised light.

Total contusion index

The contusion index is a measure based on the product of the extent (rated 1–3) and depth (rated 1–4) of contusions in any particular region of the brain. Therefore, a higher numerical index indicates more severe damage. The assessment is carried out both macroscopically and microscopically because there is usually an area of non-haemorrhagic necrosis deep to the haemorrhage, which cannot be assessed macroscopically. By combining the contusion indices from different areas of the same brain it is possible to calculate a total contusion index for that brain.37

Statistical analysis

Median values between two groups were compared using the Mann–Whitney test and confidence intervals (CI) and among three groups using the Kruskal–Wallis test. Proportions were compared using Fisher’s exact test. Confidence intervals for differences in proportions were calculated using a normal approximation although it is acknowledged that this may be inaccurate for small samples.

RESULTS

The head injury cohort consisted of 65 males and 23 females with a median age of 29.5 years ranging from less than a year old to 79 years of age. The median survival time was 46.5 hours and ranged from four hours to 24 days.

Prevalence of CAA in traumatic brain injury

Vascular amyloid deposition, identified by Congo red staining and Aβ immunoreactivity, was present in 8/88 (9%) trauma cases examined. All eight cases displayed Aβ deposits in the walls of small arteries and arterioles in the frontal and temporal cortices and the overlying leptomeninges ranging from mild to severe CAA (fig 1). In one case (21 year old male) the deposits were only in the leptomeninges (fig 1 A, B). This case was the youngest case with CAA, who survived 10 days following a vehicle accident and showed relatively severe contusional pathology illustrated by a total contusion index of 28. His APOE genotype was ε3ε4. The clinical features of those with and without CAA are given in table 1. Head injured patients with CAA were significantly older than those without CAA (p = 0.005, 95% CI for median difference 9 to 41 years). There was no statistically significant difference between the two groups with regard to cause of injury or survival time. Seven of the eight (88%) patients with CAA had accompanying deposits of Aβ in the form of plaques in the cerebral cortex. CAA without cortical plaques was identified in one case only. Cortical Aβ plaques were less common (17/80: 21%) among the patients without CAA (p<0.001, 95% CI for difference in proportions with Aβ plaques 43% to 91%).

APOE genotypes of head injured patients with CAA

Six of the 88 trauma cases were APOE ε4 homozygotes, which corresponds to about 7% of the cohort. This was slightly higher than expected but the APOE ε4 allele is known to be associated with poor outcome following traumatic brain injury, including mortality. The APOE ε4 allele frequency was higher in the patients with CAA (11/16; 69%) than in those without (35/160; 22%), and half of patients with CAA (4/8) were APOE ε4 homozygotes compared with 2% (2/80) of those without CAA. Nearly all of the patients with CAA were APOE ε4 carriers (7/8; 88%) compared with 33/80 (41%) patients without CAA (table 1). The degree of Aβ accumulation in the media and adventitia was also more pronounced in ε4 homozygotes (fig 1E–H) than in ε4 heterozygotes (fig 1A, B). Thus CAA was detected in 7/40 (18%) ε4 carriers but in only 1/48 (2%) non-ε4 carriers (p = 0.021, 95% CI for difference in proportions with CAA 3% to 29%). Thus the relative risk of CAA in trauma cases possessing at least one ε4 allele was increased 8.4 fold compared with non-ε4 carriers. Among the patients with CAA, there were no carriers of the APOE ε2 allele.

Relation between CAA and contusion index

The relation between CAA and contusion index is shown in fig 2. The data are positively skewed and the median total contusion index for the patients with CAA was 19 compared with 14.5 for the patients without CAA (p = 0.23, 95% CI for difference in medians −5 to 14). Detailed examination of the slides revealed no obvious pattern of anatomical association between the contusions and blood vessels displaying CAA. APOE genotypes of head injured patients with CAA

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Relation between APOE genotype and contusion index

The total contusion index values were plotted in relation to APOE genotype in a dose specific manner (fig 3). Again the
association between possession of APOE e4 and moderate severe contusional and severe hypoxic brain damage, but not other forms of pathology. There is also evidence that differences in coagulation profile may contribute to the relationship between APOE e4 and haemorrhage. It is possible that rigid, amyloid-laden blood vessels may be less haemodynamically reactive, perhaps impairing reactive changes in vascular tone and increasing vulnerability to ischaemia. These observations relating to pathological mechanisms seem likely to have clinical relevance because outcome studies indicate that head injured patients who are APOE e4 carriers tend to fare worse than non-carriers of APOE e4,24 and are also more prone to post-traumatic seizures.

Possession of APOE e2 appears to protect against Alzheimer’s disease in which Aβ deposition is a major pathological feature, both in plaques and in the cerebral vasculature. Remarkably, patients with APOE e2 who do develop CAA seem to be particularly prone to spontaneous haemorrhages5–10 perhaps because of susceptibility to the development of CAA-associated vasculopathy. In the present study, in keeping with these observations, none of the cases with CAA were APOE e2 carriers.

An alternative interpretation of our results revolves around CAA being a consequence of the trauma, particularly in the younger cases and in cases of dementia pugilistica41–42 perhaps reflecting deposition along perivascular drainage pathways as a result of increased Aβ in the brain. Weller et al43 have proposed that the impairment of the periarterial fluid drainage pathway may be responsible for the development of CAA in Alzheimer’s disease and in older people as it may reflect the failure to eliminate Aβ. Other hypotheses revolve around conditions that favour Aβ fibrillogenesis (for example hypoxia, acidosis, altered proteolytic environment, impaired axonal transport) and the ability of smooth muscle cells to clear Aβ.44 Animal models of CAA have provided a

### Table 1 Clinical detail of cases with and without cerebral amyloid angiopathy (CAA)

<table>
<thead>
<tr>
<th>Cause of injury (n (%))</th>
<th>Median (range) age in years</th>
<th>Median (range) survival time in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CAA</td>
<td>CAA</td>
<td>No CAA</td>
</tr>
<tr>
<td>Road traffic accident</td>
<td>4 (50)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Assault</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Median (range) contusion index</td>
<td>19 (0–54)</td>
<td>14.5 (0–46)</td>
</tr>
<tr>
<td>APOE genotype (n (%))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>e3</td>
<td>5/16 (31)</td>
<td>110/160 (69)</td>
</tr>
<tr>
<td>e4</td>
<td>11/16 (69)</td>
<td>35/160 (22)</td>
</tr>
<tr>
<td>APOE allele frequency (n/N (%))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>e3</td>
<td>5/16 (31)</td>
<td>110/160 (69)</td>
</tr>
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</table>
different perspective and also raise the possibility that CAA might occur as a consequence of head injury. Increased expression of cytokines is a feature of the response to head injury and increased expression of transforming growth factor (TGF)-β in transgenic mice results in the development of CAA.46 In addition, focal lesioning of the basal nucleus of Meynert in rabbits, the source of the cholinergic innervation of the cerebral cortex and vasculature, results in the formation of CAA accompanied by parenchymal plaques, within a few days.47 It is therefore of relevance that in humans the basal nucleus of Meynert is particularly susceptible to damage in severe head injury, particularly in the presence of raised intracranial pressure, brain swelling, and internal herniations resulting in a variety of haemorrhagic, ischaemic, and mechanical disturbances.48 There is evidence that the basal nucleus of Meynert, specifically, may be severely damaged in fatal head injury.49

In conclusion, CAA occurs in a small proportion of head injured patients, predominantly in APOE ε4 carriers. Although the CAA is likely to have been pre-existing in some of the older patients, the presence of leptomeningeal CAA in a 21 year old patient supports the concept that this pathology may have arisen de novo as a consequence of the trauma. However, although it seems there may be an association between APOE ε4 homozygosity and the severity of contunial damage, it remains unclear whether the CAA provides an anatomical substrate for the poor clinical outcome seen in these patients.

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