PAPER

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

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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-β 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 CAA 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.

The apolipoprotein E (*APOE*) gene has three common alleles (ε2, ε3, and ε4) producing corresponding isoforms of the protein: ApoE2 (Cys 112, Cys 158), ApoE3 (Arg 112, Cys 158), and ApoE4 (Arg 112, Arg 158). Accumulated evidence suggests that inheritance of an *APOE* ε4 allele renders some individuals more susceptible to a poor outcome following traumatic brain injury,[1–3] spontaneous intracerebral haemorrhage,[4–6] and possibly subarachnoid haemorrhage[7 8] but not apparently, after ischaemic stroke.[9 10] This varying influence of *APOE* genotype on outcome in different forms of acute brain injury raises the possibility that the underlying mechanism may involve vascular factors and enhanced susceptibility to haemorrhage.

Cerebral amyloid angiopathy (CAA) is a pathological condition characterised by the deposition of amyloid in cerebral cortical and leptomeningeal blood vessels. The classification of CAA is based on the specific protein deposited as amyloid and on whether the disease is inherited or sporadic.[11–13] The hereditary forms of the disease are very uncommon whereas sporadic CAA, where there is aggregation of amyloid β-protein (Aβ), is relatively common.[14 15] Sporadic CAA is strongly age related, affecting over a third of individuals above 60 years of age,[16–18] and it is present in 80–90% of cases with late onset Alzheimer's disease.[19–22] CAA manifests as circumferential deposition of Aβ in the media and adventitia with consequent thickening of the walls of blood vessels. This induces degeneration of the smooth muscle cells in the media of arterioles and small arteries, which weakens their walls rendering them brittle and unable to sustain large fluctuations in the cerebral blood flow.[12 13 23] CAA is usually clinically asymptomatic but occasionally patients present with spontaneous superficially located haemorrhages, which may be multiple or recurrent. Both symptomatic and asymptomatic CAA have been shown to be strongly associated with possession of *APOE* ε4.[20]

On the basis of these observations, we hypothesised that *APOE* ε4 carriers who sustain a head injury may be more likely to have CAA and may develop more frequent and severe haemorrhagic contusions as a result of their vascular pathology. This mechanism could explain, in part, why *APOE* ε4 is associated with poor outcome after traumatic brain injury.

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[24] and had been determined by amplifying genomic DNA extracted from formalin-fixed tissue using a published method.[25]

Immunohistochemistry

Paraffin sections were pretreated in 80% formic acid solution for eight minutes and incubated overnight at 4 ℃ 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 diaminobenzidene (DAB) as the chromogen. Sections were counterstained with Meyer's haematoxylin. The alkaline

Abbreviations: *APOE*, apolipoprotein E; CAA, cerebral amyloid angiopathy; Aβ, amyloid β-protein

method of Congo red[26] 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.[27]

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.

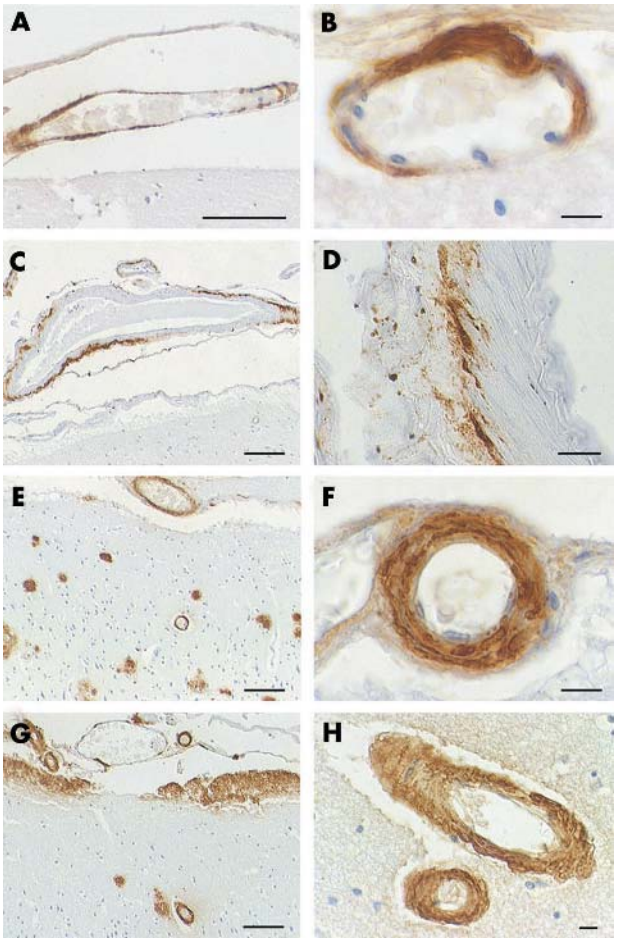

Figure 1 (A–H) Immunoperoxidase labelling for amyloid-β (Aβ) peptide illustrating cerebral amyloid angiopathy (CAA) severity in post-mortem tissue of head injured patients. CAA was present in 9% of trauma cases with nearly all showing Aβ plaques in the cortical grey matter of the frontal and temporal lobes (C–H). The exception was a 21 year old male with a survival time of 10 days who had partial deposition of Aβ only in the leptomeninges (A, B). In the other seven cases, the severity of CAA varied from partial deposition of Aβ (C, D) in small blood vessels (capillaries and arterioles) to complete Aβ laden blood vessels in the leptomeninges (F) and parenchyma (H), which was often associated with *APOE* ε4 homozygosity (E–H). In these patients, a proportion of the CAA almost certainly pre-dates the trauma. Bars = 100 μm in A, C, E, and G and 10 μm in B, D, F, and H.

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.

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

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

	CAA (n = 8)	No CAA (n = 80)
Median (range) age in years	58 (21–75)	26.5 (0–79)
Cause of injury (n (%))		
Fall	4 (50)	29 (36)
Road traffic accident	4 (50)	45 (56)
Assault	0	6 (8)
Median (range) survival time in hours	25.5 (16–240)	48 (4–576)
Median (range) contusion index	19 (0–54)	14.5 (0–46)
Aβ plaques (n (%))	7 (88)	17 (21)
APOE genotype (n (%))		
E2,2	0	2 (2)
E2,3	0	7 (9)
E2,4	0	4 (5)
E3,3	1 (12)	38 (48)
E3,4	3 (38)	27 (34)
E4,4	4 (50)	2 (2)
APOE allele frequency (n/N (%))		
ϵ2	0	15/160 (9)
ϵ3	5/16 (31)	110/160 (69)
ϵ4	11/16 (69)	35/160 (22)

data were positively skewed. Non-*APOE* ϵ4 carriers (n = 48) and *APOE* ϵ4 heterozygous cases (n = 34) had similar median total contusion index values (14.5 and 12, respectively). *APOE* ϵ4 homozygotes (n = 6) had a median total contusion index of 25, approximately double that of the other two groups: however, the small number of homozygotes was such that the difference between the medians of the three groups was not statistically significant (p = 0.057).

DISCUSSION

The results of the present study show that CAA is present in a relatively small proportion (9%) of patients who have had a fatal head injury. It tended to be present in older patients (median age 58 years; range 21–75), which is consistent with the known association between CAA and ageing.[17 18] Among head injured patients, CAA was considerably more likely to occur in those with a genetic predisposition conferred by possession of an *APOE* ϵ4 allele. Again this is consistent with previous studies, which have shown a strong association between CAA and *APOE* ϵ4.[20] Of particular interest is the possibility that the presence of CAA in patients who experience a head injury may influence the clinical outcome. For example, blood vessels laden with amyloid may be more prone to rupture after trauma, resulting in haemorrhage.[28 29] As this was a post-mortem study the only clinical outcome measure available was the survival time of the patients. The head injured cases with CAA had shorter survival time (median 25.5 hours; range 4 hours to 10 days) than trauma cases without CAA (median 48 hours; range 4 hours to 24 days) maybe indicating worse outcome, but because of the small number of cases and wide range in survival time this was not statistically significant.

In this study, the median total contusion index in the *APOE* ϵ4 homozygous head injured cases was approximately double that of the non-*APOE* ϵ4 carriers and the heterozygotes although the small number of homozygotes meant that this was not statistically significant. There was no obvious association between the presence of CAA and the severity or extent of contusions, although again the number of cases with CAA was relatively small.

Clinical studies of survivors of head injury have shown that intracranial haematomas in carriers of *APOE* ϵ4 have significantly larger volume than in non-carriers of *APOE* ϵ4.[30] In addition a study of pathological features of traumatic brain injury in relation to *APOE* genotype found a significant

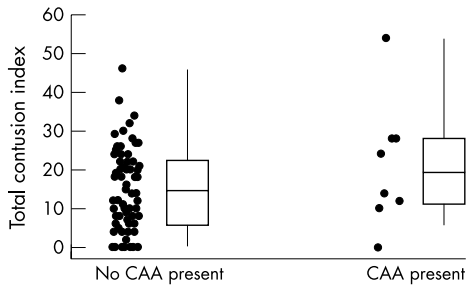

Figure 2 Dotplot (with x-axis random jitter to distinguish data points) and boxplot (showing the minimum, lower quartile, median, upper quartile and maximum values) of the total contusion index for those without and with cerebral amyloid angiopathy (CAA).

association between possession of *APOE* ϵ4 and moderate severe contusional and severe hypoxic brain damage, but not other forms of pathology.[31] There is also evidence that differences in coagulation profile may contribute to the relationship between *APOE* ϵ4 and haemorrhage.[32] 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* ϵ4 carriers tend to fare worse than non-carriers of *APOE* ϵ4[1–3 24] and are also more prone to post-traumatic seizures.[33]

Possession of *APOE* ϵ2 appears to protect against Alzheimer's disease[34] in which Aβ deposition is a major pathological feature, both in plaques and in the cerebral vasculature. Remarkably, patients with *APOE* ϵ2 who do develop CAA seem to be particularly prone to spontaneous haemorrhages[25 35–38] perhaps because of susceptibility to the development of CAA-associated vasculopathy.[35 39] In the present study, in keeping with these observations, none of the cases with CAA were *APOE* ϵ2 carriers.

An alternative interpretation of our results revolves around CAA being a consequence of the trauma, particularly in the younger cases[40] and in cases of dementia pugilistica[41 42] perhaps reflecting deposition along perivascular drainage pathways as a result of increased Aβ in the brain. Weller *et al*[43] 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

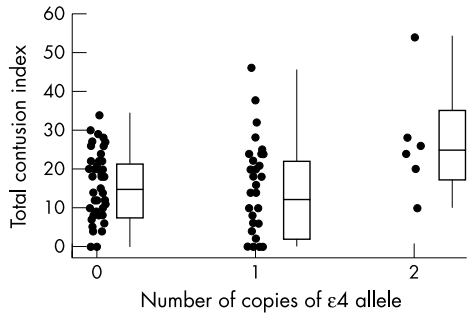

Figure 3 Dotplot (with x-axis random jitter to distinguish data points) and boxplot (showing the minimum, lower quartile, median, upper quartile and maximum values) of the total contusion index for those with 0, 1, and 2 copies of the *APOE* ϵ4 allele.

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.[45] 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.[46] 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.[47] There is evidence that the basal nucleus of Meynert, specifically, may be severely damaged in fatal head injury.[48]

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 contusional damage, it remains unclear whether the CAA provides an anatomical substrate for the poor clinical outcome seen in these patients.

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Competing interests: JARN and DIG are named applicants on the following patent: Method of prognosing chronic neurodegenerative pathology following a head injury. UK patent application 9415073.7 filed jointly by SmithKline Beecham and University of Glasgow on 27 July 1994; PCT worldwide application PCT/EP95/02827 filed on 13 July 1995; awarded 5 May 1998 (US5747260).

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