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There is no evidence of an association in children and teenagers between the apolipoprotein E ε4 allele and post-traumatic brain swelling

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Traumatic brain injury (TBI) is an important cause of mortality and disability in children and teenagers. A particular feature of the neuropathology at *post-mortem* is brain swelling. The cause of the swelling in some cases is not known, while in others it is associated with traumatic axonal injury or hypoxia. Apolipoprotein E (*APOE*) ε4 allele is known to be an important genetic determinant of outcome in children after TBI. We hypothesized a relationship between possession of *APOE* ε4 and diffuse traumatic brain swelling. A total of 165 cases aged between 2 and 19 years were identified from the department's tissue archive. *APOE* genotype was determined by polymerase chain reaction (PCR) in 106 cases. Bilateral swelling was present in 44 cases (11 with *APOE* ε4), unilateral swelling in 25 cases (7 with *APOE* ε4) and in 36 cases (9 with *APOE* ε4) there was no evidence of brain swelling. There was no significant relationship between possession of *APOE* ε4 and the presence of cerebral swelling ($\chi^2 = 0.09$, df = 2, $P = 0.96$). The 95% confidence interval for difference in proportions with swelling in those with and without the *APOE* ε4 is −19% to 22%. Thus, a significant relationship was not found between diffuse brain swelling and possession of *APOE* ε4, and in this cohort of patients there was an identifying cause of the brain swelling in all cases.

Keywords: apolipoprotein E polymorphism, brain swelling, traumatic brain injury

Introduction

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity in children and young adults [1–4]. One feature that has been identified as characterizing TBI in this group of patients is diffuse swelling of the brain in the absence of an identifiable cause [5,6]. Diffuse cerebral swelling was originally described as being present in 70% of cases of fatal head injury in children compared with 17% of adults [5]. The precise cause of the brain swelling is uncertain but one hypothesis concerns an altered vascular reactivity caused either by the mechanical loading of trauma per se and/or by an altered response to secondary factors such as hypoxia.

After about the age of 12 months the general characteristics of TBI in children, teenagers and adults is similar in that both focal (surface contusions, intracranial haematomas) and diffuse [hypoxic damage, diffuse traumatic axonal injury (TAI)] pathologies singly or in combination and in varying proportions may be present [7,8].

The apolipoprotein E (*APOE*) gene has three common alleles (ε2, ε3 and ε4) producing corresponding isoforms

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of the protein. There is some evidence that inheritance of the *APOE* ε4 allele increases the possibility of a worse outcome after TBI [9–12], spontaneous intracerebral haemorrhage [13–15] but not apparently after ischaemic stroke [16]. This varying influence of *APOE* genotype on outcome after different forms of acute brain injury raises the possibility that an underlying mechanism may involve vascular factors. Further, recent evidence from clinical imaging studies has suggested that cerebral swelling related to focal traumatic contusions is greater in carriers of *APOE* ε4 than noncarriers of ε4 [17], and it has also been shown that *APOE* knockout mice develop more marked cerebral oedema after experimental trauma than wild-type mice [18].

Recent clinical studies indicate that the association of *APOE* ε4 with poor outcome after head injury is stronger in children and young adults than in the middle-aged or elderly (Teasdale *et al.* – personal communication).

The current study is based on information obtained at post-mortem over a 40-year period. During this time the admission policy of the Department of Neurosurgery has changed as have the investigation and management of the cases after admission. Brain swelling is a complex event and may reflect the maturity of the blood–brain barrier and various mechanisms that among others may be genetically determined. Therefore this study was designed to test the hypothesis as to whether there is a relationship between the polymorphisms of *APOE* and brain swelling as determined at *post-mortem* regardless of all other factors. Therefore with this background, we hypothesized that the brain swelling in childhood and young adulthood after trauma may be associated with possession of *APOE* ε4 allele.

Materials and methods

There were 165 suitable cases identified from the departmental tissue archive between 1962 and 2000. These cases met the following inclusion criteria – aged between 2 and 19 years, managed by the Department of Neurosurgery at the Institute of Neurological Sciences, and having undergone a full *post-mortem* and a detailed neurohistological assessment from paraffin embedded material.

The cases aged less than 2 years of age were not included as the mechanism and neuropathology of this age group is different from that of adults [7]. This study was approved by the Research Ethics Committee of the Southern General Hospital, South Glasgow University Hospitals NHS Trust, Glasgow.

All brains were fixed in 4% formol saline for a minimum of 3 weeks before dissection, after which a full macroscopic and microscopical examination was undertaken. Blocks for neurohistology were taken from the cerebral hemispheres at the level of the lateral geniculate bodies (bilateral parasagittal including corpus callosum, parietal cortex at junction between the anterior cerebral and middle cerebral arterial boundary zones, the insulae, thalami including internal capsules, and the hippocampi), each cerebellar hemisphere including the dentate nuclei, and the brainstem at the levels of the midbrain, pons and the medulla. Eight-μm-thick sections were cut from each block and stained with haematoxylin and eosin (H&E), and by a method that combines Luxol fast blue and Cresyl violet (LFB/CV).

A proforma was drawn up for the entry of data.

Brain swelling was identified by diffuse swelling of the cerebral hemispheres and a corresponding reduction in the ventricular system. Unilateral swelling was present if in addition to the diffuse enlargement of one cerebral hemisphere there were a reduced ventricular size ipsilateral to the affected hemisphere and a shift of the midline structures to the contralateral side by more than 3 mm.

Raised intracranial pressure was considered to be present if there were internal herniae identified by wedges of pressure necrosis [19] and associated infarcts in the distribution of the anterior cerebral or pericallosal artery, the posterior cerebral artery, the posterior inferior cerebellar artery, and in the brainstem.

Skull fractures were documented as being either present or absent, and intracranial haematomas (greater than 2.0 cm thick or 25 ml in volume) were coded according to anatomical compartment (extradural, subdural and intracerebral). Contusions were assessed quantitatively using the total contusion index: this assessed the extent and depth of contusions in various anatomical locations, producing a contusion score for each hemisphere, which is then combined and interpreted as absent, minimal, moderate or severe [20]. Diffuse TAI was graded [21]. Grade 1 lesions had widespread axonal damage in the corpus callosum, the cerebral hemispheres and brainstem, grade 2 lesions in addition had focal lesions in the corpus callosum, and grade 3, in addition to the features of grade 2, had a lesion in the rostral brainstem. Diffuse TAI was diagnosed using Palmgren silver impregnation on the 1962–1993 inclusive cases ($n = 117$): thereafter ($n = 48$)

by immunohistochemistry using an antibody to β-APP (monoclonal, Boehringer Mannheim, 1 : 3000) and in some cases using an antibody to reactive microglia/macrophages (monoclonal, Dako, 1 : 200). The antibodies were detected using the ABC kit (Vector stain, Vector Laboratories, Peterborough, UK). For the purposes of this study only the 48 cases diagnosed diffuse TAI by immunohistochemistry have been included in the results.

Ischaemic damage when present, was assessed as severe, moderate or mild [22]. Severe damage comprised those cases in which the lesions were diffuse, multifocal or large within arterial territories, moderate when the ischaemic damage was limited to the arterial boundary zones, singly or in combination with subtotal infarction in the distribution of the cerebral arteries, or if there were 5–10 subcortical lesions in the brain.

APOE genotyping was performed, 'blind' to the histological assessments, using a hot start polymerase chain reaction (PCR) method that has been optimized for archival formalin-fixed paraffin-embedded tissue [23].

Statistical analysis

The relationship between the *APOE* ε4 genotype and brain swelling was examined using a chi-squared test of association and quantified using 95% confidence intervals for the difference in proportions with swelling (bilateral or unilateral). With the numbers involved there would be over 80% power to detect a difference of 0.3 in the proportions with brain swelling in the two groups.

Table 1. Characteristics of the 165 cases of traumatic brain injury in children and young adults (2–19 years)

	Number (%)
Male	126/165 (76%)
Age (years) median : range : Q1–Q3	13 : 2–19 : 8–17
Cause of injury	
RTA	126/162 (78%)
Fall	19/162 (12%)
Assualt	13/162 (8%)
Other	4/162 (2%)
Skull fracture	121/165 (73%)
Raised ICP	136/162 (84%)
Ischaemic damage	
None	27/147 (18%)
Mild	35/147 (24%)
Moderate	30/147 (20%)
Severe	55/147 (37%)
Brain swelling	
None	57/163 (35%)
Unilateral	38/163 (23%)
Bilateral	68/163 (42%)
Haematoma	
None	102/165 (62%)
Extradural only	14/165 (8%)
Subdural only	24/165 (15%)
Extradural and subdural	1/165 (1%)
Intracerebral	10/165 (6%)
Burst lobe	13/165 (8%)
Subdural and burst lobe	1/165 (1%)
Total contusion index median : range : Q1–Q3	14 : 0–48 : 6–20
Survival (days) median : range : Q1–Q3	3 : 1–150 : 1–4
Diffuse traumatic axonal injury	
None	8/48 (17%)
Grade 1	5/48 (10%)
Grade 2	5/48 (10%)
Grade 3	30/48 (63%)

Q1, lower quartile; Q3, upper quartile; RTA, road traffic accident; ICP, intracranial pressure.

Results

The principal features of the 165 cases studied are given in Table 1. There were 126 males and 39 females with a median age of 13 (range 2–19) years and a survival of between 1 h and 5 months (median 3 days). The most common cause of injury was road traffic accident (78%).

It was possible to determine *APOE* genotype from the formalin-fixed paraffin-embedded *post-mortem* tissue in 106/165 (64%) cases: 27/106 (25%) were in possession of the *APOE* ε4 allele. The principal neuropathological findings of these 106 cases are given in Table 2 and are comparable to those in the 165 cases. Diffuse bilateral brain swelling was present in 44 cases, unilateral swelling in 25 cases and in 36 cases there was no evidence of brain swelling. Of the 44 cases with bilateral brain swelling *APOE* ε4 allele was present in 11 (25%) cases compared with 7 (28%) with unilateral hemispheric swelling, and in 9 (25%) cases where swelling was not a feature. There was no significant relationship between possession of *APOE* ε4 allele and the presence of cerebral swelling ($\chi^2 = 0.09$, df = 2, $P = 0.96$). The 95% confidence interval for difference in proportions with swelling in those with and without the *APOE* ε4 is −19% to 22%.

Discussion

The 165 cases in this study were identified from the tissue archives of the Department of Neuropathology between 1962 and 2000, the inclusion/exclusion criteria reflect-

Table 2. Characteristics of the 106 cases in which *ApoE* Genotype (ε4 present or not) was determined

	Total *Number (%)*	*ε4 present* *Number (%)*	*ε4 absent* *Number (%)*
	106 (100%)	27 (25%)	79 (65%)
Male	79/106 (75%)	15/27 (56%)	62/79 (78%)
Age (years) median : range : Q1–Q3	14 : 2–19 : 8.5–17	13 : 4–19 : 7–17	15 : 2–19 : 9–17
Cause of injury			
RTA	85/103 (83%)	20/26 (77%)	65/77 (84%)
Fall	8/103 (8%)	2/26 (8%)	6/77 (8%)
Assualt	8/103 (8%)	4/26 (15%)	4/77 (5%)
Other	2/103 (2%)	0/26	2/77 (3%)
Skull fracture	82/106 (77%)	19/27 (70%)	63/79 (80%)
Raised ICP	87/105 (83%)	22/26 (85%)	65/79 (82%)
Ischaemic damage			
None	19/99 (19%)	3/26 (12%)	16/73 (22%)
Mild	27/99 (27%)	8/26 (31%)	19/73 (26%)
Moderate	22/99 (22%)	4/26 (15%)	18/73 (25%)
Severe	31/99 (31%)	11/26 (42%)	20/73 (27%)
Brain swelling			
None	36/105 (34%)	9/27 (33%)	27/78 (35%)
Unilateral	25/105 (24%)	7/27 (26%)	18/78 (23%)
Bilateral	44/105 (42%)	11/27 (41%)	33/78 (42%)
Haematoma			
None	70/106 (66%)	18/27 (67%)	52/79 (66%)
Extradural only	7/106 (7%)	1/27 (4%)	6/79 (8%)
Subdural only	18/106 (17%)	6/27 (22%)	12/79 (15%)
Extradural and subdural	1/106 (1%)	1/27 (4%)	0/79
Intracerebral	3/106 (3%)	1/27 (4%)	2/79 (3%)
Burst lobe	6/106 (6%)	0/27	6/79 (8%)
Subdural and burst lobe	1/106 (1%)	0/27	1/79 (1%)
Total contusion index median : range : Q1–Q3	14 : 0–38 : 6–18	10 : 0–30 : 4–16	14 : 0–38 : 8–18
Survival (days) median : range : Q1–Q3	3 : 1–150 : 1–4.75	3 : 1–150 : 1–5.5	2 : 1–9 : 1–3
Diffuse traumatic axonal injury			
None	8/44 (18%)	2/8 (25%)	6/36 (17%)
Grade 1	5/44 (11%)	2/8 (25%)	3/36 (8%)
Grade 2	5/44 (11%)	0/8	5/36 (14%)
Grade 3	26/44 (59%)	4/8 (50%)	22/36 (61%)

Q1, lower quartile; Q3, upper quartile; RTA, road traffic accident; ICP, intracranial pressure.

ing the ages over which brain swelling is a measurable feature that is recognized at *post-mortem* and not withstanding the fact that these cases may not be representative of the complete spectrum of head injury seen in children and teenagers. However, they do represent a particular cohort of patients who died after TBI and as such represent one end of a spectrum of brain injury seen in patients admitted to the Department of Neurosurgery.

Diffuse cerebral swelling occurs after head injury in children and less commonly in teenagers [6]. Historical literature has suggested that it is a not uncommon finding at *post-mortem* [5], in children occurring in 70% of fatalities compared with 17% in adults and similarly brain swelling was found in six of 11 cases (54%) surviving for longer than 60 min [24]. CT imaging studies demonstrate small ventricles and subarachnoid cisterns with compression or absence of the basal cisterns in the absence of a mass lesion [25]. Repeat imaging some 7–10 days later showed that the ventricles and cisterns usually return to normal size. However, in severely injured children the swelling may increase with the onset of the syndrome of 'malignant brain oedema' the outcome of which may be death [6].

At *post-mortem* diffuse brain swelling in children has been variously associated with diffuse TAI, contusion, ischaemic damage and intracranial haematoma [5,24]. However, none of these pathologies may be present and the high CT density of brain tissue sometimes seen after TBI has been interpreted as evidence of cerebral hyperaemia [25] with an increase in cerebral blood volume [2].

However, hyperaemia has not been demonstrated in all cases although it was present in 28 of 32 severely head-injured children at some time after admission to hospital [26,27].

TBI is a major cause of mortality and morbidity in children and young adults [2,28]. Of those who survive outcome is dependent on a number of factors one of which might be apolipoprotein E genotype (*APOE*: Gene, apoE: protein). *APOE* is a polymorpic gene with three alleles: ε2, ε3 and ε4. Previous studies have associated possession of the ε4 allele with an unfavourable outcome after acute TBI with 57% of patients who possessed *APOE* ε4 having an unfavourable outcome (dead, vegetative state or severe disability) compared with 27% of patients who did not possess ε4 [12].

Other clinical studies have shown that possession of *APOE* ε4 is associated with a poor clinical outcome after intracerebral haemorrhage [13,14,17], cerebral ischaemia after cardiopulmonary resuscitation [29] and cardiac bypass surgery [30,31] but not apparently after ischaemic stroke [32].

The cellular and molecular mechanisms that are influenced by *APOE* genotype in response to acute brain injury are complex and are not completely known. The *APOE* ε4 allele is associated with protection from oxidative stress [33,34], excitotoxicity and increased intracellular calcium [35], decreased immunosuppressive effect on microglial proliferation [36], increased risk of atherosclerosis [37], altered blood coagulation [38], and decreased tumour necrosis factor α production [39]. Either singly or in combination these effects of the *APOE* gene may result in a greater amount of lipid peroxidation, increased capillary permeability with oedema formation [18], erythrocyte diapedesis and parenchymal bleeding.

In view of these data and the need to establish relevant experimental models of TBI in children [40] we tested the hypothesis in a group of children and teenagers that either unilateral or bilateral hemispheric swelling might be determined by *APOE* genotype. The original cohort of 165 cases was identified from the Department's large archive of head-injured material [41] using criteria of age, survival, cause of injury, and full *post-mortem* and neuropathological examination to establish the nature and distribution of any pathologies. The cohort was subsequently reduced to 106 cases because of difficulty in determining the *APOE* genotype from the paraffin-embedded material [8] using an established technique. The ε4 allele was present in 25% (27/106) cases an allelic prevalence of 28.5% for the Scottish population [42]. It is accepted that a limitation of this study is that the patient cohort has been selected by death and that a bias towards the worst outcome has been introduced. However, recent clinical studies provide a degree of clinical correlation with the *post-mortem* work [17,43].

The patient sample size is sufficient to detect a difference of 30% in the proportion with swelling with 80% power. The actual difference observed was 1%**.** Given the number and complexity of the effects of *APOE* perhaps the results are not surprising. Perhaps more likely is that *APOE* is but one gene that responds to acute brain injury and in as yet are undermined way effects the outcome.

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