The response of the brain to traumatic injury and genetic influences.

Colin Smith

BSc MB ChB MRCPath

Submitted for the degree of MD to the University of Glasgow

Work undertaken in the Academic Unit of Neuropathology

Division of Clinical Neurosciences

July 2004

Acknowledgements

I would like to thank Professors David Graham and James Nicoll for their helpful guidance and supervision. I would also like to thank the following;

Mrs Janice Stewart for expertise and assistance with genotyping of cases.

Mrs Mary-Ann McKinnon for invaluable technical assistance with cutting and staining of tissue sections.

Dr Lilian Murray for advice on statistical analysis.

I would like to thank all the members of staff and research students in the Academic Unit of Neuropathology for their support and contributions to discussions related to the research topic.

This work was partially funded by a Clinical Research Fellowship from the Scottish Council for Postgraduate Medical and Dental Education, and by funding from both the Medical Research and National Institute of Health (NIH) USA.

Contents

List of figures and tables

Graph number	**Graph title**	**Page**

Publications

1. Graham DI, Smith C. (2001) The pathology of head injury. *CPD Bulletin-Cellular Pathology* **3;** 148- 151.
2. Smith C, Graham DI, Murray L, Nicoll JAR (2003) Tau immunohistochemistry in acute brain injury. *Neuropath Appl Neurobiol* **29**; 496-502.
3. Smith C, Nicoll JAR, Graham DI (2004) Head Injury and Dementia. In, The Neuropathology of Dementia (eds. Esiri MM, Morris JH, Trojanowski J) 2nd Ed., Cambridge University Press.
4. Gentleman SM, Leclercq PD, Moyes L, Graham DI, Smith C, Griffin WS, Nicoll JA (2004) Long-term intracerebral inflammatory response after traumatic brain injury. *Forensic Sci Int* 146: 97-104.
5. Smith C, Graham DI, Murray L, Nicoll JAR (2004) Association of APOE ε4 and cerebrovascular pathology in traumatic brain injury. Submitted.

Presentations at Professional Societies

Oral Presentations

1. Tau imunohistochemistry in acute brain injury. Smith C, Graham DI, Nicoll JAR (2001) 100th Meeting of the British Neuropathological Society, London.
2. Association of APOE polymorphisms and pathological features in traumatic brain injury. Smith C, Graham DI, Murray L, Nicoll JAR (2002) 102nd Meeting of the British Neuropathological Society, London.

3. Possession of the *IL-1A* allele 2 and the neuroinflammatory response after head injury. Smith C, Graham DI, Murray L, Nicoll JAR, Gentleman SM, Leclercq PD (2003) 104th Meeting of the British Neuropathological Society, Glasgow.

Poster Presentations

1. GAP-43 Immunoreactivity following traumatic brain injury- evidence for neuronal regeneration? Smith C, Graham DI, Nicoll JAR (2001) 77th Annual Meeting of the American Association of Neuropathologists, Chicago.

List of abbreviations

Aβ;	β-amyloid protein
Aβ-PP;	β-amyloid precursor protein
AD;	Alzheimer's disease
APC;	antigen presenting cell
AOI;	Area of interest
apoE;	apolipoprotein E
APOE;	gene encoding apolipoprotein E
DNA;	deoxyribonucleic acid
GCS;	Glasgow Coma Scale
GOS;	Glasgow Outcome Scale
IL-1;	interleukin-1
IL-1A;	gene encoding interleukin-1α
IL-1B;	gene encoding interleukin-1β
IL-8;	interleukin-8
IL-18;	interleukin-18
MAP;	microtubule-associated protein
MHC;	major histocompatability complex
PCR;	polymerase chain reaction
PNS;	peripheral nervous system
RTA;	road traffic accident
TAI;	traumatic axonal injury
TBI;	traumatic brain injury
TCI;	total contusion index

Summary

Traumatic brain injury (TBI) remains a significant cause of morbidity and mortality, and is one of the commonest causes of disability in young people. Increasingly evidence is emerging to suggest that TBI results in long-term neurodegeneration and is a risk factor for the subsequent development of Alzheimer's disease (AD) in later life. The mechanisms underlying the association between head injury and AD are unknown, although the response to TBI and the pathology of AD have some features in common not only in terms of a cellular and protein response but also striking parallels in the genetic influences.

Statement of aims: This studied aimed to assess the potential contribution of cytoskeletal pathology and neuroinflammation to long-term outcome after head injury, and influence of genetic factors in determining outcome.

Methodology: Archived human brain tissue was used for this study. Both cytoskeletal pathology and neuroinflammation were assessed by immunohistochemistry. Cytoskeletal pathology was assessed semi-quantitatively while neuroinflammation was assessed using image analysis. Genotyping of cases used a PCR based technique. Standard statistical methods were used to assess the data.

Neurofibrillary tangles were not seen in acute fatal TBI cases but they may be an important feature of neurodegeneration in long-term survivors. Cytoskeletal neurodegenerative pathology has been demonstrated after repetitive mild head injury in humans and a single head injury in animals, and is a major component of the neurodegenerative pathology associated with AD. This study assessed *tau*-associated

cytoskeletal pathology in individuals dying after a single episode of TBI. Subtle alterations in *tau* immunoreactivity, for example in oligodendrocytes, were present in some head injury cases but not controls. However, neurofibrillary tangles did not appear more prevalent after TBI when compared with age-matched controls. Although alterations in *tau* immunoreactivity may occur which warrant further study, neurofibrillary tangles were not more prevalent after a single fatal episode of TBI.

Possession of APOE ε4 was associated with more severe vascular related pathology after fatal TBI (contusions, global cerebral ischaemia) and this may be relevant to the relatively poor outcome from TBI in patients with APOE ε4 identified in clinical studies. APOE has genetic polymorphisms which may increase susceptibility to AD. Possession of *APOE* ε4 has been shown to increase susceptibility to AD. In addition possession of *APOE* ε4 is associated with a worse outcome after TBI. This study demonstrated that possession of *APOE* ε4 is associated with the greatest incidence of moderate/severe contusional injury and severe ischaemic brain damage in fatal cases of TBI.

There does appear to be an increased neuroinflammatory response after TBI particularly in cases with diffuse traumatic axonal injury. This increased neuroinflammatory response may be associated with greater neurotoxicity. Neuroinflammation has been implicated as a potential cause of neurodegeneration both in AD and in the response to brain injury. There have been few studies looking at the neuroinflammatory response after acute head injury or in long-term survivors.

Possession of IL-1A allele 2 is not associated with a greater neuroinflammatory response. Interleukin-1 (IL-1) is the main controller of neuroinflammation and homozygosity for *IL-1A* allele 2 has been shown to increase

susceptibility to AD. This study has demonstrated no association between possession of *IL-1A* allele 2, either heterozygosity or homozygosity, and an increased inflammatory response (identified by expression of MHC class II molecules) after TBI. However, the neuroinflammatory response is most pronounced in cases with diffuse white matter damage (diffuse traumatic axonal injury).

Conclusion: This study has demonstrated that possession of *APOE* ε4 is associated with the greatest incidence of moderate/severe contusional injury and severe ischaemic brain damage in fatal cases of TBI and that this may account for the over-representation of *APOE* ε4 in severe outcome after head injury. With regard to neurodegeneration after head injury there is increased neuroinflammation, particularly in cases with diffuse TAI, but this did not appear to be modified by genetic influences although the number of cases studied was small. Cytoskeletal pathology was limited to subtle glial alterations although the study only looked at a survival of less than 12 months.

1 Introduction

1.1 The mechanisms of traumatic brain injury and its significance in society

1.1.1 Incidence of traumatic brain injury

Traumatic brain injury remains a significant cause of morbidity and mortality throughout the world. In the United Kingdom more than 150 000 patients are admitted to hospital each year with a head injury. Of this group more than 80% are classified as having a mild head injury, as defined by the Glasgow Coma Scale (GCS). The GCS (Teasdale and Jennett 1974, Teasdale and Jennett 1976) provides a means of quantifying the level of consciousness after traumatic brain injury based on the clinical features of verbal performance, eye opening and motor response. Using this scale three levels of severity of head injury are defined; mild (score 13-15), moderate (score 9-12), and severe (score 3-8).

1.1.2 Outcome after traumatic brain injury

Approximately 1-2% of patients admitted to hospital after traumatic brain injury die as a consequence of their injuries. The majority of fatalities are within the severe head injury group, with 40% of the cases resulting in death at 6 months (Murray *et al* 1999). The outcome is modified by the type and severity of injury and may be influenced by the pre-morbid state such as age, nutritional status and pre-existing disease (Vollmer 1993).

Among survivors of traumatic brain injury of all grades chronic disability may have a physical component although it is predominantly the cognitive and behavioural problems which provide the greatest challenge (Jennett *et al* 1981). Outcome may be assessed by the Glasgow Outcome Scale (GOS) (Jennett and Bond 1975) which defines four outcome states; death/ vegetative state, severe disability, moderate disability, and good recovery. The GOS is based predominantly on assessment of

social re-integration after traumatic brain injury involving a structured questionaire-based interview. This has recently been modified as the extended GOS (Teasdale *et al* 1998). Predictors of neurobehavioural outcome in adults include age (greater than 50 years is a poor prognostic factor), the acute GCS, abnormal brain stem reflexes, subacute ventricular enlargement, neurological deficit, and the duration of post-traumatic amnesia (Capruso and Levin 2000).

Recent studies have indicated that the incidence of moderate and severe disability in young people and adults one year after mild head injury is similar to that seen in survivors of moderate and severe head injury (Thornhill *et al* 2000). In addition there is evidence (discussed below) that traumatic brain injury may be associated with continuing cognitive decline in later years and with an increased incidence of Alzheimer's disease (AD). The mechanisms underlying the association between head injury and AD are unknown, although, as shall be discussed, the response to traumatic brain injury and the pathology of AD have some features in common not only in terms of a cellular and protein response but also striking parallels in the genetic influences.

1.1.3 The pathology of traumatic brain injury

In order to attempt to clarify the mechanisms underlying post-traumatic cognitive deficit a basic understanding of the pathology of traumatic brain injury is helpful.

Blunt force head injury results in both focal and diffuse pathologies involving the skull and the underlying brain and its coverings. Focal lesions can take the form of skull fractures, cerebral contusions, focal ischaemic lesions secondary to raised intracranial pressure, and intracranial haematomas. Diffuse lesions may take the form of cerebral ischaemia or cerebral swelling, or may develop as a consequence of

rotational forces (diffuse traumatic axonal injury and diffuse vascular injury) (Graham *et al* 1995a). The primary injury is related to mechanical damage, and can be focal or diffuse, or a combination of both. It is related to the effects of both the impact and inertial forces on the skull and brain. Delayed secondary events such as diffuse traumatic axonal injury and cerebral ischaemia develop over a period of hours or days after the traumatic episode. The secondary events may be related to neurochemical alterations and the associated cellular and molecular alterations induced by trauma (Graham *et al* 2000).

1.2 The effects of head injury on cognitive function

1.2.1 Concussion and vegetative state

Concussion refers to an immediate, usually reversible episode of brain dysfunction after traumatic brain injury. A clinical spectrum is recognised ranging from mild concussion, in which consciousness is often preserved, to severe diffuse axonal injury resulting in the vegetative state (Gennarelli 1993). The anatomical basis of concussion syndromes is currently considered to be diffuse traumatic axonal pathology and, in particular, axonal disruption resulting in disconnection between areas involved in consciousness; cerebral cortex, brainstem reticular activating areas, thalamus and hypothalamus (Gennarelli 1993). The vegetative state refers to a group of patients who have loss of meaningful cognitive function and awareness, but retain spontaneous breathing and periods of wakefulness. The neuropathological basis of the vegetative state has been explored in a study that examined 49 patients in the vegetative state, 35 of whom had experienced traumatic brain injury (Adams *et al* 2000). In the trauma-related cases diffuse traumatic axonal injury of grade 2 or 3 was found in 71% of cases, and thalamic pathology in 80% of cases. In cases with minimal brainstem and cerebral cortical pathology, thalamic pathology was always present.

Therefore, damage to the thalamic nuclei and/or the afferent and efferent white matter pathways of the thalamus appear to play a major role in the genesis of the vegetative state after head injury. White matter (Wallerian) degeneration is a consequence of severe diffuse traumatic axonal injury. The axonal loss results in gliosis and macrophage activation, which may be under genetic control as discussed later. In contrast, the structural basis of moderate disability after traumatic brain injury is more likely to be a focal lesion rather than diffuse brain pathology, usually an evacuated intracranial haematoma (Adams *et al* 2001). In a study of 30 severely disabled patients 50% had focal brain pathology only. Some severely disabled patients did show diffuse brain pathology similar to vegetative state patients, and it may be that there is a greater quantitative amount of damage in the vegetative cases (Jennett *et al* 2001). In assessment of the pathology of moderate and severe disability case selection may be important. It must be remembered that autopsy based studies may not be a true reflection of the clinical spectrum associated with both moderate and severe disability.

1.2.2 Long term outcome from head injury and chronic neurodegeneration

1.2.2.1 Clinical studies

Mild head injury (acute GCS 13-15) is associated with a higher than expected incidence of disability (GOS moderate or severe disability) at one year post injury (Thornhill *et al* 2000). Of major interest in the context of this discussion are the longer term effects on cognition many years after the injury, and the possible relationship between traumatic brain injury and AD.

There is a considerable epidemiological literature examining the relationship between traumatic brain injury and the development of AD in later life. Many of these take the form of retrospective case-control studies and are therefore subject to recall

bias. A number have also reported an association between traumatic brain injury and AD (French *et al* 1985, Graves *et al* 1990, van Duijn *et al* 1992, Mayeux *et al* 1993, Rasmussen *et al* 1995, Salib *et al* 1997, O'Meara *et al* 1997, Nemetz *et al* 1999, Guo *et al* 2000) although some do not reach statistical significance (Chandra *et al* 1987, Amaducci *et al* 1986). In particular, the study by Mayeux *et al* in 1993 reported an almost 4x increased risk of developing AD after traumatic brain injury when compared to age matched controls. Guo *et al* (2000) studied 2233 individuals who met the criteria for probable or definite AD, and compared them with 14668 controls (first-degree relatives or spouses) as part of the MIRAGE (Multi-Institutional Research in Alzheimer Genetic Epidemiology) project. They reported that traumatic brain injury was a risk factor for AD and that the risk was proportional to the severity of the injury. For example, comparison of probands with unaffected spouses yielded odds ratios for AD of 9.9 for head injury with loss of consciousness and 3.1 for head injury without loss of consciousness. Comparison of probands with their parents and sibs were 4.0 for head injury with loss of consciousness and 2.0 for head injury without loss of consciousness. At age 93 years the lifetime risk of developing AD was 77.2% for those with and 40.1% for those without a history of head injury. Other retrospective case-control studies, however, have not confirmed that there is an association between traumatic brain injury and AD (Broe *et al* 1990, Ferini-Strambi *et al* 1990, Li *et al* 1992, Mendez *et al* 1992, Fatiglioni *et al* 1993).

To try and address the problems of recall bias inherent in case-control studies a number of prospective studies have been designed. Again, however, there is conflicting data. Corkin *et al* (1989) performed neuropsychological assessment of 57 World War 2 veterans with a penetrating head injury at two time points 30 years apart, and compared their performance with 27 veterans who experienced a peripheral

nerve injury only and who were assessed over the same 30 year period. They found that a penetrating head injury exacerbated the decline in cognitive performance over time when compared with the peripheral injury group. Schofield *et al* (1997) reported a community based longitudinal study of ageing in north Manhattan. 271 participants without significant cognitive impairment at the time of enrolment were interviewed in relation to previous head injury and associated loss of consciousness. Patients were then followed-up for 5 years with annual evaluations. They reported that previous traumatic brain injury was a 3x risk factor for AD. Plassman *et al* (2000) examined 1776 World War 2 navy and marine veterans, with military medical records. 548 had a history of non-penetrating traumatic brain injury, 1228 did not. All individuals were assessed for AD. They found in this group that moderate head injury (Frankowski scale, Frankowski *et al* 1985) resulted in 2.3 x increased risk of AD while severe head injury resulted in a 4 x increased risk. Against this data, however, there are a number of prospective studies which have failed to demonstrate an association between traumatic brain injury and AD (Katzman *et al* 1989, Aronson *et al* 1990, Williams *et al* 1991, Breteler *et al* 1995). Launer *et al* (1999), as part of the European Studies of Dementia (EURODEM), analysed four European population-based prospective studies, with individuals aged 65 years or older at time of recruitment. This large study did not find an association between traumatic brain injury and AD. Mehta *et al* (1999) reported the prospective population-based Rotterdam study, which looked at 6645 individuals aged 55 years or older and who did not have dementia when recruited. This study found that mild traumatic brain injury was not associated with an increased risk of AD, although the follow-up period was short being on average 2.1 years after initial assessment.

Meta-analysis has been used to review case-control studies. Mortimer *et al* (1991) studied 7 case-control studies and reported a relative risk of developing AD of 1.82 for head injury with loss of consciousness. The relative risk, however, only reached significance for males. Fleminger *et al* (2003) studied 15 case-control studies and showed an odds-ratio (OR) of 1.58. Again, however, this study showed that the association between head injury and AD was only significant for males (males OR 2.26, females OR 0.92).

There are many difficulties in assessing the relationship between traumatic brain injury and AD as the conflicting results presented above clearly illustrate. Retrospective case-control studies have both recall and selection bias. The prospective studies have a lesser degree of recall bias and do not rely on the recollections of cognitively impaired individuals. However, some of the prospective studies have only a short follow up period, 5 years in many cases, and this may bias the outcome. Comparisons between studies are difficult due to differences in definitions of severity of brain injury, post injury outcome status, and clinical definitions of AD. Also the age at the time of the injury and the age at the time of assessment are likely to be important variables.

1.2.2.2 Boxers and *dementia pugilistica*

While the data relating to long term associations between traumatic brain injury and AD is currently conflicting, the association between mild repetitive head injury and cognitive impairment has been established in the literature for many years. The "punch drunk" state was first described by Martland in 1928 and was renamed *dementia pugilistica* by Millspaugh in 1937. *Dementia pugilistica* has been extensively reviewed (Bruton 1997) and a summarised account will be presented here. This condition is described in boxers who have competed in many bouts over a long

period of time. Clinically, they develop a degree of intellectual deterioration often with an associated movement disorder, usually parkinsonism but in some cases predominantly ataxia. The largest study of this disorder clinically (Roberts 1969) examined 224 ex-boxers using neurological examination, electroencephalogram, and simple psychometric testing. He found that 17% had varying degrees of movement disorder involving the cerebellar, pyramidal and extrapyramidal systems. Minor degrees of intellectual dysfunction were seen in several of the ex-boxers, although only two required long term care as a result of their cognitive impairment. Roberts (1969) concluded that the occurrence of encephalopathy increased significantly with the number of bouts and the length of the boxer's career. He also concluded, however, that the rate of cognitive decline was not greater than that associated with ageing alone. More recent studies (Casson *et al* 1984, McLatchie *et al* 1987, Brooks *et al* 1987, Murelius and Haglund 1991, Heilbronner *et al* 1991) suggest that full blown *dementia pugilistica* is now rarely seen, although mild cognitive and movement disorders are still associated with boxing.

While *dementia pugilistica* was initially described in relation to boxing, cases have been described in National Hunt jockeys (Foster *et al* 1976). In addition, there is a considerable literature relating to the risks of repetitive mild traumatic head injury and other sports such as soccer (Matser *et al* 1999, Kirkendall *et al* 2001), rugby union and Australian rules football (McIntosh *et al* 2000), American football (Maroon *et al* 2000), and ice hockey (Biasca *et al* 1993). In the absence of large prospective studies the risk of cognitive impairment and movement disorders secondary to repetitive mild traumatic brain injury in relation to these contact sports, remains uncertain.

The largest pathological assessment of *dementia pugilistica* was the examination of the brains of 15 boxers, 11 of whom were diagnosed with *dementia pugilistica* in life (Corsellis *et al* 1973). This followed on from previous case reports (Brandenburg and Hallervorden 1954, Grahmann and Ule 1957, Constantinidis and Tissot 1967) and the descriptions by Mawdsley and Ferguson (1963, 1965) of the brains of four ex-boxers. Corsellis *et al* (1973) reported four principal features of the brain in *dementia pugilistica* ;

(1) abnormalities of the septum pellucidum, (2) cerebellar damage, (3) degeneration of the substantia nigra, and (4) cerebral cortex pathology.

(1) A fenestrated cavum septum pellucidum was seen in 77% of ex-boxers but in only 3% of non-boxers. One third of the non-boxers who had a fenestrated cavum septum pellucidum had evidence of a previous head injury. The degree of separation of the two leaflets of the septum pellucidum may be related to repetitive injury being most pronounced in the ex-boxers.

(2) Ataxia may be a feature of *dementia pugilistica*. Corsellis *et al* (1973) described cortical scarring of the inferior aspects of the lateral cerebellar hemispheres adjacent to the tonsils in 10 of the 15 ex-boxers brains studied. Histologically there was gliosis and loss of both Purkinje cells and granule cells.

(3) Parkinsonism is a common feature of *dementia pugilistica* and pathology of the substantia nigra appears to be the underlying cause. Pigmented cell loss is often marked, both within the substantia nigra and the locus coeruleus, and neurofibrillary tangles can be seen in some of the remaining neurons. Lewy bodies are not a feature (Corsellis *et al* 1973).

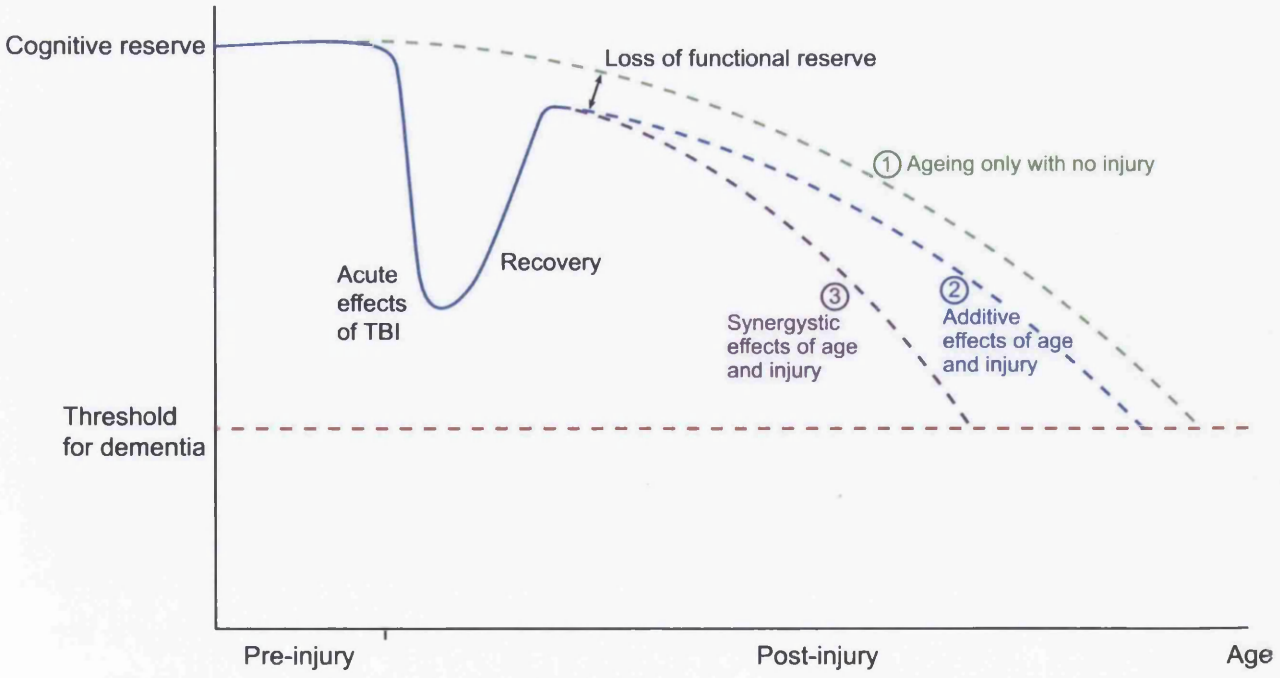
(4) Gross cortical pathology, a common feature of acute traumatic brain injury in the form of contusions, does not appear to be a significant feature of *dementia*

pugilistica (Corsellis *et al* 1973, Adams and Bruton 1989). However, diffuse microscopic cortical pathology is a feature of *dementia pugilistica* (see below).

1.3 Pathological mechanisms which may underlie long term neurodegeneration after head injury

Over-representation of late cognitive decline in survivors of traumatic brain injury may simply reflect the additive effects of the acute damage and later age-related functional compromise. From this viewpoint the acute injury acts to decrease the "functional reserve" of the brain and subsequent age-related neurodegeneration is more likely at an earlier age to result in a breach of the threshold of impairment required to manifest as dementia. However, there are remarkable parallels in the pathological processes involved both in the response to traumatic brain injury and AD (see below). It is possible that a component of the acute response to traumatic brain injury acts as a "trigger" to initiate a positive feedback loop that smoulders away to become manifest in later years as frank neurodegenerative pathology and dementia (Nicoll *et al* 1995, Griffin *et al* 1998) (figure 1).

Figure 1; A graphical representation of a postulated cognitive reserve and how head injury may increase the risk of cognitive decline. The broken green line [1] represents the "normal" situation. There is loss of cognitive function with ageing until a threshold point is crossed (broken red line) resulting clinically in dementia. After an episode of traumatic brain injury there is a significant decline in cognitive function which recovers, the degree of recovery being dependent on the severity of the head injury. Recovery is, however, not complete resulting in a loss of functional reserve. After this point cognitive decline may be as for normal ageing (broken blue line [2]) with the dementia threshold being crossed earlier due to loss of functional reserve, or there may a continued synergistic effect of mechanisms initiated by the head injury which accelerates cognitive decline (broken purple line [3]).



Detailed classical neuropathological descriptions of cohorts of long-term survivors of traumatic brain injury including immunohistochemical studies are lacking. However, some of the processes that are believed to be involved in chronic neurodegeneration, including AD, have been explored in the context of both acute injury and long term survival after trauma.

1.3.1 Cytoskeletal neurodegenerative pathology

Tau is a microtubule –associated protein (MAP) which is found predominantly in axons of the nervous system. Microtubules have a range of important functions required for normal neuronal function, including maintenance of cell structure and transport of vesicles along axons. MAPs are required for the normal functioning of microtubules. Six isoforms of the protein exist in humans, the isoforms being produced by alternative splicing of the gene. In neurodegenerative conditions in which tau is implicated (tauopathies) the protein is abnormally phosphorylated (hyperphosphorylation) resulting in accumulation of the abnormal protein within the neuronal cell body (neurofibrillary tangles) and processes (neuropil threads).

Cytoskeletal pathology after diffuse traumatic brain injury has been examined experimentally using a pig model with injury induced *via* controlled head rotational acceleration (Smith *et al* 1999). Head-injured pigs were examined at days 1, 3, 7 and 10 post-injury, and compared to control animals without head injury. Within the experimental group tau and neurofilament accumulations were identified immunohistochemically within the white matter, co-localised with damaged axons (Aβ-PP immunoreactive), and within neuronal perikarya in the cerebral cortex. Smith *et al* (2003) demonstrated neurofilament and Aβ accumulations within the white matter of patients dying after a single episode of TBI. To date neuronal perikaryal inclusions have not been demonstrated in humans after a single episode of traumatic

brain injury although cleaved forms of tau protein are markedly elevated in the CSF of brain-injured patients (Zemlan *et al* 1999). However, in cases of repetitive head injury cytoskeletal pathology is observed. Neurofibrillary tangles were reported in ex-boxers by Corsellis *et al* (1973) scattered throughout the cerebral cortex and the brainstem, being most prominent in the medial temporal cortex. Recently, Geddes *et al* (1999) examined the brains of four young individuals (age range from 23-28 years) with a history of repetitive head injury (two boxers, one footballer, and one mentally subnormal patient with a history of self inflicted head banging) and a frontal lobectomy specimen from an individual with intractable complex partial seizures with recurrent minor head injury. They identified widespread neocortical neurofibrillary tangles and neuropil threads not seen in age matched controls which in areas showed a perivascular distribution. Schmidt *et al* (2001) have compared the molecular profiles of the neurofibrillary tangles in *dementia pugilistica* and AD. They found that *dementia pugilistica* and AD had a common tau isoform and phosphorylation profile. They concluded that the mechanisms underlying both these conditions might be similar.

1.3.2 Amyloid deposition

Diffuse β-amyloid protein (Aβ) plaques have been identified in approximately 30% of individuals who die shortly after a single episode of severe traumatic brain injury (Roberts *et al* 1991, Roberts *et al* 1994, Graham *et al* 1995). This is a higher proportion than in non-head injury controls. Another group (Adle-Biassette et al 1996), however, has not confirmed this observation. Most of the deposits consist of Aβ42 (Gentleman *et al* 1997, Horsburgh *et al* 2000a), which is believed to be of pathological significance in AD. Smith *et al* (2003) have demonstrated Aβ accumulation within damaged axons in human brains after a single episode of fatal

TBI. They postulate that damaged axons can act as a reservoir of Aβ which may then be involved in plaque formation.

The distribution of the plaques does not correlate with focal traumatic lesions such as contusions but may be an expression of a diffuse acute phase response (e.g. hypoxia, acidosis, oedema, reduced cerebral blood flow) (Graham *et al* 1995b). For example, the Aβ deposits may be the result of increased production or altered distribution of Aβ-PP, increased cleavage of Aβ-PP in a proteolytic environment to produce Aβ, an alteration in the balance of production of $A\beta_{40}$:$A\beta_{42}$, extracellular conditions which favour the precipitation of amyloid fibrils, or decreased removal or drainage of Aβ. Tracer studies in experimental animals and observations on human brains suggest that Aβ is removed from the brain by passing along the peri-arterial interstitial fluid drainage pathways (Weller and Nicoll 2003).

In the study by Corsellis *et al* (1973) neurofibrillary tangles were found in the almost complete absence of senile plaques when examined using silver (Bielschowsky) and Congo red stains. However, when this was re-examined using immunohistochemistry with formic acid pre-treatment for Aβ, extensive immunoreactive plaque-like structures were seen in most cases of *dementia pugilistica* (Roberts *et al* 1990) although the neuritic plaques characteristic of AD were absent. In the study by Geddes *et al* (1999) of repetitive mild head injury Aβ plaques were not seen despite using both a modified Bielschowsky silver stain and Aβ immunohistochemistry with formic acid pre-treatment. They concluded that neurofibrillary tangle formation in the absence of Aβ deposition is an early consequence of repetitive head injury and that, because of their striking perivascular distribution, neurofibrillary tangle formation may be related to damage to blood vessels.

The relationship between Aβ deposition and genetic polymorphisms is discussed in a later section.

1.3.3 Neuronal Loss

Neuronal loss after traumatic brain injury has been reported in the neocortex, the hippocampus, the cerebellum and the thalamus (Adams *et al* 1985, Kotapka *et al* 1992, Ross *et al* 1993). In the acute phase, neuronal loss is related to contusions or as a consequence of cerebral ischaemia, and bilateral hippocampal neuronal loss has been documented in 85% of cases in one study (Adams *et al* 1985). The mechanisms of cell death have been extensively studied and the processes of necrosis and programmed cell death have been considered to be separate mechanisms, although this view is being increasingly challenged and shared molecular pathways have been identified in both processes. The role of programmed cell death after traumatic brain injury has been reviewed by Raghupathi *et al* (2000) and Royo *et al* (2003). Cell death has been identified *in situ* after traumatic brain injury in both animal models and in human material using the terminal deoxynucleotidyl transferase mediated dUTP nick end-labelling (TUNEL) technique (Rink *et al* 1995, Smith *et al* 1997). This technique identifies DNA fragmentation, a feature common to both necrosis and programmed cell death. Differentiation between necrosis and programmed cell death is possible by assessing other mechanisms seen in programmed cell death such as caspase activation, and identification of the morphological expression of programmed cell death, apoptosis. TUNEL positive neurons and oligodendroglial cells have been reported in human traumatic brain injury; Clark *et al* (1999) demonstrated elevated levels of bcl-2 and caspase 3, increased cleavage of both caspases 1 and 3, and cells with the morphological appearances of apoptosis in 8 patients who had contusions removed surgically between 1-9 days after an episode of traumatic brain injury. Smith

et al (2000) and Shaw *et al* (2001) studied a number of brain areas in human post-mortem tissue of 18 patients who survived between 6 hours and 10 days after traumatic brain injury. TUNEL-positive cells were seen in both grey and white matter, peaking between 25 and 48 hours although still identifiable at 10 days post injury. There was a mixture of both apoptotic and necrotic morphology in neurons, although white matter TUNEL-positive cells more consistently showed an apoptotic morphology. They concluded that in human frontal lobe contusions both apoptosis and necrosis contributed to post-traumatic pathology, and that multiple cell types, including neurons, were involved.

Recent experimental studies suggest that the cellular pathology initiated by an episode of acute traumatic brain injury may indeed be progressive. Rats subjected to severe lateral fluid-percussion brain injury were studied for up to 12 months and showed long term cognitive and neurological motor dysfunction (Pierce *et al* 1998). Bramlett *et al* (1997) and Smith *et al* (1997) demonstrated cell loss from neocortex, thalamus and hippocampus with associated gliosis and ventriculomegaly in rats after fluid-percussion induced injury. Bramlett *et al* (1997) demonstrated tissue loss up to 8 weeks while Smith *et al* (1997) demonstrted continuing tissue loss up to 12 months. Recent studies in human cases have demonstrated TUNEL-positive cells up to 12 months after traumatic brain injury (Williams *et al* 2001). The majority of the cells were present in the white matter and were considered to be closely associated with Wallerian degeneration. Long-term DNA fragmentation therefore appears to be a feature of traumatic brain injury in man.

1.3.4 Cholinergic brain pathways

The nucleus basalis of Meynert within the basal forebrain provides cholinergic innervation of the cerebral cortex and the hippocampus, and damage to this pathway

can result in attention, memory and emotional dysfunction (Everitt and Robbins 1997). Abnormalities within the cholinergic projection system have been postulated to contribute both to the altered mental state in AD (Geula and Mesulam 1994) and to the neurobehavioural sequelae which persist after a head injury (Cardenas *et al* 1994).

In rats there is a reduction in the number of choline acetyltransferase [ChAT] positive neurons after experimentally-induced traumatic brain injury (Schmidt and Grady 1995), and alterations of cholinergic innervation of the cerebral cortex and hippocampus have been detected (Dixon *et al* 1995, Dixon *et al* 1997).

Patients who die acutely as a consequence of traumatic brain injury have reduced levels of cortical ChAT when compared to age-matched controls (Dewar and Graham 1996, Murdoch *et al* 1998). Recently, neuronal damage has been demonstrated within the nucleus basalis of Meynert in eight of twelve fatally head injured patients, with a median survival time of 27 hours (Murdoch *et al* 2002). Neuronal damage was a result of both mechanical distortion (tissue herniation) and focal ischaemia. The authors concluded that damage to cholinergic neurons may contribute to the dysfunction of memory and cognition in survivors of traumatic brain injury, although studies of the nucleus basalis of Meynert in long-term survivors has not been undertaken.

1.3.5 Neuroinflammation

Recent studies have focussed attention on "neuroinflammation" as a potential underlying mechanism both in AD and in the response to brain injury (Engel *et al* 2000, Nicoll *et al* 2000, Griffin *et al* 1998). The principal mediator of inflammatory processes in the CNS is the microglial cell. Microglia have a variety of functions including antigen presentation, synthesis and secretion of cytokines and phagocytosis. These cells are a source of several of the proteins upregulated both in AD and after

traumatic brain injury, including apolipoprotein E, and pro-inflammatory cytokines such as interleukin 1 (IL-1). This raises the question that patients who sustain a head injury may have a microglial response which plays a role *both* in influencing their outcome following injury *and* their increased susceptibility to AD later in life.

1.3.5.1 Morphological changes associated with microglial activation

Microglia form an extensive network throughout the brain and spinal cord "sensing" the microenvironment, with each cell providing surveillance of a non-overlapping field. When stimulated they pass through several stages which can be distinguished morphologically. In the adult CNS microglia are in a functionally quiescent state, and morphologically are ramified. When activated the microglia hypertrophy and express several cell surface markers, including the MHC class II antigen (Streit *et al* 1989). The monoclonal antibody CR3/43 is directed against the β-chain of the MHC class II antigen and can be used to detect activated microglia (Graeber *et al* 1994). After this phase the microglia may become phagocytic, transforming into intrinsic brain macrophages (Streit and Kreutzberg 1988). The monoclonal antibody PG-M1 is directed against CD 68, a transmembrane glycoprotein involved in lysosomal transport, although the precise function of this protein remains uncertain (Holness and Simmons 1993). CD 68 immunoreactivity can be used to detect phagocytic microglia and morphologically they have a rounded appearance with loss of the extensive ramifying cellular processes. Microglia can migrate within the CNS to areas of injury, and a number of chemokines have been described which can induce microglial motility (Rezaie and Male 2002), including Aβ (Davis *et al* 1992).

1.3.5.2 The acute response to injury

After brain injury cytokines are released and microglia are activated, the degree of activation reflecting the severity of the injury. In animal models of ischaemia microglial activation is seen within 20 minutes of reperfusion (Morioka *et al* 1991), and this is associated with the production of both Aβ-PP (Banati *et al* 1993) and apolipoprotein E (Uchihara *et al* 1995). Microglial activation in itself will lead to cytokine release, including IL-1, possibly secondary to elevated levels of ATP released from damaged cells (Di Virgilio 1995), with activation of purinergic P2X7 receptors on microglia (Ferrari *et al* 1997). IL-1 is actually a family of three related proteins two of which act as agonists (IL-1α and IL-1β) and one as an antagonist (IL-1ra) at the IL-1 receptors. IL-1α is active in both the precursor (pro-IL-1α) and mature forms while pro-IL-1β is inactive. Activation requires the intracellular enzyme IL-1β converting enzyme (ICE) (Mrak and Griffin 2001). Functional differences between IL-1α and IL-1β in the CNS are poorly defined but IL-1β is the first to be released (Davies *et al* 1999) and induces expression of other cytokines including IL-6, TNFα and TGFβ (Allan and Rothwell 2001). IL-1 is thought to orchestrate the inflammatory responses within the brain after an injury, resulting in a number of responses including; (a) microglial proliferation (Ganter *et al* 1992), (b) induction of neuronal production of Aβ-PP (Goldgaber *et al* 1989), and (c) astrocytic activation with upregulation of astrocytic-derived proteins (Das and Potter 1995). The IL-1 receptor IL-1RI binds both IL-1α and IL-1β (Greenfeder *et al* 1995) although IL-1 may also bind to other less specific receptors (Rothwell *et al* 2000). By binding to receptors IL-1 has been shown to enhance neurotoxicity *in vivo* by inducing cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS) (Serou *et al* 1999). A recent study of mixed cultures of activated glia and neurons suggested that

inflammatory neurodegeneration may be mediated by glial nitric oxide (NO) (Bal-Price and Brown 2001). They proposed that NO produced by activated microglia or astrocytes inhibits the mitochondrial function of surrounding neurons, causing glutamate release from neurons (and possibly from astrocytes). Activation of NMDA receptors by glutamate triggers massive influx of Ca^{2+} into neurons, leading to cell death. Other potential mechanisms of NO mediated neuronal death include mitochondrial damage (Heales *et al* 1999) and poly(ADP-ribose) synthetase activation (Zhang *et al* 1994).

Further cellular injury may be produced by glial activation and release of neurotoxins such as Aβ-PP (Allan and Rothwell 2001). Hypothalamic corticotrophin releasing factor (CRF) is also released and may be neurotoxic although the mechanisms remain uncertain (Roe *et al* 1998).

1.3.5.3 Chronic microglial activation

IL-1 is expressed in increased quantities in the cerebral cortex within hours of traumatic brain injury (Griffin *et al* 1994), and chronic overexpression of IL-1 is found in AD (Griffin *et al* 1989). Griffin *et al* (1998) have proposed a "Cytokine Cycle" in which traumatic brain injury, or other forms of brain injury, can, in susceptible individuals, initiate an overexuberant sustained inflammatory response which can result in neurodegeneration. Aβ-PP and the astrocyte-produced molecule S-100β are upregulated in response to increased IL-1 levels, and the situation is similar in AD (Griffin *et al* 1989, Mrak *et al* 1996). Aβ-PP is not only upregulated in acute traumatic brain injury, but there is increased intraneuronal processing of the molecule (Buxbaum *et al* 1992) potentially resulting in Aβ production and deposition. The relation between IL-1 and Aβ-PP is uncertain, but increased levels of IL-1 may result in sustained Aβ-PP, and therefore Aβ, production. Positive feedback of this

interaction may be provided by soluble fragments of Aβ-PP (sAPP) which are produced by the processing of Aβ-PP. sAPP promotes microglial activation by a mechanism that is modulated by apolipoprotein E in an isoform specific fashion (Barger and Harmon 1997).

IL-1 positive microglial cells lie in close relation to Aβ-PP positive neurons and dystrophic neurites in the brains of head-injured patients (Griffin *et al* 1994) and are also found in close apposition to neurofibrillary tangle-containing neurons in AD (Sheng *et al* 1997). IL-1 is known to be trophic to neurons in low concentrations, but at higher concentrations IL-1 has a neurotoxic effect, inducing overexpression and phosphorylation of both neurofilaments and tau (Sheng *et al* 2000). The IL-1 mediated pathological effects of microglia on tau phosphorylation in neurons are through a p38-MAPK signalling pathway (Li *et al* 2003).

1.4 Evidence for genetic influences on outcome after head injury

1.4.1 *APOE* polymorphisms

The response to brain injury and AD have in common not only a cellular and protein response but there are striking parallels in the genetic influences. Apolipoprotein E (apoE) is a protein, initially described in 1973 (Shore and Shore, 1973), which in the circulation plays an important role in the transport of lipids. There is a polymorphism of the apolipoprotein E gene (*APOE*, gene; apoE, protein) of which there are 3 common alleles (ε2, ε3 and ε4) resulting in three common protein isoforms (E2, E3 and E4). In the human CNS apoE has been demonstrated in both neurons and glia (Horsburgh *et al* 2000b), with marked increases in intraneuronal apoE after brain injury (Horsburgh *et al* 1999a). The function of apoE after brain injury is uncertain but apoE may be retained within the parenchyma of the CNS and may have a

protective role which is isoform dependent. Decreases in cerebrospinal fluid apoE concentration have been detected after TBI in humans (Kay *et al* 2003).

Possession of *APOE* ε4 allele is the major genetic susceptibility factor for sporadic AD (Saunders *et al* 1993). In addition, the *APOE* polymorphism influences neuropathological findings in patients who die from head injuries (Nicoll *et al* 1995). This study examined the brains of 90 cases who died within 2 weeks of a head injury. Aβ deposits were found in 23 patients and the frequency of the *APOE ε4* allele within this group was significantly greater than that seen in either control populations without neurological disease or in AD. In addition all cases homozygous for the *APOE ε4* allele had Aβ deposition. Furthermore, the density of these plaques was related to *APOE* genotype, with greater numbers of plaques being associated with the *APOE ε4* allele in an allele dose dependant manner (i.e. homozygotes having greater numbers of plaques than heterozygotes) (Horsburgh *et al* 2000a). The initial interpretation of these findings by Nicoll *et al* (1995) was that in genetically susceptible individuals (i.e. those with an *APOE ε4* allele) traumatic brain injury appears to act as a trigger for Aβ deposition. However, there are alternative explanations for these observations (Roses and Saunders 1995); Aβ deposits may pre-date the injury, and patients with ε4, who are more likely to have age-related deposits, may have a higher mortality from traumatic brain injury and therefore be selected for an autopsy-based study. Until it is possible to image Aβ plaques during life it may not be possible to resolve this uncertainty. Subsequent clinical studies have indeed shown that head-injured patients (and patients with spontaneous intracerebral haemorrhage) who possess *APOE* ε4 have a poorer outcome than non-carriers of *APOE* ε4 (Alberts *et al* 1995, Teasdale *et al* 1997, McCarron *et al* 1998). Jordan *et al* (1997) studied 30 professional boxers and assessed their cognitive status in relation to *APOE* genotype

and number of professional bouts. They concluded that possession of an *APOE ε4* allele is associated with increased severity of chronic neurological deficits in high-exposure boxers. A recent neuropathological study compared Aβ deposits in long term survivors of traumatic brain injury (survival time up to 20 years) with age-matched and *APOE* genotype matched controls (MacFarlane *et al* 1999). They found Aβ deposits were more common in ε4 patients in both the long-term survivors and the control groups, but were not more common among long term survivors than controls. Activated microglial cells are involved in the deposition of Aβ, as discussed above, and when deposited Aβ is bound by apoE in an isoform specific manner (Strittmatter *et al* 1993). In addition, possession of the *APOE* ε4 allele is associated with greater Aβ deposition (Schmechel *et al* 1993) and an increase in the number of activated microglial cells (Egensperger *et al* 1998) in the brains of patients with AD.

However, Millar *et al* (2003) studied the long-term neuropsychological outcome after head injury and found no clear differences in individuals who possess the ε4 allele. This study did provide additional data for a late decline after head injury. Comparison using the Glasgow outcome scores demonstrated a neuropsychological decline after a 6 month assessment and found that improvement in condition after this assessment was uncommon. Therefore, this study suggested that although possession of the *e4* allele was not related to neuropsychological outcome, there was a late (after 6 months) decline which may be related to other genetic polymorphisms, such as those of IL-1. The cohort was considered too young (mean age 42.1 years) to assess the risk of AD.

In AD there is evidence that patients with *APOE* ε4 have increased microglial activity compared to patients without *APOE* ε4 (Egensperger *et al* 1998). Further investigations relating *APOE* genotypes and the neuroinflammatory response have

shown that response targeted-replacement mice expressing human *APOE* ε4 genes express greater levels of systemic and brain pro-inflammatory cytokines (TNFα and IL-6) after administration of lipopolysaccharide (LPS) when compared to animals expressing *APOE* ε3 genes (Lynch *et al* 2003). These results suggest that apoE has an isoform specific effect on modulation of the neuroinflammatory response after injury. Multiple sclerosis is a demyelinating condition in which neuroinflammation is a key feature. *APOE* polymorphisms have been demonstrated to modify disease severity with possession of *APOE* ε4 being associated with severe disease (Fazekas *et al* 2001, Schmidt *et al* 2002) and *APOE* ε2 being associated with less severe disease (Schmidt *et al* 2002), particularly in women (Kantarci *et al* 2004). While these findings suggest that apoE isoforms do modify neuroinflammation in humans the findings are disputed by other investigators (Savettieri *et al* 2003).

1.4.2 *IL-1A* and *IL-1B* polymorphisms

A further genetic polymorphism has recently been suggested to confer susceptibility to AD and this also implicates neuroinflammatory processes. Interleukin 1 (IL-1) exists in two distinct forms (IL-1α and IL-1β with *IL-1A* and *IL-1B* genes respectively) and polymorphisms have been identified in each of these genes (both have an allele 1 and an allele 2). An association has been demonstrated between the *IL-1A* 2,2 genotype and AD (Nicoll *et al* 2000, Grimaldi *et al* 2000). Nicoll *et al* studied 232 pathologically confirmed cases of Alzheimer's disease and found the *IL-1A* 2,2 genotype in almost 13% of cases as compared to 6.6% of age-matched and *APOE*-matched controls. In addition they found that homozygosity for allele 2 of both *IL-1A* and *IL-1B* conferred an even greater risk, although homozygosity for allele 2 of *IL-1B* alone was not significant. A gene dose dependent association of *IL-1A* allele 2 polymorphism and AD has been demonstrated (Combarros *et al* 2002) with the risk of

developing AD for a heterozygote having an odds ratio of 1.4 increasing to 3.1 for homozygotes. Some studies, however, have not demonstrated an association between *IL-1A* allele 2 and AD (Bertram *et al* 2000, Green *et al* 2002).

1.4.3 Genetic influence overview

Although there is currently a lack of definitive information about *APOE* genotype, *IL-1* genotype and microglial activation in traumatic brain injury, the observations outlined above raise the possibility that microglial activation ("neuroinflammation") may be under genetic influence. Specifically, they raise the question that individuals with the relevant alleles (*APOE* ε4 or *IL-1*A allele 2) who sustain a head injury may have a relatively overexuberant microglial response which is associated *both* with a poorer outcome from injury *and* greater susceptibility to later AD. If this were the case it is possible to speculate that simple anti-inflammatory medication may have a role in the long-term management of the head-injured patient in much the same way that non-steroidal anti-inflammatory drugs (NSAIDs) may be useful in protecting against AD (McGeer *et al* 1990, McGeer *et al* 1996).

2 Hypotheses

In light of this background information the present study was designed to test potential cellular and genetic mechanisms which may underlie the association between head injury and AD. This study tested the hypotheses;

Neurofibrillary pathology

- That *tau* accumulation occurs in humans in the acute phase after TBI.

Neuroinflammation

- That there is a sustained neuroinflammatory response after TBI which may, in the long-term, have significant neurodegenerative effects.

Genetic

- That head-injured patients with *APOE* ε4 are selectively predisposed to one or more of the different pathological features that constitute the response to TBI, and that this underlies the association of *APOE* ε4 with poor clinical outcome.
- That possession of *IL-1A* allele 2 (heterozygosity or homozygosity) results in an increased neuroinflammatory response.
- The neuroinflammatory response may further be modified by possession of *IL-1B* allele 2 or *APOE* ε4.

3 Methods

3.1 Case selection

3.1.1 Traumatic brain injury archive

The departments of Neurosurgery and Neuropathology in Glasgow have a long standing research interest in TBI. One of the key resources in Glasgow, and which has been key to much of the published research in this field, is the extensive and probably unique archive of tissue retained in the Neuropathology department, accumulated from cases of fatal traumatic brain injury. Tissue was retained for diagnostic purposes and subsequently used for research. Detailed examination and retention of tissue from the brains of patients dying after an episode of trauma dates back to 1968. From 1968 until 1999 approximately 1400 cases were examined. Many of these cases were neurosurgical in-patients and as such have detailed clinical information. Autopsy information is generally detailed; neuropathologists undertook many of the autopsies although, more recently, most of the autopsies were undertaken by staff of the University Department of Forensic Medicine and Science who then made the brains available for neuropathological examination. In all these cases there was a macroscopic examination of the brain; in the great majority they were then sampled in a standardised way for microscopic examination. The tissue was processed to paraffin blocks, as detailed below, and the paraffin blocks were retained in the archive of the Neuropathology department as part of the patient record. Every case has a unique identifying code number. Following the diagnostic process the range of pathologies seen in each case were entered into a database. Using this database, cases could be selected on the range of pathologies present. The database is cross-referenced with the unique identifying code for each case and the archived paraffin blocks could be retrieved for research purposes.

3.1.2 Previous cohorts published using cases from the archive

Previous studies have been published analysing the distribution of brain injury in fatal non-missile TBI in the West of Scotland using the archive. Adams *et al* (1980) provided detailed neuropathological data on 151 cases of fatal TBI between 1968-1972, and this cohort was again assessed along with 112 cases accrued between 1981-1982 (Graham *et al* 1989). A larger cohort of 635 cases, accrued between 1968-1982, was used to assess the prevelance of raised intracranial pressure (Adams and Graham, 1976), ischaemic brain injury (graham *et al* 1989), contusions (Adams *et al* 1985), and diffuse TAI (Adams *et al* 1989). As a result of these detailed neuropathological studies there has been greater clinico-neuropathological correlation and an understanding of the structural basis of clinical outcomes, such as the vegetative state (Adams *et al* 2000), severe disability (Jennett *et al* 2001), and moderate disability (Adams *et al* 2001).

3.1.3 Identification of cases

The current study utilised cases from the paraffin-embedded tissue archive of the Glasgow Neuropathology department. Initially all cases that died from TBI during the 13-year period 1987-1999 within the archive were selected. These cases were identified from the database as all had been coded as "acute head injury". The unique identifying number allowed all paraffin blocks to be retrieved from the archive along with all neuropathological reports. While many of the cases had been managed by the Department of Neurosurgery, Institute of Neurological Sciences, some patients had died at District General Hospitals or at the scene of the injury. Requirements for inclusion into this cohort were;

Coded as acute head injury in the database, allowing cases to be studied "blind" to the clinical data.

Each case had detailed clinico-neuropathological information available.

Each case had a full autopsy examination.

Standardised set of paraffin blocks were available in the archive.

Cases had been reported by Professors DI Graham, JH Adams or JAR Nicoll.

A total of 259 cases were identified and they are detailed in appendix 2.

3.1.3.1 Identification of cases used in the study of cytoskeletal pathology

A total of 45 acute TBI cases with varying survival times ranging from <24 hours up to one month were included in this study. They were grouped on the basis of age and survival times (tables 1-3). In general, the acute TBI cases had all experienced a severe head injury (Glasgow Coma Scale [GCS] 8 or less) with only one case having a moderate injury (GCS 9-12). Four cases had a mild head injury (GCS 13-15) and died of pathology not directly related to the brain injury. In 12 cases GCS was not recorded as patients either died rapidly before hospital admission or were not formally assessed. In all of these cases the pathology suggested a head injury was a major feature of the autopsy findings. The mechanisms of injury varied with road traffic accidents (RTA) being more common in the <20 year old group, and falls more common in the >50 year old group. 15 cases with no significant neurological impairment or neuropathological abnormality were used as controls (table 4). These cases were all hospital autopsies which were fully consented. Tissue was retained for diagnostic purposes and subsequently used for research after examination.

The issue of consent for these cases is discussed below.

Table 1; Details of TBI cases aged < 20 years used in the cytoskeletal pathology study

	Age (years)	Documented survival	Severity of head injury (admission GCS)	Mechanism of head injury	Main pathology
Survival <24 hours	8 wks	4 hours	Severe (NA)	RTA	DVI
	1.5	22 hours	Severe (NA)	Fall	TAI
	3	21 hours	Severe (5)	RTA	TAI, swelling
	9	10 hours	Severe (3)	RTA	TAI, swelling
	14	7 hours	Severe (3)	RTA	DVI
Survival 24 hours- 1 week	3	48 hours	Severe (NA)	RTA	Brain swelling
	5	3 days	Severe (NA)	RTA	TAI, swelling
	7	48 hours	Severe (6)	RTA	Brain swelling
	12	7 days	Severe (5)	RTA	ICH, TAI
	14	48 hours	Severe (3)	RTA	TAI, swelling
Survival 1 week- 1 month	5	9 days	Severe (5)	RTA	Acute LSDH
	15	8 days	Severe (4)	RTA	TAI, swelling
	15	8 days	Severe (4)	RTA	TAI, swelling
	17	14 days	Severe (5)	RTA	TAI
	19	21 days	Severe (3)	RTA	TAI

Legend: NA= Not available
RTA= Road traffic accident
DVI= Diffuse vascular injury
TAI= diffuse traumatic axonal injury
ICH= intracerebral haemorrhage
R/LSDH= right/left subdural haemorrhage

Table 2; Details of TBI cases aged 20- 49 years used in the cytoskeletal pathology study

	Age (years)	Documented survival	Severity of head injury (admission GCS)	Mechanism of head injury	Main pathology
Survival <24 hours	23	8 hours	Severe (3)	RTA	TAI
	25	20 hours	Severe (4)	RTA	Global ischaemia due to raised ICP
	30	16 hours	Severe (3)	RTA	Acute LSDH
	34	23 hours	Severe (3)	Fall	Acute LSDH
	39	12 hours	Severe (3)	RTA	DVI
Survival 24 hours- 1 week	23	3 days	Mild (15)	Assault	Extracranial pathology
	26	24 hours	Severe (3)	Fall	Contusions, swelling
	26	4 days	Severe (6)	Fall	Contusions, swelling
	37	24 hours	Severe (3)	Fall	Acute RSDH
	40	5 days	Severe (4)	Assault	Acute LSDH
Survival 1 week- 1 month	21	9 days	Severe (NA)	RTA	TAI, acute LSDH
	23	28 days	Severe (3)	Assault	TAI
	27	11 days	Moderate (10)	Fall	TAI, R burst lobe
	30	18 days	Mild (15)	Fall	Extracranial pathology
	35	10 days	Mild (15)	Fall	Extracranial pathology

Legend: NA= Not available
RTA= Road traffic accident
DVI= Diffuse vascular injury
TAI= diffuse traumatic axonal injury
ICH= intracerebral haemorrhage
R/LSDH= right/left subdural haemorrhage
ICP= intracranial pressure

Table 3; Details of TBI cases aged > 50 years used in the cytoskeletal pathology study

	Age (years)	Documented survival	Severity of head injury (admission GCS)	Mechanism of head injury	Main pathology
Survival <24 hours	51	12 hours	Severe (3)	Fall	Acute RSDH
	59	20 hours	Severe (3)	Fall	Bilateral acute SDH
	66	21 hours	Severe (4)	RTA	Acute LSDH
	71	12 hours	Severe (NA)	Fall	Acute LSDH
	88	7 hours	Severe (NA)	Fall	Acute LSDH
Survival 24 hours- 1 week	53	2.5 days	Severe (NA)	Fall	Acute RSDH
	56	4 days	Severe (5)	Fall	Acute RSDH, TAI
	60	8 days	Severe (NA)	Fall	ICH
	68	3 days	Severe (4)	RTA	Acute LSDH, TAI
	73	7 days	Severe (NA)	RTA	Contusions, swelling
Survival 1 week- 1 month	53	11 days	Severe (3)	Fall	Acute LSDH
	59	17 days	Severe (6)	Fall	LSDH
	60	10 days	Mild (14)	Fall	ICH, TAI
	79	8 days	Severe (NA)	RTA	Acute LSDH
	83	18 days	Severe (NA)	RTA	ICH, TAI

Legend: NA= Not available
RTA= Road traffic accident
DVI= Diffuse vascular injury
TAI= diffuse traumatic axonal injury
ICH= intracerebral haemorrhage
R/LSDH= right/left subdural haemorrhage

Table 4; Details of control cases used in the cytoskeletal pathology study

	<20 years		**20-50 years**		**>50 years**	
	Age	**Cause of death**	**Age**	**Cause of death**	**Age**	**Cause of death**
Non-head injured controls with no neurological disease	4	Congenital heart disease	20	Drug overdose	50	Metastatic carcinoma
	8	Viral infection	21	Septic shock	59	Disseminated Langerhan's histiocytosis
	10	Burns	28	Drug overdose	68	Ruptured aortic aneurysm
	18	Leukaemia	33	Ischaemic heart disease	69	Congestive cardiac failure
	18	Systemic Hodgkin's disease	38	Congestive cardiac failure	71	Pneumonia

3.1.3.2 Identification of cases used in the study of neuroinflamation

A subset of cases were selected from the main cohort to form a smaller group used to study the neuroinflammatory response after TBI. A total of 55 TBI cases with varying survival times ranging from <24 hours up to 4 years were included in this study (tables 5-7). In general, these cases had all experienced a severe head injury (Glasgow Coma Scale [GCS] 8 or less) with only three cases having had a moderate injury (GCS 9- 12). Three cases had had a mild head injury (GCS 13-15) and died of pathology not directly related to the brain injury. In 11 cases GCS was not recorded as patients either died rapidly before hospital admission or were not formally assessed. In all of these cases the pathology suggested a head injury was a major feature of the autopsy findings. In four of the longer survivors there was no data relating to the GCS at time of admission. The mechanisms of injury varied with road traffic accidents (RTA) being more common in the <20 year old group, and falls more common in the >50 year old group.

20 cases with no significant neurological impairment or neuropathological abnormality were used as controls (table 8). These cases were all hospital autopsies which were fully consented. Tissue was retained for diagnostic purposes and subsequently used for research.

The issues relating to consent for these cases is discussed below.

Table 5; Details of TBI cases aged < 20 years used in neuroinflammatory study

	Age (years)	Documented survival	Severity of head injury (admission GCS)	Mechanism of head injury	Main pathology
Survival <24 hours	1.5	22 hours	Severe (NA)	Fall	TAI
	3	21 hours	Severe (5)	RTA	TAI, swelling
	9	10 hours	Severe (3)	RTA	TAI, swelling
	14	7 hours	Severe (3)	RTA	DVI
Survival 24 hours- 1 week	3	48 hours	Severe (NA)	RTA	Brain swelling
	5	3 days	Severe (NA)	RTA	TAI, swelling
	7	48 hours	Severe (6)	RTA	Brain swelling
	12	7 days	Severe (5)	RTA	ICH, TAI
Survival 1 week- 1 month	5	9 days	Severe (5)	RTA	Acute LSDH
	15	8 days	Severe (4)	RTA	TAI, swelling
	15	8 days	Severe (4)	RTA	TAI, swelling
	17	14 days	Severe (5)	RTA	TAI
	19	21 days	Severe (3)	RTA	TAI
Survival 1-3 months	18	2.5 months	Severe (4)	RTA	TAI
Survival >3 months	NA	Nil	Nil	Nil	NA

Legend: NA= Not available
RTA= Road traffic accident
DVI= Diffuse vascular injury
TAI= diffuse traumatic axonal injury
ICH= intracerebral haemorrhage
R/LSDH= right/left subdural haemorrhage

Table 6; Details of TBI cases aged 20- 49 years used in neuroinflammatory study

	Age (years)	Documented survival	Severity of head injury (admission GCS)	Mechanism of head injury	Main pathology
Survival <24 hours	23	8 hours	Severe (3)	RTA	TAI
	25	20 hours	Severe (4)	RTA	Global ischaemia secondary to
	30	16 hours	Severe (3)	RTA	Acute LSDH
	34	23 hours	Severe (3)	Fall	Acute LSDH
Survival 24 hours- 1 week	23	3 days	Mild (15)	Assault	Extracranial pathology
	26	24 hours	Severe (3)	Fall	Contusions, swelling
	26	4 days	Severe (6)	Fall	Contusions, swelling
	37	24 hours	Severe (3)	Fall	Acute RSDH
	40	5 days	Severe (4)	Assault	Acute LSDH
Survival 1 week- 1 month	21	9 days	Severe (NA)	RTA	TAI, acute LSDH
	23	28 days	Severe (3)	Assault	TAI
	27	11 days	Moderate (10)	Fall	TAI, R burst lobe
	35	10 days	Mild (15)	Fall	Extracranial pathology
Survival 1-3 months	45	30 days	Severe (5)	RTA	Global ischaemia secondary to
Survival >3 months	43	10 months	Severe (6)	Assault	TAI
	38	4 years	NA	Fall	Extracranial pathology
	28	9 months	Severe (5)	RTA	TAI

Legend: NA= Not available
RTA= Road traffic accident
DVI= Diffuse vascular injury
TAI= diffuse traumatic axonal injury
ICH= intracerebral haemorrhage
R/LSDH= right/left subdural haemorrhage
EDH= extradural haematoma
MI= myocardial infarct

Table 7; Details of TBI cases aged > 50 years used in neuroinflammatory study

	Age (years)	Documented survival	Severity of head injury (admission GCS)	Mechanism of head injury	Main pathology
Survival <24 hours	51	12 hours	Severe (3)	Fall	Acute RSDH
	59	20 hours	Severe (3)	Fall	Bilateral acute SDH
	66	21 hours	Severe (4)	RTA	Acute LSDH
	71	12 hours	Severe (NA)	Fall	Acute LSDH
	88	7 hours	Severe (NA)	Fall	Acute LSDH
Survival 24 hours- 1 week	53	2.5 days	Severe (NA)	Fall	Acute RSDH
	56	4 days	Severe (5)	Fall	Acute RSDH, TAI
	68	3 days	Severe (4)	RTA	Acute LSDH, TAI
	73	7 days	Severe (NA)	RTA	Contusions, swelling
Survival 1 week- 1 month	53	11 days	Severe (3)	Fall	Acute LSDH
	59	17 days	Severe (6)	Fall	LSDH
	60	10 days	Mild (14)	Fall	ICH, TAI
	79	8 days	Severe (NA)	RTA	Acute LSDH
Survival **1-3 months**	50	7 weeks	Severe (4)	Fall	pneumonia RSDH
	51	2 months	Severe (NA)	Fall	LSDH Global ischaemia
	76	6 weeks	Moderate (9)	Assault	RSDH
	76	5 weeks	NA	Fall	RICH TAI
Survival **>3 months**	56	4 months	NA	NA	AD
	65	5 months	Severe (6)	Assault	Pneumonia TAI
	67	12 months	Severe (6)	RTA	Pneumonia Cerebral swelling
	70	6 months	NA	Assault	PTE eEDH
	75	11 months	Severe (5)	Assault	Pneumonia TAI
	78	4 months	Moderate (10)	RTA	LSDH
	79	4 months	Severe (NA)	RTA	eLSDH

Legend: NA= Not available
RTA= Road traffic accident
DVI= Diffuse vascular injury
TAI= diffuse traumatic axonal injury
ICH= intracerebral haemorrhage
(e)R/LSDH= (evacuated) right/left subdural haemorrhage
RICH= right intracerebral haematoma
AD= Alzheimer's disease
eEDH= evacuated extradural haematoma
PTE= pulmonary thrombo-embolus

Table 8; Details of control cases used in the neuroinflammatory study

	<20 years		20-50 years		>50 years	
	Age	**Cause of death**	**Age**	**Cause of death**	**Age**	**Cause of death**
Non-head injured controls with no neurological disease	18	Leukaemia	20	Drug overdose	50	Metastatic carcinoma
	18	Systemic Hodgkin's disease	21	Septic shock	55	Gastric lymphoma PTE
			24	Drug overdose	59	Disseminated Langerhan's histiocytosis
			28	Drug overdose	60	Bronchial carcinoma
			33	Ischaemic heart disease	64	Sarcoidosis
			35	Malignant teratoma	69	Acute pyelonephritis
			43	Hodgkin's lymphoma	69	Congestive cardiac failure
			44	Myocardial infarct	70	Breast carcinoma
			47	T cell lymphoma	71	Pneumonia

Legend: PTE= pulmonary thrombo-embolus

3.1.4 Issues related to organ retention

The retention of organs at post-mortem examination and the use of retained tissues in medical research has been the focus of much public and political attention over the past five years. The practise of removing and retaining organs at autopsy examination had been widespread and long-standing in the United Kingdom. While this was considered to be part of the normal autopsy examination by many health care professionals it became clear that many relatives who consented to autopsy examination after a death were unaware of the possibility of organ retention for diagnostic purposes and, in some cases, use of the retained tissues in research. The issue came to light during an inquiry into paediatric heart surgery at Bristol Royal Infirmary (Kennedy 2001). At this inquiry it was disclosed that organs (malformed hearts) had been retained as part of the diagnostic process. Subsequent investigations focussed on Liverpool (Alder Hey Children's Hospital) where the practises of an individual paediatric pathologist were scrutinised (Redfern 2001). It became apparent that there was inadequate information being provided to relatives and that the whole process of consent for autopsy examination had to be addressed. In Scotland Professor Shelia McLean headed the inquiry, the findings being published in 2001 (McLean 2001). The medical profession responded to the recommendations made by these various reports and have improved the process of seeking and documenting informed consent such that relatives are fully aware of any retained tissues and give specific consent allowing the use of any retained tissues in medical research and education.

Much of the discussion relating to retained organs focussed on autopsies that had been performed in cases outwith the authority of the Procurator Fiscal (or Coroner in England, Wales and Northern Ireland). There was, however, considerable concern relating to retention of organs in cases which had been instructed by the Procurator

Fiscal (or Coroner) and the subsequent use of tissues for medical research and education. A particularly high profile case resulted in a review of the practises by Her Majesty's Inspector of Anatomy (Metters 2003) which coincided with a review of the Coroners system in England and Wales (Luce 2003). There remains uncertainty in Scotland with regard to the use in medical research of tissue retained for diagnostic purposes under the authority of the Procurator Fiscal. In particular archived material which was retained in good faith using the appropriate mechanisms available at the time was problematic. There is a clear willingness that research which will benefit the general public should continue and as such, following a 12 month moratorium on research using human tissues during which time relatives could reclaim retained tissue if they so wished, research continues. The Metters report makes the following points; prior to 1991 Research Ethical Committee (REC) approval was not required for research using human material. After 1991 and up to the late 1990's REC approval was required for research using tissues from the recently dead, although this was not clearly defined. The current situation is that all research using human materials requires REC approval. This normally requires anonymisation of the tissues recruited to the studies. Clearly in many situations, such as this current study, reference to clinical data is imperative and complete anonymisation would negate any value of the research. Therefore the concept of coded anonymised studies exists, whereby the cases are all coded with only the principal investigator having access to clinical details for each coded case. This current study was submitted to, and approved by, the Southern General Hospital Research Ethics Committee (SGHREC). All future studies related to this archived material require separate submissions to be made to the SGHREC.

3.2 Processing of tissues

3.2.1 Fixation and sampling of brains

The brains had been fixed in 4% formal saline for a minimum of 3 weeks prior to dissection after which a standardised brain cut and histological sampling was undertaken. The brains were cut at a standardised 1-cm thickness in the coronal plane. Full macroscopic and microscopic examination was undertaken in each case. Blocks for histology were taken from the cerebral hemispheres at the level of the lateral geniculate bodies (bilateral parasagittal including corpus callosum, bilateral parietal convexities including watershed regions, bilateral thalami including internal capsules, bilateral hippocampi), cerebellar hemispheres including dentate nucleus, midbrain, pons at the level of the nucleus coeruleus, and medulla.

3.2.2 Processing and staining of histology sections

The tissue was processed in a VIP tissue processor (Bayer Diagnostics, Newbury, UK) using a 60-hour cycle and embedded in paraffin wax. Eight micrometre thick sections of the paraffin blocks were cut and stained with haematoxylin and eosin (H&E) and luxol fast blue/ cresyl violet (LFB/CV).

3.3 Generation of pathological data

Archival data which had been gathered prospectively during the years 1987-1999 inclusive according to a uniform protocol (see appendix 1) was logged into a database for each case. The data included age and length of survival after the episode of TBI. Pathological data included both focal and diffuse pathologies. The focal pathologies were skull fractures, intracranial haemorrhages, raised intracranial pressure and associated infarcts, contusions and cerebral infection; the diffuse pathologies were diffuse traumatic axonal injury (TAI), ischaemic brain damage and brain swelling. In addition information was available relating to systemic injuries and

to the post- mortem interval (PMI) for each case. The data was gathered from the neuropathological reports and from microscopic examination of cases where required.

3.3.1 Skull fractures and intracranial haemorrhages

Skull fractures were documented as being either present or absent, and intracranial haemorrhages were recorded in relation to the anatomical compartment involved (extradural, subdural, intracerebral). If known the size of the intracranial haematomas was documented and whether there had been neurosurgical intervention resulting in evacuation of the haematoma. The criteria for coding are outlined in appendix 1.

3.3.2 Traumatic axonal injury

Traumatic axonal injury (TAI) was documented as being absent or present, and if present was graded as grade 1, 2 or 3 (Adams *et al* 1989). Grade 1 lesions had widespread axonal damage in the corpus callosum and the cerebral hemispheres. Grade 2 lesions, in addition, had focal haemorrhagic lesions in the corpus callosum, and in grade 3 there was in addition a haemorrhagic lesion in the rostral brain stem. The term diffuse axonal injury (DAI) was originally applied to traumatic damage exclusively. However, as immunohistochemical studies using β-APP as a marker of axonal damage have demonstrated, many brain insults can result in axonal damage. Therefore, it has been proposed that the aetiology of any axonal damage should always be indicated, and that DAI (as originally defined) should now be referred to as TAI (Geddes *et al* 2000).

Diffuse vascular injury (DVI) is a diffuse injury in which haemorrhages, which are often microscopic, are present throughout the brain. The condition is usually fatal and is thought to be due to rotational forces applied to the brain. DVI was assessed as being either absent or present.

3.3.3 Ischaemic brain damage

Ischaemic brain damage was assessed as mild, moderate or severe using a grading system developed by Graham *et al* 1989;

1. severe comprised those cases in which the lesions were diffuse, multifocal and large within arterial territories.
2. moderate when the lesions were limited to the arterial boundary zones, singly or in combination with subtotal infarction in the distribution of the cerebral arteries, or if there were 6-10 subcortical lesions.
3. mild if there were five or less subcortical lesions in the brain.

3.3.4 Raised intracranial pressure

Raised intracranial pressure was considered to be present if there were tentorial hernias (either macroscopic or microscopic), often with associated vascular complications within the distributions of the anterior choroidal artery, the pericallosal artery, the posterior cerebral artery, and the arterial supply to the cerebellum and brainstem (Adams and Graham, 1976).

3.3.5 Contusions

Contusions were graded using the total contusion index (TCI) developed by Adams *et al* (1980) and subsequently modified (Adams *et al* 1985). This assesses the extent (0-3) and depth (0-4) of contusions in a variety of anatomical locators, producing a numerical score for each hemisphere which is then combined and interpreted as absent, mild, moderate, or severe. The anatomical locators are the frontal, temporal, parietal and occipital lobes, the cortex above and below the Sylvian fissure, and the cerebellum. The maximum score for an anatomical locator is 12 (4x3=12), and the TCI has a maximum value of 144 (each side 6x12=72, 2x72=144). For this study contusional injury was mild if the TCI was less than 20, moderate if the

TCI was between 20 and 37, and severe if the TCI was greater than 37 (Graham *et al* 1988).

3.4 Genotyping

3.4.1 Polymerase Chain Reaction

Polymerase chain reaction (PCR) was introduced in 1985 (Saiki *et al* 1985) and has since revolutionised molecular analysis of tissues. PCR is a technique which allows the rapid amplification of a relatively small quantity of DNA from a tissue sample. The main principles are as follows; DNA is extracted from the tissue of interest and denatured to a single-stranded form (ssDNA). Primers are then introduced to specifically bind to a selected region of the ssDNA, the primers usually being no more than about 100 base pairs (bp) long. When the primers have bound to the ssDNA (annealed) a polymerase enzyme is activated in the presence of excess nucleotides, resulting in extension of primer to produce a new double-stranded molecule of DNA. The process is then repeated many times (usually between 30 and 40 times) resulting in the production of many copies of the section of DNA of interest. The DNA is then digested in a specific way to produce shorter segments (restriction enzyme digestion) and the fragments are separated on an electrophoresis gel.

The PCR technique can be used to examine polymorphisms of specific genes. Each gene polymorphism will have a different nucleotide structure which will result in segments of differing length after restriction enzyme digestion. These nucleotide fragments of different molecular weights will separate out in a specific way on the gel dependent on the polymorphism present.

3.4.2 Technical Difficulties

PCR requires the initial DNA sample to be in a reasonable good state of preservation. If there is DNA degradation as is often the case where there has been a

long post-mortem interval (PMI) the yield of nucleic acid after the PCR process can be unpredictable and may be of poor quality. PCR analysis in this study was complicated by the technical difficulties associated with extracting viable DNA from formalin-fixed paraffin-embedded tissues (Greer *et al* 1991). The DNA extraction method is detailed in appendix 2. Briefly, sections (10μm), had to be dewaxed using xylene and ethanol prior to digestion. Digestion is required as formalin fixation will result in increased cross-linkage between nucleotides in the DNA molecule. Proteinase K treatment is used to extract (digest) DNA from formalin-fixed material, although often the DNA can be very fragmented and there may be a low yield (Palmer 1995).

In this study successful *APOE* genotyping was one of the inclusion criteria for case selection. Genotyping was considered to be unsuccessful if no interpretable gels were produced after four attempts including extended Proteinase K digestion and use of different blocks of tissue to extract DNA. In many cases there was only access to archived brain material for each case, there being no other tissue blocks available to attempt DNA extraction.

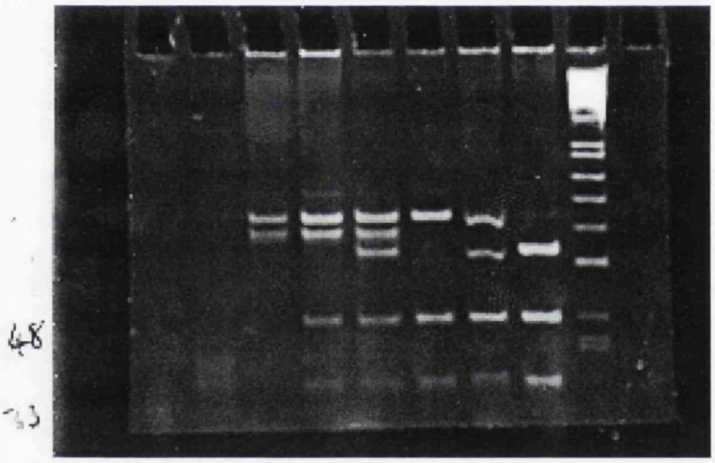
3.4.3 *APOE* genotyping

APOE genotype was determined using a previously described PCR method (Nicoll *et al* 1997). The protocol is outlined in appendix 3. The digested amplified fragments had lengths of 91 base pairs (bp), 81bp, 72bp, 48bp and 33bp. All genotypes shared fragments of 16bp and 21 bp (figure 2).

3.4.4 *IL-1A* and *IL-1B* genotyping

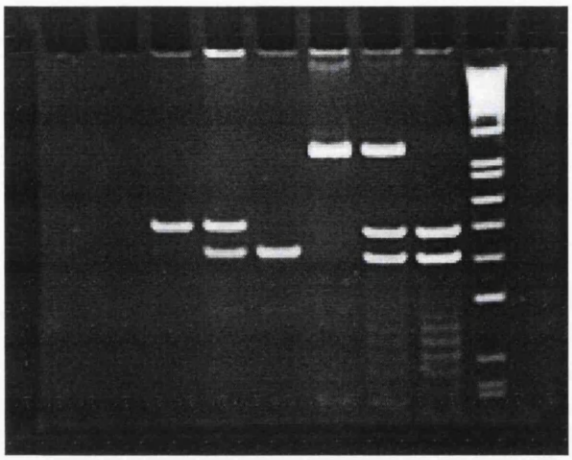
IL-1A and *IL-1B* genotyping used a previously described technique (Nicoll *et al* 2000) described in appendix 3. A number of common polymorphisms have been described for both the *IL-1A* and *IL*-1B genes. This study looked at the

polymorphisms which have been suggested to confer an increased risk for AD and have been associated with inflammatory disease (Nicoll *et al* 2000): for *IL-1A* this is a C-to-T transition at position –889 (relative to the start site for transcription); for *IL-1B* this is at position +3953. The *IL-1A* polymorphism is a promoter polymorphism and may alter the rate and quantity of gene transcription. The *IL-1B* polymorphism is a coding polymorphism which may alter the conformation of the protein thereby potentially altering function. For *1L-1A* the digested amplified fragments had lengths of 104bp and 83 bp (figure 3a). For *1L-1B* the digested amplified fragments had lengths of 182bp, 97bp and 85 bp (figure 3b).



2/2 2/3 2/4 3/3 3/4 4/4

Figure 2; a gel demonstrating the digested amplified fragments seen in *APOE* genotyping. 2/2 has bands at 91 and 81 base pairs (bp); 2/3 at 91, 81 and 48 bp; 2/4 at 91, 81 72 and 48 bp; 3/3 at 91 and 48 bp; 3/4 at 91, 72 and 48 bp; and 4/4 at 72, 48 and 33 bp. The right hand column is a molecular weight ladder.



[IL-1A] 2/2 1/2 1/1 2/2 1/2 1/1 [IL-1B]

Figure 3; a gel demonstrating the digested amplified fragments seen in *IL-1A* and *IL-1B* genotyping. The *IL-1A* genotypes are seen on the left, the *IL-1B* genotypes on the right. For *IL-1A*; 2/2 has a band at 104 base pairs (bp); 1/2 at 104 and 83 bp; 1/1 at 83 bp. For *IL-1B*; 2/2 has a band at 182 bp; 1/2 at 182, 97 and 85 bp; 1/1 at 97 and 85 bp. The right hand column is a molecular weight ladder.

3.5 Immunohistochemistry

Immunohistochemistry is a frequently used technique in which proteins can be identified by their specific binding to an antibody labelled with a marker. The antibodies used in this study were all monoclonal and therefore of greater specificity than polyclonal antibodies. Briefly immunohistochemistry involves the application of an antibody labelled with a marker to a paraffin section, with the antibody being localised by a second chemical reaction, resulting in a colour change on the paraffin section. In this study counterstaining with haematoxylin was weak to allow greater sensitivity of image analysis as discussed below (**3.6**).

3.5.1 Tau immunohistochemistry

Immunohistochemistry was undertaken for tau (monoclonal antibody, Dako 1: 15000). No pre-treatment was required and the primary antibody was applied overnight at 4°C. This antibody reacts with both the phosphorylated and non-phosphorylated forms of tau protein, and labels the tau protein of neurofibrillary tangles. The antibody was detected using the ABC kit (Vecta Stain, Vector Laboratories, Peterborough, UK) and developed with diaminobenzidine (DAB).

3.5.2 Neuroinflammation immunohistochemistry

Immunohistochemistry was undertaken for markers of microglial activation. Sections were immunostained with anti-CD68 to identify microglia with phagocytic functions (mouse monoclonal antibody to a macrophage-specific 110 kDa glycoprotein - Dako, 1:1000) and CR3/43 which labels activated microglia (mouse monoclonal antibody to class II MHC: HLA-DR, -DQ and -DP β chains - Dako, 1:800). The antibodies were detected using the ABC kit (Vecta Stain, Vector Laboratories, Peterborough, UK) and developed with diaminobenzidine (DAB).

3.6 Image analysis

Image analysis is a technique which allows capture and manipulation of digital images. Image analysis was undertaken to assess the immunostaining load within defined anatomical regions in cases of TBI and to compare these with control cases. The regions of interest were the hippocampus including the alveus, the inferior temporal gyrus including both grey and white matter, the corpus callosum at the level of the lateral geniculate body, and the grey and white matter of the cingulate gyrus again at the level of the lateral geniculate body.

3.6.1 Image capture and generation of tiled images

The morphometric study used an image analysis system consisting of a digital CCD-Camera (CoolSnap-Pro®) linking an Olympus BX 40 Light Microscope to a PC

with the image analysis software (Image-Pro® Plus, Media Cybernetics) (figure 4).

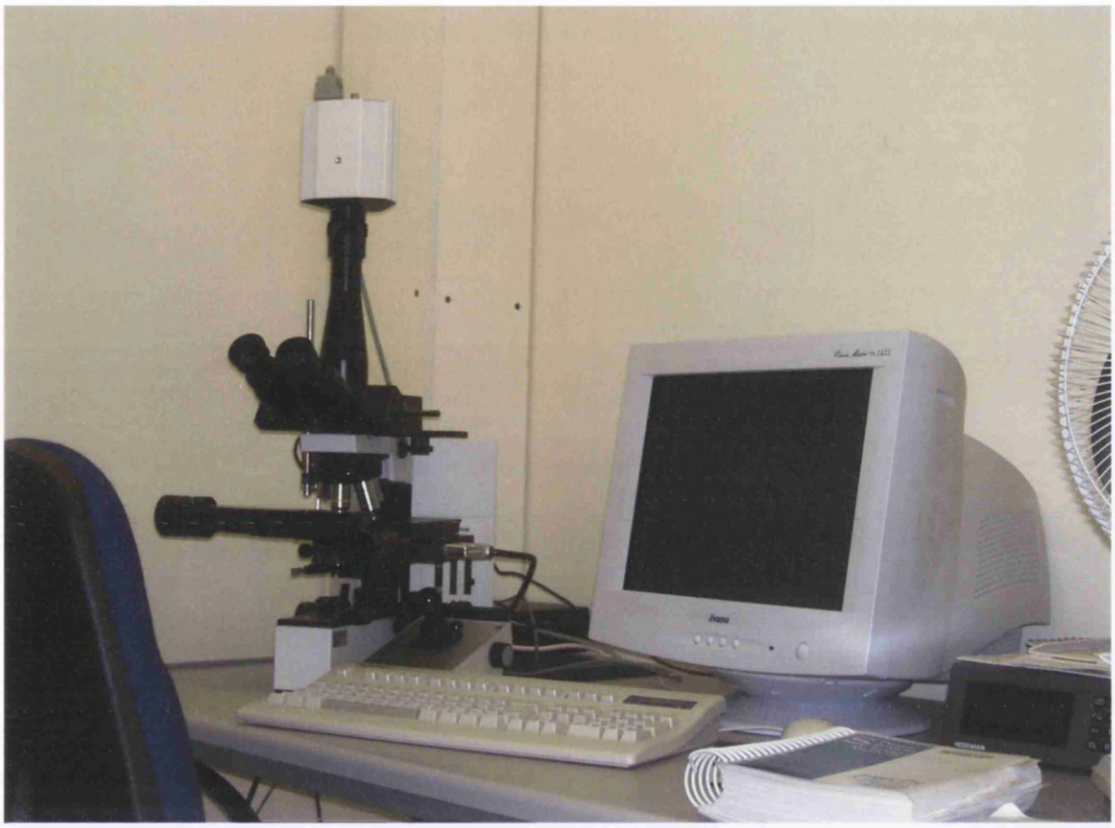
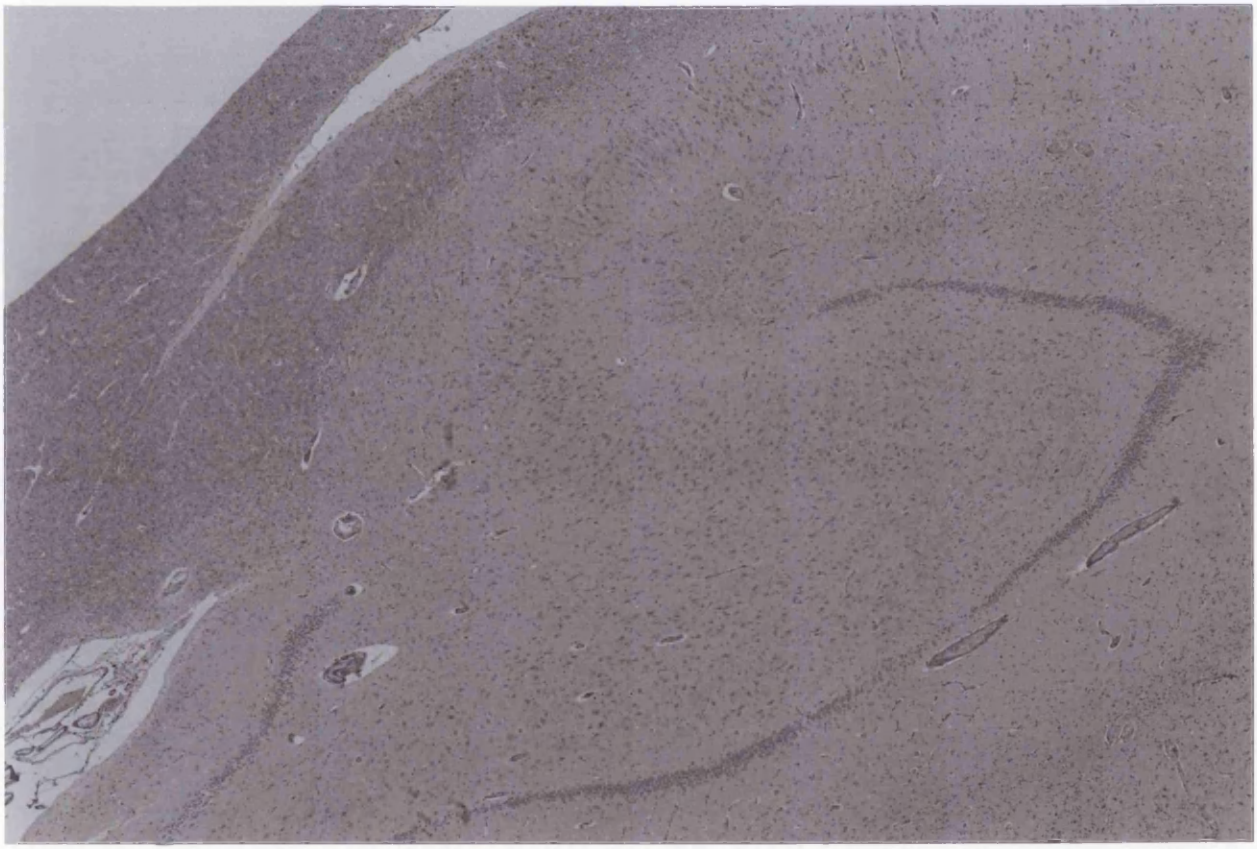


Figure 4; The image analysis system used to analyse the immunohistochemistry. The microscope is connected to the computer and converts histological slides to digital images.

Immunostained sections were placed on an automated stage (Prior®) which could move in both the X- and Y- axis. Multiple non-overlapping colour images of the area of interest were captured using x 10 stage objective lens and the images were tiled together automatically to give a large composite image (figure 5). Images were stored using the RGB 24 (red, green, blue) colour model. This system allows the production of colour images as every colour can be produced by varying levels of red, green and blue. The bitmaps (digital grids of which each individual element is called a pixel) have pixels which contain varying brightness values for each of red, green and

blue. The brightness values range from 0 to 255 for each colour and allow detailed colour images to be created digitally.

Figure 5; This is a composite tiled image of a section of hippocampus including the alveus. Non-overlapping images are captured at x10 and tiled together automatically to produce a composite image. This example is produced by tiling 63 separate images; rows of 9 images in the x-axis and 7 images in the y-axis. The image is stored as a jpeg file and can be magnified to x40 for segmentation. Areas of interest (AOI) can then be defined manually for assessment of immunoload.



Each image had a field 0.32 mm^2 (width 0.66 mm, height 0.49 mm) and contained 1447690 pixels (width 1392 pixels, height 1040 pixels). The number of images taken for each anatomical area was variable due to anatomical variation between cases, but generally in the region of 50-60 images were taken for each composite image. The composite image was stored as a data file in jpeg format. Lamp intensity, digital camera set-up and calibration parameters were kept constant throughout the capture of images.

3.6.2 Area of interest function

Using the stored image an "area of interest" (AOI) could be defined for data generation. The AOI tool allowed a freehand or geometric area to be defined; the area defined was dependent on the anatomical area being studied. For example, zones of the hippocampus required freehand areas to be defined whereas corpus callosum was more suited to a defined geometric area for analysis. Each AOI was defined in terms of pixels such that the number of pixels forming the X- and Y- axes of the shape were known. The system was calibrated such that this figure could be converted to microns (μm) or millimetres (mm).

3.6.3 Assessment of immunostaining load

The image analysis software (Image-Pro® Plus, Media Cybernetics) used in this study allowed the definition of immunoreactive profiles based on a defined threshold (segmentation). Segmentation is a process which allows the isolation of certain colours from an image as a whole. In this study the immunoreaction was developed by diaminobenzidine (DAB) which produces a brown precipitate. A manual segmentation technique was used to isolate the brown immunoreaction in the captured images. The sections were all weakly counterstained with haematoxylin to allow greater differentiation between the brown immunostaining reaction and the

bluish background. The stored images were magnified to allow greater sensitivity during the segmentation process. The colour cube-based model was used for these images. This allows a square measuring 3x3 pixels to be assessed for segmentation; this produces great sensitivity in differentiating between an area of brown immunostaining and adjacent haematoxylin counterstaining.

While this programme did allow the threshold setting to be applied as a constant to all images this was not done due to immunostaining intensity variation between batches of immunostaining. Therefore each slide had unique parameters set for segmentation based on the intensity of immunostaining. While this was more labour intensive it allowed greater sensitivity in the segmentation process. All results were generated by one analyst to remove any inter-observer variation. To assess intra-observer variation, the same field of the same slide was analysed at the start of each session, and the load scores checked to see if they were similar. This showed less than 5% variation over a ten day period.

Immunostaining load within a given AOI was determined using the "Per Area" function found in the measurements tools. This gave information regarding the ratio between the area of the counted object (the immunostained area) to that of the entire area of the pre-defined AOI.

3.7 Statistical analysis

The study was designed as an observational study. Statistical analysis varied dependent on the variables being assessed and the spread of data. The statistical assessment of each study is presented in the results section and statistical data is presented in appendix 4.

4 Results

4.1 Genotyping

Of the initial 259 cases 239 were successfully genotyped (92% success rate) for *APOE*. *IL-1A* and *IL-1B* genotyping was attempted on all of the cases which had been successfully genotyped for *APOE* (239 cases in total). For *IL-1A* 228 (95%) cases were successfully genotyped; for *IL-1B* 207 (87%) cases were successfully genotyped. Cases were considered to have been unsuccessful after four failed attempts including extended proteinase K digestion and DNA extraction from different blocks.

4.2 Association of *APOE ε4* and cerebrovascular pathology in traumatic brain injury

Of the total number of 239 cases of fatal TBI examined there were 83 *APOE ε4* carriers (35%) and 156 were non-carriers of *APOE ε4* (65%). The pathological features for each case documented on the database were assessed in relation to *APOE* ε4 allele carriage presence or absence. The power of a study refers to the chance of detecting a specified effect if it exists, in this case that possession of *ε4* was associated with specific pathological features. The study had 70% power to detect differences of approximately 15-19% in patterns of response between *ε4* carriers and non-carriers. Comparison of the prevalence of the features was made using confidence intervals (CI) for the differences in proportions. Calculations were performed using Minitab (Version 12). The CI is presented for each feature in table 9.

Differences were noted between *APOE ε4* carriers and non-carriers of *APOE ε4* in relation to contusions and ischaemic brain damage (table 9). 42% of ε4 carriers had moderate or severe contusions (*i.e.* a total contusion index of >20) compared with 29% of non-carriers of ε4 (p=0.05). With regard to ischaemic brain damage a trend

was noted between the possession of *APOE ε4* and severe ischaemic brain damage which was present in 54% of *APOE ε4* carriers and 42% of non-carriers of ε4 (p=0.08). No significant associations were demonstrated between possession of *APOE ε4* and the presence of extradural haematoma, subdural haematoma, intracerebral haematoma, skull fracture, traumatic axonal injury or evidence of raised intracranial pressure.

Table 9; The incidence of specific pathological features in relation to the presence and absence of *APOE ε4*. There is a significant association between the possession of e4 and moderate/severe contusions in fatal TBI, and a greater incidence of severe ischaemic brain damage.

Pathological Feature	*APOE ε4* carriers n=83 (35%)	*APOE ε4* non-carriers n=156 (65%)	95% Confidence Interval for difference	p-value
Moderate/severe contusions	35 (42%)	46 (29%)	0 to25%	0.05
Severe ischaemic brain damage	45 (54%)	66 (42%)	- 1 to25%	0.08
Skull fracture	61 (73%)	105 (67%)	- 6 to18%	0.31
Traumatic axonal injury	31 (37%)	68 (44%)	- 19 to 7%	0.35
Extradural haemorrhage	13 (16%)	13 (8%)	-4 to 14%	0.31
Subdural haemorrhage	52 (60%)	98 (63%)	-13 to13%	0.98
Intracerebral haemorrhage	31 (37%)	47 (30%)	-5 to 20%	0.26
Raised intracranial pressure	58 (70%)	98 (63%)	-5 to 20%	0.27

4.3 Cytoskeletal pathology

Four patterns of tau immunoreactivity were seen (figure 6);

1/ neuronal perikaryal immunoreactivity.

2/ neuropil threads with a pattern similar to that seen of the dendritic neuropil threads seen in AD.

3/ glial cell immunoreactivity with associated punctate staining in white matter.

4/ diffuse neuropil staining although thte cell processes involved could not be established by immunohistochemistry.

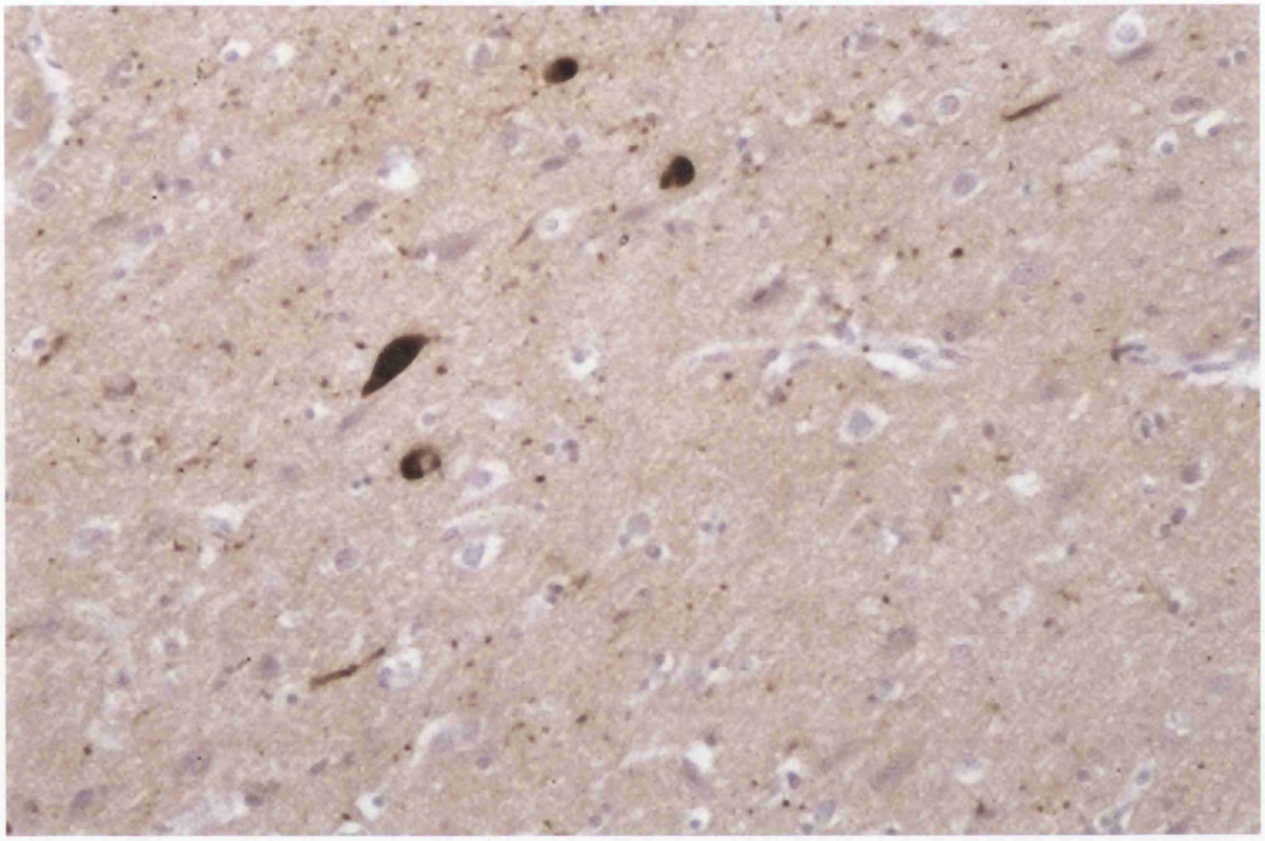


Figure 6a; Photomicrograph from the medial temporal cortex demonstrating both neuronal perikaryal immunoreactivity and neuropil threads. Tau immunoreactive fibrillary structures (neurofibrillary tangles) are seen in 4 neuronal cell bodies, and neuropil threads are numerous. Tau immunostaining x20.

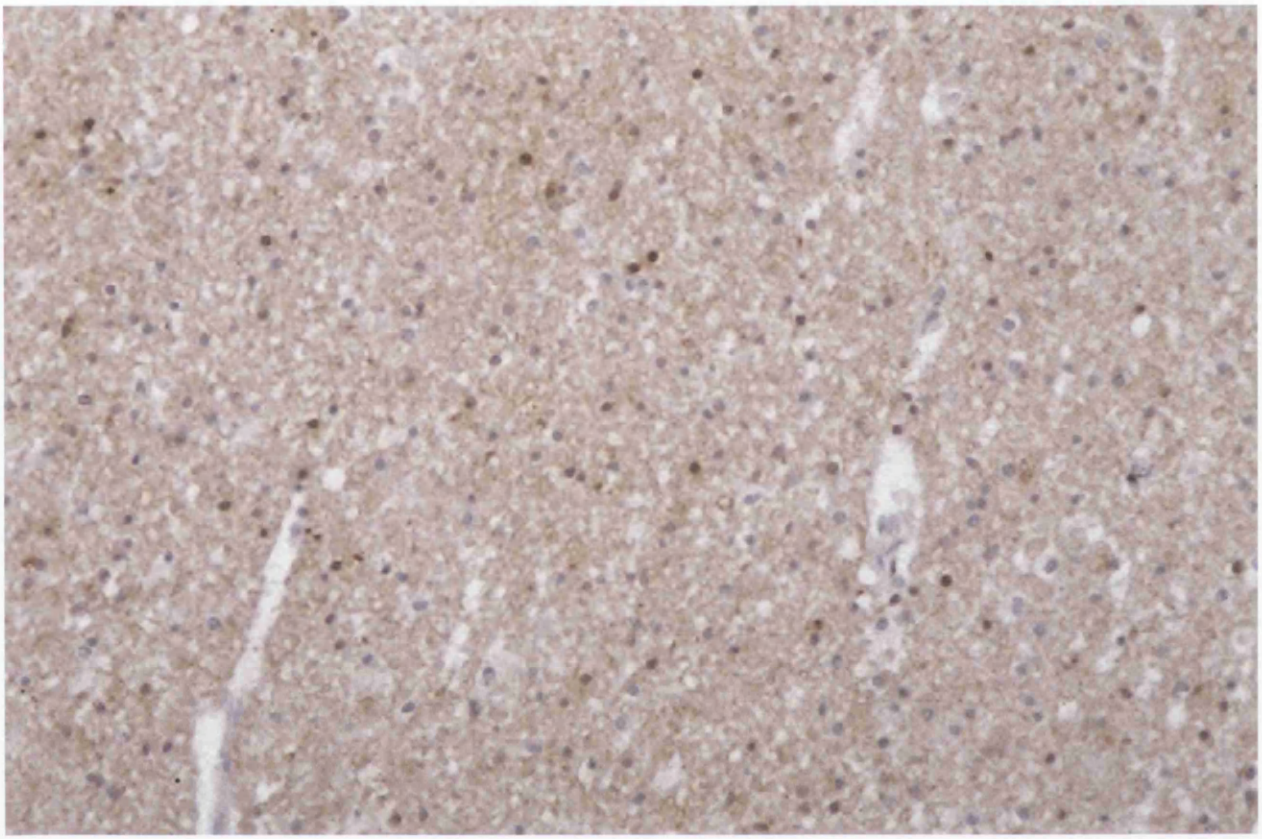


Figure 6b; Photomicrograph from cerebral white matter showing tau immunoreactivity within glial cell bodies. In addition there is a punctate pattern of staining within the white matter. Tau immunostaining x20.

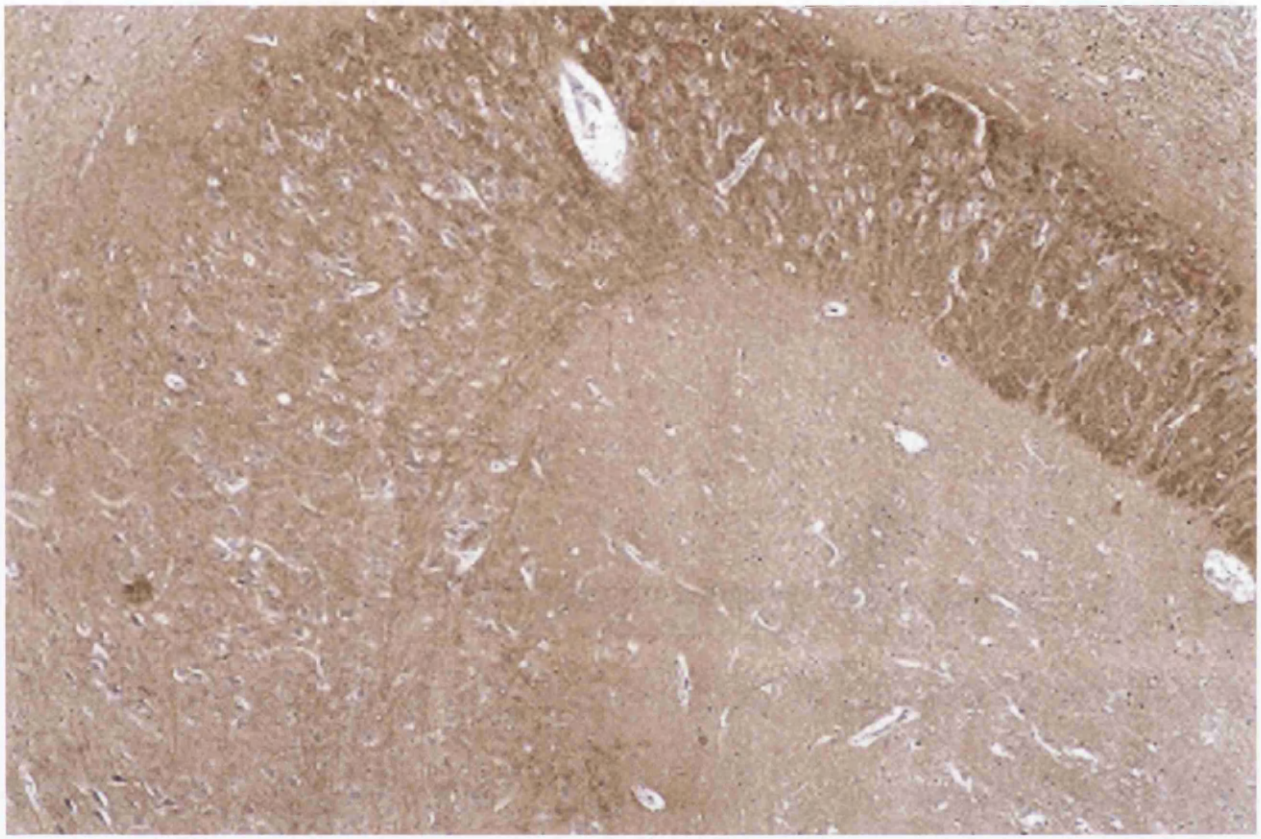


Figure 6c; Photomicrograph from the hippocampus showing diffuse tau immunoreactivity in the stratum pyramidalis of the cornu ammonis. Tau immunostaining x20.

Neuronal perikaryal immunoreactivity was seen in the form of fibrillary structures (neurofibrillary tangles). These were identified by anatomical region and analysed semi-quantitatively as total number per x10 field. Neuronal perikaryal immunoreactivity was seen in both TBI and control cases and, in both groups, increased with age (table 10).

Neuropil threads were assessed as being either present or absent. Neuropil threads, like neuronal perikaryal immunoreactivity, were seen in both TBI and control cases and, in both groups, increased with age.

Glial cell immunoreactivity was assessed as either present or absent. Glial tau immunoreactivity was seen in 9 of 45 (20%) TBI cases and in only 1 of 15 (6.7%) control cases.

Diffuse neuropil staining was assessed as either present or absent. This pattern of immunoreactivity was seen at all ages in both TBI and control cases.

The number of cases examined in this part of the study was small so no formal statistical analysis was carried out. If the trend is maintained with an increased sample size then this may indicate increased glial tau immunoreactivity with ageing.

Table 10; Comparison of tau immunoreactivity staining patterns at different ages and survival times

	<20 years				**20-50 years**				**>50 years**			
Tau immunoreactivity staining pattern	perikaryal	threads	glia	neuropil	perikaryal	threads	glia	neuropil	perikaryal	threads	glia	neuropil
Non- head injury controls with no neurological disease	0/5	0/5	1/5	1/5	1/5	1/5	0/5	1/5	4/5	1/5	0/5	3/5
Survival <24 hours	0/5	0/5	1/5	2/5	0/5	0/5	2/5	3/5	3/5	2/5	0/5	0/5
Survival 24 hours- 1 week	0/5	0/5	1/5	1/5	1/5	1/5	0/5	3/5	2/5	2/5	2/5	3/5
Survival 1 week- 1 month	0/5	0/5	0/5	2/5	0/5	0/5	1/5	1/5	3/5	2/5	2/5	2/5

4.4 Neuroinflammation

4.4.1 Immunohistochemistry

CD 68 and CR3/43 immunohistochemistry was undertaken on all 55 pre-selected cases for the neuroinflammation study. Microglial cells were seen with differing morphological appearances; these ranged from resting ramified cells through to rounded phagocytosing amoeboid cells (figure 7). There was considerable variation in CD 68 and CR3/43 immunoreactivity within cases and a range of staining intensities is presented in figure 8.

Figure 7a; ramified microglial cells. CR3/43 immunostaining x40

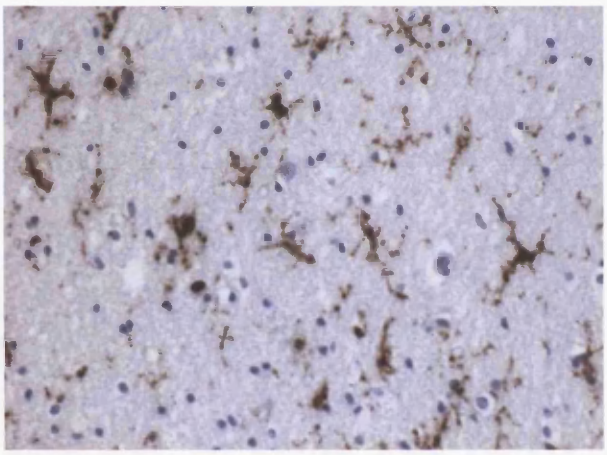
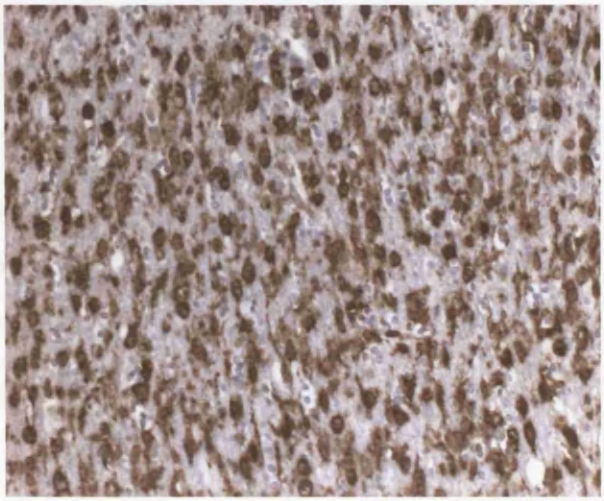


Figure 7b; amoeboid (phagocytosing) microglial cells. CD 68 immunostaining x40.



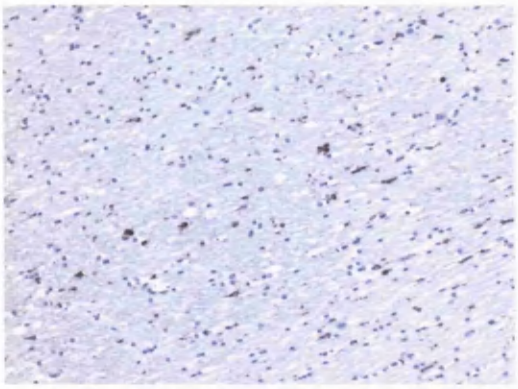


Figure 8a- low intensity

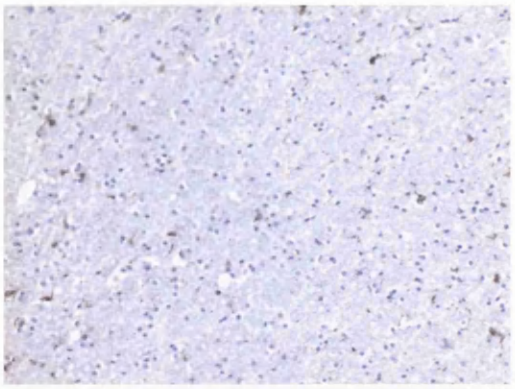


Figure 8b- low intensity

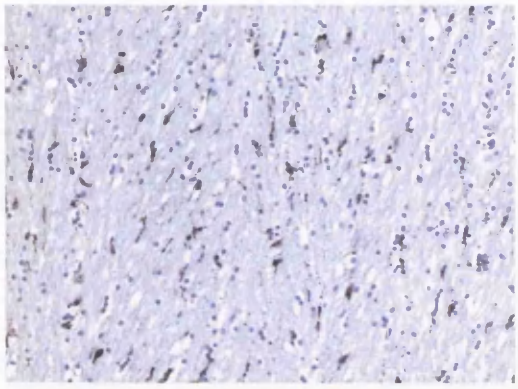


Figure 8c- medium intensity

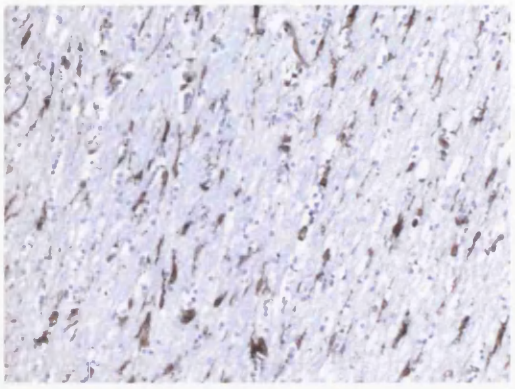


Figure 8d- medium intensity

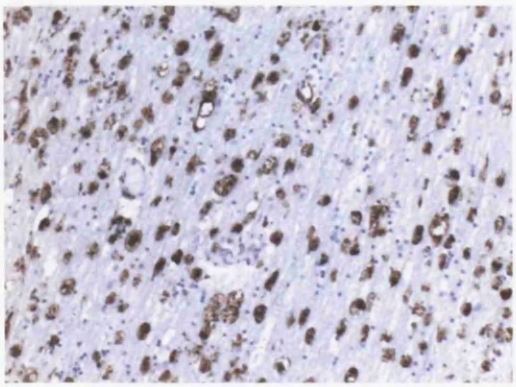


Figure 8e- high intensity

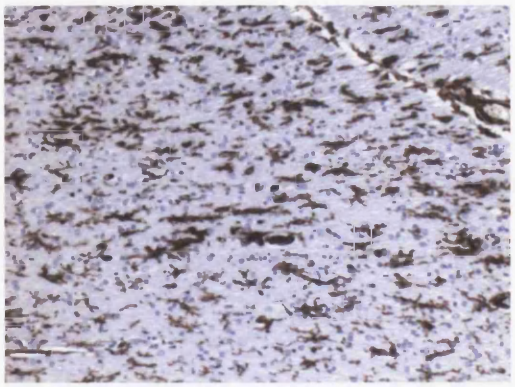


Figure 8f- high intensity

Figure 8; Examples of the variation of immunostaining seen between cases. The left column (figures 8 a, c and e) was stained with CD 68; the right column (figures 8 b, d and f) was stained with CR3/43. All figures are x10.

4.4.2 Control cases

An average of the CR3/43 immunoload in the control cases for each anatomical region is shown in table 11 and for CD 68 in table 12. For statistical analysis the immunoloads were summarised for each case by averaging the individual values of each anatomical region. Statistical analysis compared all control cases under the age of 50 (n=11) against all control cases over the age of 50 (n=9). Median values for each group were analysed using a non-parametric test (Mann-Whitney test). There was no significant difference observed between the two groups for CR3/43 (p=0.29). For CD 68, however, there was a reduction in the immunoload with ageing and this reached statistical significance (p=0.05) [see appendix 9.4.1].

Table 11; CR3/43 immunoreactivity in selected anatomical areas in the control group. There is an increase with ageing seen in the temporal lobe and hippocampus, sites commonly involved in Alzheimer's disease. Comparison of the overall immunoloads between those less than 50 years old and those greater than 50 years old, however, showed no significant difference. The numbers refer to the immunostaining intensity presented as a percentage of the total area. The values presented are the median values of the dataset. Mean values were not used due to the small number of cases studied and the skewed distribution of values.

	cc	cg	cw	tg	tw	alveus	hippo
0-19	5.86	1.07	2.70	0.46	1.00	0.69	0.52
20-49	2.35	0.48	1.70	0.17	0.60	1.84	1.01
50+	5.35	1.15	2.05	1.01	2.94	2.19	2.18

Legend: cc= corpus callosum cg= cingulate grey matter
cw= cingulate white matter tg= temporal grey matter
tw= temporal white matter hippo= hippocampus

Table 12; CD68 immunoreactivity in selected anatomical areas in the control group. Comparison of the overall immunoloads between those less than 50 years old and those greater than 50 years old showed a significant reduction in the immunoload in the older age group. The numbers refer to the immunostaining intensity presented as a percentage of the total area. The values presented are the median values of the dataset. Mean values were not used due to the small number of cases studied and the skewed distribution of values.

	cc	cg	cw	tg	tw	alveus	hippo
0-19	0.08	0.24	0.46	0.63	1.23	3.80	2.58
20-49	1.15	0.27	0.57	0.06	0.18	0.09	0.08
50+	0.68	0.34	0.98	0.10	0.29	0.19	0.20

Legend: cc= corpus callosum cg= cingulate grey matter
cw= cingulate white matter tg= temporal grey matter
tw= temporal white matter hippo= hippocampus

4.4.3 Control v TBI cases

The next part of the study looked to see if there was a difference in the neuroinflammatory response between the control group and the TBI group. For statistical analysis the immunoloads were summarised for each case by averaging the individual values of each anatomical region. Statistical analysis compared all control cases (n=20) against all TBI cases (n=55). Cases were not separated by age. Median values for each group were analysed using a non-parametric test (Mann-Whitney test). A significant difference was seen with CD 68 there being a significant increase in CD 68 immunoload in the TBI cases (p= 0.03). There was no significant difference in CR3/43 immunoload between TBI and control cases (p= 0.42) [see appendix 9.4.2].

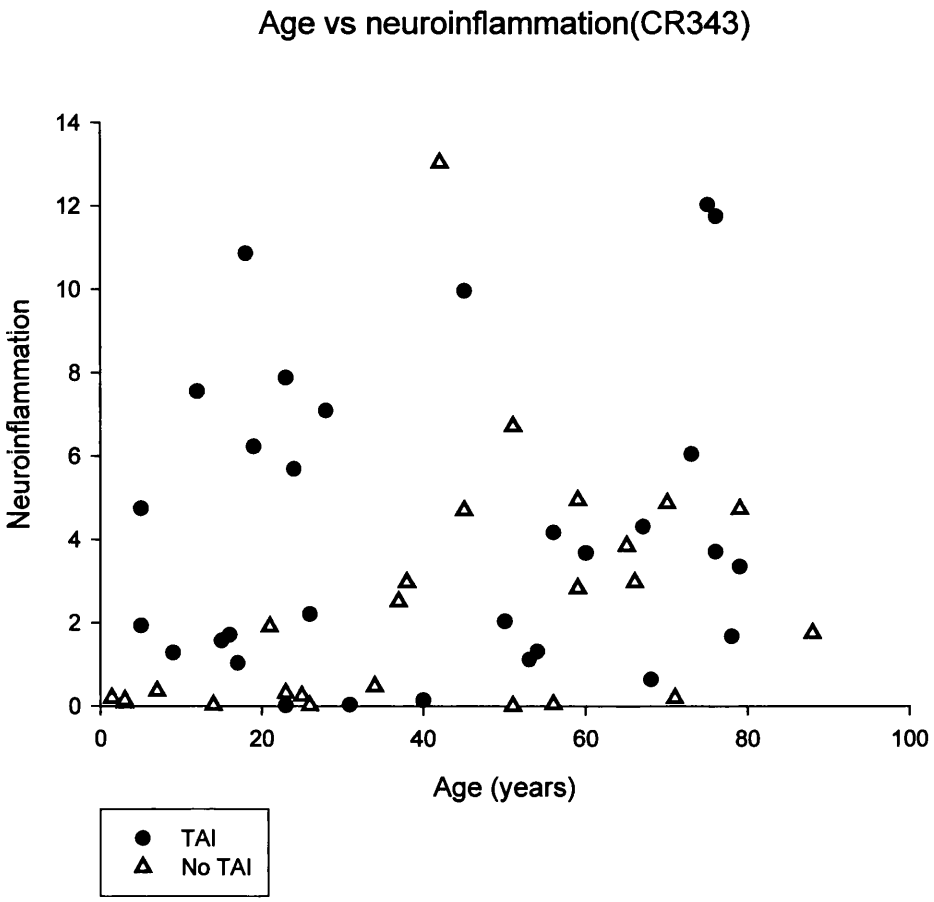
4.4.4 Traumatic axonal injury

Within the group of 55 TBI cases 30 had a pathological diagnosis of diffuse TAI. Image analysis of CR3/43 of diffuse TAI cases when compared to non-TAI cases showed an increased immunoload in regions related to the central white matter; corpus callosum ($p=0.01$) and cingulate white matter (parasagittal white matter) ($p=0.02$) (table 13). In addition there was a significant increase in immunoload in the cingulate grey matter ($p=0.02$). These include white matter regions which consistently show significantly more axonal damage in diffuse TAI. CD 68 did show a similar increase in cases of diffuse TAI although these were less pronounced than those seen for CR3/43 (table 14). The increased CD 68 immunoreactivity was significant in the cingulate grey ($p=0.05$) and white matter ($p=0.04$). The statistical methods appear in the appendix in sections 9.4.3 and 9.4.4.

Table 13; CR3/43 immunostaining. Comparison between cases diagnosed with and without diffuse traumatic axonal injury (TAI). The numbers refer to the immunostaining intensity presented as a percentage of the total area. The values presented are the median values of the dataset. Mean values were not used due to the small number of cases studied and the skewed distribution of values.

	cc	cg	cw	tg	tw	alveus	hippo
+TAI (n=30)	4.76	1.11	3.38	0.34	2.35	2.16	0.70
-TAI (n=25)	1.00	0.10	0.24	0.13	0.84	1.15	0.30
p-values	**0.01**	**0.02**	**0.02**	0.11	0.11	0.45	0.50

Legend: cc= corpus callosum
cg= cingulate grey matter
cw= cingulate white matter
tg= temporal grey matter
tw= temporal white matter
hippo= hippocampus
TAI= traumatic axonal injury

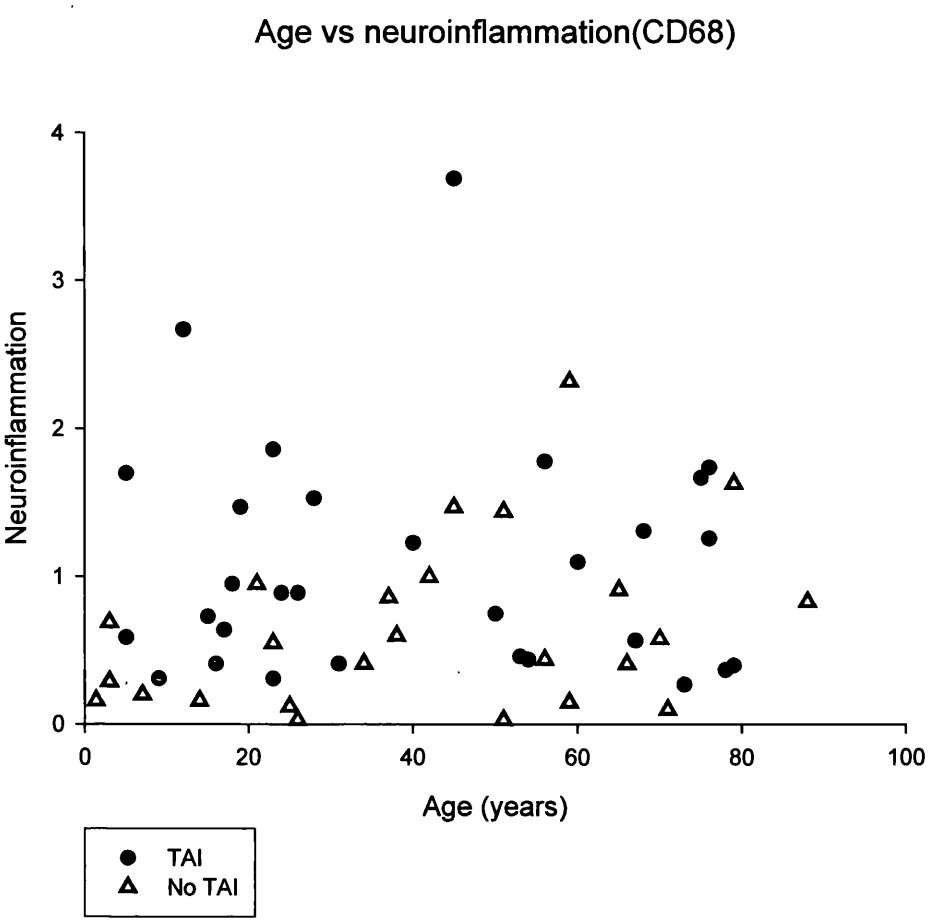


Graph 1: The average neuroinflammatory response is charted against age for each case, with cases of TAI being separated from cases of non-TAI.

Table 14; CD68 immunostaining. Comparison between cases diagnosed with and without diffuse traumatic axonal injury (TAI). The numbers refer to the immunostaining intensity presented as a percentage of the total area. The values presented are the median values of the dataset. Mean values were not used due to the small number of cases studied and the skewed distribution of values.

	cc	cg	cw	tg	tw	alveus	hippo
+TAI (n=30)	1.46	0.52	0.93	0.24	0.74	0.61	0.21
-TAI (n=25)	0.93	0.22	0.58	0.10	0.27	0.45	0.21
p-value	0.08	**0.05**	**0.04**	0.23	0.09	0.29	0.47

Legend: cc= corpus callosum
cg= cingulate grey matter
cw= cingulate white matter
tg= temporal grey matter
tw= temporal white matter
hippo= hippocampus
TAI= traumatic axonal injury



Graph 2: The average neuroinflammatory response is charted against age for each case, with cases of TAI being separated from cases of non-TAI.

4.4.5 Genotype data

4.4.5.1 *IL-1A* and *IL-1B* genotypes

For *IL-1A* the results were as follows;

	Controls	Trauma cases
1,1	9 (47.5%)	30 (56%)
1,2	9 (47.5%)	20 (35%)
2,2	1 (5%)	5 (9%)

This gave an allele frequency of 0.71 and 0.29 for alleles 1 and 2 respectively in the control cases and 0.73 and 0.27 for the trauma cases. A chi-squared test demonstrated no significant difference between the control group and the TBI group regarding possession of *IL-1A* allele 2 (p= 0.59) [see appendix 9.4.5]. For assessment of the neuroinflammatory response the immunoloads were summarised for each case by averaging the individual values of each anatomical region. Median values of the immunoloads for each polymorphism in the control and TBI groups are shown in table 15. The neuroinflammatory response was assessed in individuals who possessed any copies of *IL-1A* allele 2 and in individuals who were *Il-1A* allele 2 homozygotes. There was no significant difference in the median neuroinflammatory response in individuals who possessed any copies of *IL-1A* allele 2 and those who had no copies when control and TBI cases were considered together and when TBI cases were considered against other TBI cases (see table 16). The statistical methods appear in the appendix in sections 9.4.6 (any copy of allele 2) and 9.4.7 (homozygotes).

For *IL-1B* the results were as follows;

	Controls	Trauma cases
1,1	10 (67%)	30 (69%)
1,2	3 (20%)	11 (26%)

2,2	2 (13%)	2 (5%)

This gave an allele frequency of 0.77 and 0.23 for alleles 1 and 2 respectively in the control cases and 0.83 and 0.17 for the trauma cases. A chi-squared test demonstrated no significant difference between the control group and the TBI group regarding possession of *IL-1B* allele 2 (p= 0.82) [see appendix 9.4.5]. For assessment of the neuroinflammatory response the immunoloads were summarised for each case by averaging the individual values of each anatomical region. Median values of the immunoloads for each polymorphism in the control and TBI groups are shown in table 15. The neuroinflammatory response was assessed in individuals who possessed any copies of *IL-1B* allele 2 and in individuals who were *IL-1B* allele 2 homozygotes. There was no significant difference in the median neuroinflammatory response in individuals who possessed any copies of *IL-1B* allele 2 and those who had no copies when control and TBI cases were considered together and when TBI cases were considered against non-TBI cases (see table 16). The statistical methods appear in the appendix in sections 9.4.6 (any copy of allele 2) and 9.4.7 (homozygotes).

Table 15; Immunoloads, as determined by image analysis, for each genotype. The numbers are medians of the average neuroinflammatory response for each individual. The values presented are the median values of the dataset. Mean values were not used due to the small number of cases studied and the skewed distribution of values.

IL-1A	**Genotype (%)**			**Allele frequency**		**CR3/43 immunoload**			**CD 68 immunoload**		
	1,1	**1,2**	**2,2**	**1**	**2**	**1,1**	**1,2**	**2,2**	**1,1**	**1,2**	**2,2**
Controls (n)	47.5 (9)	47.5 (9)	5 (1)	0.71	0.29	2.72	1.34	1.59	0.34	0.24	0.93
Trauma cases (n)	56 (30)	35 (19)	9 (5)	0.73	0.27	1.98	2.84	4.71	0.64	0.64	1.47

IL-1B	**Genotype (%)**			**Allele frequency**		**CR3/43 immunoload**			**CD 68 immunoload**		
	1,1	**1,2**	**2,2**	**1**	**2**	**1,1**	**1,2**	**2,2**	**1,1**	**1,2**	**2,2**
Controls (n)	67 (10)	20 (3)	13 (2)	0.77	0.23	2.23	1.43	1.88	0.30	0.29	0.69
Trauma cases (n)	69 (29)	26 (12)	5 (2)	0.83	0.17	2.05	4.73	0.67	0.75	1.46	0.37

Table 16; Statistical analysis could not demonstrate any significant difference in median intensity of the neuroinflammatory response between *IL-1A* and *IL-1B* allele 2 homozygotes and heterozygotes. Statistical analysis was carried out on all cases (TBI and control 2,2 cases against TBI and control non-2,2 cases) and on TBI only cases (TBI 2,2 cases against TBI non-2,2 cases).

		CD 68	CR3/43
IL-1A 2,2	All cases (n=6 vs 68)	p=0.10	p=0.21
	TBI cases (n=5 vs 50)	p=0.21	p=0.24
IL-1B 2,2	All cases (n=4 vs 54)	p=0.53	p=0.19
	TBI cases (n=2 vs 41)	p=0.19	p=0.16
IL-1A allele 2 any copy	All cases (n=35 vs 39)	p=0.75	p=0.72
	TBI cases (n=25 vs 30)	p=0.25	p=0.24
IL-1B allele 2 any copy	All cases (n=18 vs 40)	p=0.19	p=0.40
	TBI cases (n=13 vs 30)	p=0.22	p=0.25

4.4.5.2 *APOE* genotypes

For *APOE* the results were as follows;

	Controls	Trauma cases
2,2	1 (5%)	0
2,3	1 (5%)	9 (16%)
2,4	0	1 (2%)
3,3	13 (65%)	32 (58%)
3,4	3 (15%)	12 (22%)
4,4	2 (10%)	1 (2%)

This gave an allele frequency of 0.08, 0.75 and 0.17 for alleles 2, 3 and 4 respectively in the control cases and 0.09, 0.77 and 0.14 for the trauma cases. The neuroinflammatory response was assessed in individuals who possessed any copy of *APOE*ε4 or any copy of *APOE*ε2 in the control and TBI groups. Possession of *APOE*ε4 was not associated with any statistically significant difference in the median neuroinflammatory response in either control or TBI groups. In addition there was no significant difference in the neuroinflammatory response between TAI and non-TAI cases. Similar results were obtained for *APOE*ε2. The statistical methods appear in the appendix in sections 9.4.8 and 9.4.9.

Table 17; No significant differences in the neuroinflammatory response could be demonstrated based on possession of any copy of either *APOE*ε4 or *APOE*ε2. Statistical analysis was performed on control cases, TBI cases and TAI cases, comparing the neuroinflammatory response in those cases with any copy of either *APOE*ε4 or *APOE*ε2 against that in those cases which did not possess a copy.

		Control	TBI	TAI
Any copy of *APOE*ε4	CR343	p=0.12	p=0.14	p=0.34
	CD68	p=0.12	p=0.47	p=0.27
Any copy of *APOE*ε2	CR343	p=0.41	p=0.75	p=1.00
	CD68	p=0.34	p=0.37	p=0.53

5 Discussion

A limitation of this study relates to the fact that the pathology of only the most severe outcome from TBI group could be assessed (i.e. a fatal outcome). A United Kingdom based study found a fatal outcome occurs in only 5-10% of the total hospitalised TBI population, although approximately 50% of TBI related deaths occur before the patient can be transferred to hospital (Jennett and MacMillan 1981). Many of these initial deaths are due to very severe injuries and occur almost instantaneously while improvements in trauma care have reduced the numbers of deaths occuring several hours after the injury (Wyatt *et al* 1995). A USA based report found that annually 1.5 million people sustained a head injury of which 230000 were hospitalised and survived (15%) and 50000 (3%) died from their injuries (CDC report 1999). Therefore this autopsy-based study is only addressing a small proportion of the head injured population. An important question is whether the association identified in this study is of relevance to survivors of TBI.

5.1 Cytoskeletal pathology in acute brain injury

This study has assessed tau immunoreactivity in the hippocampus and adjacent temporal lobe in cases of fatal TBI with survival times ranging from <24 hours up to one month. In humans, unlike the previously reported animal studies, tau immunoreactivity is a feature of control brains, that is brains with neither clinical nor gross neuropathological abnormalities. The control brains demonstrated perikaryal, neuropil thread and diffuse neuropil tau immunoreactivity. Tau perikaryal and neuropil thread immunoreactivity increased with age, while diffuse tau neuropil immunoreactivity was seen at all ages. Tau glial immunoreactivity, however, did appear to be associated with acute TBI and immunoreactivity could be detected in cases with a survival of <24 hours. There was no demonstrable perikaryal

accumulation of phosphorylated tau after acute TBI in these human studies, a finding reported in the pig model (Smith *et al* 1999). Smith *et al* (1999) used controlled head rotational acceleration to induce injury, resulting in diffuse traumatic axonal pathology. A range of antibodies was used to assess tau pathology (Tau-2, PHF-1, PHF-6, PHF-13.5). These antibodies recognised different epitopes of tau;

Tau-2 recognises 52-68 kDa phosphorylated and non phosphorylated tau. The antibody stains neurofibrillary tangles, neuropil threads and neuritic plaques, astrocytes and ribosomes.

PHF-1 recognises an epitope in phosphorylated tau at position serine 396 in human tau and serine 387 in rodent tau.

PHF-6 and PHF-13.5 have not been fully characterised.

The current study has examined human material derived from cases of fatal TBI due to different causes of primary insults (road traffic accident, fall, assault) producing mixed focal and diffuse pathologies, including diffuse TAI. It is probable that the loading conditions determine the brain response, and that the highly controlled laboratory situation accentuates a specific brain response. The current study used only a single antibody to detect tau (Dako monoclonal antibody); this antibody identifies an epitope in the C-terminus region of human tau which contains the four repeated sequences involved in microtubule binding. This antibody will react with both phosphorylated and non-phosphorylated forms. It may be that a wider panel of antibodies, such as that used by Smith *et al* (1999) may be required to detect specific cytoskeletal abnormalities. There are difficulties in replicating the study by Smith *et al* (1999) in that the antibodies used in that study do not work on formalin-fixed paraffin-embedded human material. In addition, there may be species differences, such that cytoskeletal disruption, if present in man, may develop over a different time

course. Smith *et al* (1999) demonstrated axonal tau immunoreactivity at 3 days post-trauma, and consistently found expression up to 10 days post-trauma (latest time-point examined) in animals. The current study included up to one month post-trauma, but longer survival times may need to be examined to fully assess the possibility of tau expression after TBI.

The study by Newman *et al* (1995), although looking at TBI in man, utilised a different antibody against tau from this study. While the current study utilised an antibody that reacts with both phosphorylated and non-phosphorylated forms of tau, Newman *et al* (1995) used Alz50, an antibody which selectively binds specific conformations of the tau protein (Carmel *et al* 1996). The different antibodies may identify different stages in the temporal *evolution* of neurofibrillary pathology.

The cases of mild TBI reported by Geddes *et al* (1999) were all subjected to repetitive relatively mild blows to the head. These cases clearly differ from the mixed neuropathologies seen in most cases of human TBI, but demonstrate that in this rather specific type of insult TBI is associated with tau pathology. In cases of fatal TBI after a single blow to the head structural cytoskeletal disturbances involving tau may be initiated. The pathological response to human TAI is known to develop over a period of time (Gentleman *et al* 1995), and the cytoskeletal response may be modified by co-existent primary and secondary pathologies. Future studies may have to look at survival times beyond one month to fully assess the role of tau in the response to TBI, and the relationship between a single episode of TBI and the development of Alzheimer-type pathology.

In man, genetic influences may play an important role in modifying the outcome after TBI. Possession of *APO* ε4 is associated with a worse outcome after TBI (Teasdale *et al* 1997), and with an increased severity of chronic neurological

deficits in high-exposure boxers (Jordan *et al* 1997), with high-exposure being defined as participation in at least 12 professional bouts. The role of *APOE* polymorphisms in modifying cytoskeletal responses after TBI was not addressed in the current study but may be an area of future research.

5.2 Association of APOE ε4 and cerebrovascular pathology in traumatic brain injury

Among the pathological features which are present in fatal cases of TBI this study has identified an association between possession of *APOE ε4* and the severity of contusions and a trend for an association with severe ischaemic brain damage. These findings suggest that cerebrovascular and haematological mechanisms may underlie, at least in part, the association of *APOE ε4* with poor outcome after TBI. A recent study, performed in the same institute, of CT scans of survivors of TBI (Liaquat *et al* 2002) showed that although patients with *APOE ε4* were no more likely to have intracranial haemorrhages than-non carriers of *APOE ε4*, if haemorrhages were present then they were of greater volume in those patients with *APOE ε4*. This study, therefore, provides a degree of clinical correlation with our autopsy based work, and suggests that our findings may well be relevant to survivors of TBI.

Although prospective clinical studies of outcome after TBI have identified *APOE ε4* carriers as more likely to fall into poor outcome or poor recovery groups (Teasdale *et al* 1997, Sorbi *et al* 1995), they have not yet specifically addressed the question of whether *APOE ε4* carriers are more likely to have a fatal outcome. The previous clinical studies have looked at the prevalence of ε4 carriers in poor outcome (severe, vegetative, or fatal) after TBI (Teasdale *et al* 1997) or in the vegetative state patients only (Sorbi *et al* 1995). The present study, looking at only the fatal

outcome group, did not find an over-representation of ε4 carriers, the *APOE ε4* carriage rate (35%) being similar to that of all head-injured patients admitted to the same institution (33%, n=984, Teasdale et al, unpublished observations). Therefore, although *APOE ε4*-associated vascular pathology may influence the outcome in survivors it seems unlikely to significantly increase the probability of a fatal outcome after TBI. However, the situation after spontaneous intracerebral haemorrhage appears to be different; there is evidence that among patients with stroke due to spontaneous intracerebral haemorrhage *APOE ε4* carriers are substantially more likely to die in hospital (McCarron *et al* 1999, McCarron *et al* 2003).

These findings point towards an important role for apoE in cerebrovascular and haematological mechanisms which are of relevance in the response to an episode of brain injury. More specifically, existing evidence indicates that apoE may play an important role in relation to both blood vessel wall integrity and coagulation of blood.

One role of apoE is as a lipid transport protein and apoE is therefore involved in the transport of the fat soluble vitamins together with lipids, from the small intestine to the liver. This mechanism is suggested to underlie the relatively low levels of plasma vitamin K in *APOE ε4* carriers (Shearer 1995). Vitamin K is required by the liver for the synthesis of clotting factors and prothrombin times have been reported to vary with *APOE* genotype (Giraud *et al* 1998). Prolonged clotting times were also identified in *APOE ε4* carriers after stroke (Weir *et al* 2001) providing further evidence that *APOE* genotype is of relevance to the coagulation cascade. The possibility that contusions in head-injured patients with *APOE ε4* are more severe as a result of relatively deficient clotting mechanisms provides the basis for a testable hypothesis.

A further mechanism of possible relevance to the findings of this study relates to the increased prevalence of atherosclerosis and cerebral amyloid angiopathy in carriers of *APOE ε4* (Horsburgh *et al* 2000b). Such vascular pathology might pre-date the head injury and promote contusional haemorrhage by increasing vascular fragility and decreasing the capacity for reactive vasoconstriction. Post mortem studies have confirmed the association of *APOE ε4* with cerebral amyloid angiopathy in patients who died from TBI and have suggested that this is associated with increased severity of contusions (Leclercq *et al* 2002).

Animal models, using *APOE* knockout and transgenic mice, have provided further information about apoE mechanisms and the response of the brain to injury (Horsburgh *et al* 2000b). ApoE deficient mice were found to have larger infarcts than wild-type mice (Laskowitz *et al* 1997) and a greater extent of neuronal damage after controlled ischaemia (Horsburgh *et al* 1999b), which can be ameliorated by continuous intracerebral infusion of apoE (Horsburgh *et al* 2000c). Using transgenic mice differences have been demonstrated between the response to ischaemia and excitotoxicity in mice with human *APOEε3* and *APOEε4* genes, such that the *APOEε4* mice have larger lesions (Horsburgh *et al* 2000d, Buttini *et al* 1999) than *APOEε3* mice (Buttini *et al* 2000). These animal studies correlate with the findings of this study that there is a trend for greater ischaemic damage after fatal TBI in individuals who possess *APOEε4*.

Further elucidation of potential vascular and haematological mechanisms which may underlie the role of apoE in response to brain injury could result in the development of new therapeutic interventions which may modify the outcome after TBI.

5.3 Neuroinflammation

This study has shown that there is a significant up-regulation of the neuroinflammatory response as identified with CD 68 immunoreactivity after TBI when compared to non-trauma controls. Within the group of TBI cases a difference was seen between TAI cases and non-TAI cases with both CD 68 and CR3/43 being up-regulated in TAI cases.

After TBI the increase in CD 68 immunoreactivity signifies increased phagocytic activity. This phagocytic activity may be a function of both microglia and macrophages and is a response to damaged tissue. The tissue damage may be diffuse (ischaemia or TAI) or may be focal in response to lesions such as contusions or other focal infarcts. In TAI cases, however, the neuroinflammatory response was not due to phagocytic activity alone. Indeed, the dominant response was increased expression of MHC class II, as assessed by CR3/43 immunoreactivity. The increased neuroinflammatory response was seen within one week of injury and persisted for many months after the injury.

MHC class II molecules are used to present a processed antigen to T-lymphocytes resulting in lymphocyte activation, acting as a form of immunosurveillance. The majority of cells expressing MHC class II in the central nervous system are microglia although increasingly evidence is being generated supporting the view that astrocytes can express these molecules in some situations (Dong and Benveniste 2001). Assessing the resting levels of MHC class II expression in human brains is difficult as even control (non-trauma) brains will have been subjected to some form of agonal event which may have resulted in microglial activation. A further confounding factor is the increasing realisation that inflammation within the CNS can be modified by systemic inflammatory responses (Perry *et al*

2003). Clinical studies of AD patients have demonstrated further impairment of cognitive function after systemic infection that persists for up to 2 months and is associated with elevated serum levels of IL-1β (Holmes *et al* 2003). The cases used as controls in this study were defined as having no neurological disease during life and no significant neuropathology demonstrated at autopsy. However, on review some of these cases died after pneumonia and others had systemic haematological malignancies or inflammatory conditions. It is possible, therefore, that some of the control cases had a significant neuroinflammatory response to systemic disorders such as infection and malignancy. Systemic disorders did not appear to affect the CNS in a predictable fashion such that some cases of systemic haematological malignancy were associated with increased microglial reaction while others were not. Future studies assessing the neuroinflammatory response may need to apply more stringent definitions to their control tissue.

Despite these limitations the age matched control cases used in this study have results which suggest that MHC class II expression is normally low in the human brain and that while there is an age-related increase in MHC class II expression in temporal and hippocampal regions this did not reach statistical significance. These are regions frequently affected in AD and MHC class II expressing microglia have been described in relation to neuritic plaques (Haga *et al* 1989, McGeer *et al* 1988) in AD, a condition predominantly associated with ageing.

Activation of microglial cells can be produced by a variety of mechanisms. In a study of patients with a severe head injury IL-8 was noted to be markedly upregulated acutely, with a x1000 increase in CSF when compared to peripheral blood levels (Kushi *et al* 2003). The authors, therefore, postulated a role of this cytokine in initiating the neuroinflammatory response. S100B activation of microglia and other

glial cells after ischaemia was investigated in S100B transgenic, knockout and wild-type mice (Wainwright *et al* 2004). The transgenic mice showed significantly increased mortality compared with knockout and wild-type mice, and also exhibited greater cerebral injury and volume loss in the ischaemic hemisphere after an 8-day recovery period. The neuroinflammatory response was greatest in the transgenic mice.

Cultured microglial cells have been shown to express alpha 7 nicotinic acetylcholinergic receptors (Shytle *et al* 2004) and acetyl choline and nicotine pre-treatment can inhibit the lipopolysaccharide induced microglial response. An alpha 7 selective nicotinic antagonist can attenuate this inhibitory effect. Therefore, the intrinsic cholinergic system within the CNS may modulate the neuroinflammatory response. As discussed earlier, this system is frequently damaged in severe TBI and may result in loss of inhibition of the neuroinflammatory response. A second receptor system which may modulate the microglial response is the purinergic receptor group P2X. Activation of the ionotrophic $P2X_7$ microglial receptor by extracellular ATP increases diacylglycerol lipase activity and inhibits monoacylglycerol lipase (Witting *et al* 2004). This results in increased levels of 2-arachidonoylglycerol being produced by microglia. This molecule is currently thought to be instrumental in co-ordinating the neuroinflammatory response. 2-arachidonoylglycerol, via cannabinoid receptors, can reduce excitotoxicity damage by reducing glutamate release (Marsicano *et al* 2003), reduce cerebral oedema by reducing cerebral blood flow (Parmentier-Batteur *et al* 2002), and inhibiting the production of neurotoxic agents by microglia (Klegeris *et al* 2003).

As discussed, microglia may act in a phagocytic capacity or as antigen presenting cells (APC). Both of these functions may be beneficial in the response to TBI. In the peripheral nervous system (PNS) macrophages mount a phagocytic

response post-injury. The phagocytosis of damaged tissue is considered to be a prerequisite prior to any attempt at axonal regeneration. Within the CNS microglia perform a phagocytic function but appear to be unable to mount a response sufficient to allow axonal regeneration. The optic nerve crush model in the rat has been used to study microglial responses. After crush injury in both PNS and CNS there is a significant increase in IL-18, a cytokine involved in microglial activation (Menge *et al* 2001). Although the response in the CNS was similar to that seen in the PNS the phagocytic response in the CNS was considered to be insufficient to allow successful CNS regeneration. Other processes have been described which may underlie the difference in axonal recovery between the CNS and PNS. In the adult CNS axonal growth is inhibited by the interaction between myelin-associated proteins, such as the extracellular domain of Nogo-A (Nogo-66), oligodendrocyte myelin glycoprotein (OMgp) and myelin-associated glycoprotein (MAG), and an axonal membrane receptor, Nogo receptor (NgR) (Prinjha *et al* 2000). An additional transducing factor p75, a neurotrophin receptor, is required for this inhibitory effect to be active. p75 knockout mice are no longer responsive to myelin and blocking the p75-NgR interaction also reduces the activities of these inhibitors (Wang *et al* 2002). Similar mechanisms are not found in the peripheral nervous system and may account for the differences between central and peripheral axonal responses to injury. It is currently uncertain how, after injury to the central nervous system, microglial function may interact with this intrinsic inhibitory mechanism such that axonal growth is inhibited. In addition there may be further inhibitory signals from the surrounding microenvironment.

The most pronounced increase in MHC class II expression after TBI was seen in the central white matter regions (corpus callosum and cingulate gyrus) of cases

which were diagnosed pathologically as having diffuse traumatic axonal injury. As expected, given that there would be Wallerian degeneration secondary to axonal disruption there was an increase in phagocytic capacity in these regions, although the phagocytic response was less pronounced than the MHC class II expression. These findings again focus interest on the white matter as a region of great importance in the long-term response to TBI. TUNEL positive cells, both oligodendrocytes (Williams *et al* 2001) and macrophages/microglia (Wilson *et al* 2004), have been detected in the white matter of TBI cases many months after the injury.

One interpretation of these findings is that axonal disruption may continue for many months after the initially forces associated with TBI have been applied and that there is little, if any, axonal recovery; the neuroinflammatory response may contribute to or be secondary to this. There has been speculation in the medical literature for many years that multiple sclerosis can be initiated or exacerbated by trauma (reviewed in Compston *et al* 1998). Multiple sclerosis is presumed to be an auto-immune condition with myelin degradation resulting in axonal degeneration. Inflammation is a prominent feature with both overexpression of microglial MHC class II and an influx of T lymphocytes, with antigen being presented to the infiltrating lymphocytes by MHC class II expressing microglia. Early expression of MHC class II after crush injury in rat optic nerve has been correlated with a less severe injury (Shaked *et al* 2004). Different strains of rats with known variation in response to crush injury were studied. Sprague-Dawley (SPD) rats (a strain relatively resistant to CNS injury) were compared with Lewis rats (a strain susceptible to injury) at different time points post-injury. SPD rats had an early transient activation of MHC class II expression in microglia which was not seen in the Lewis rats. The authors postulate that the less severe injury in the strain with early MHC class II expression is related to T-

lymphocyte responses which have been described to be protective in some instances (Yoles *et al* 2001a,b). Auto-immune T-lymphocyte responses underlie the demyelination associated with MS. While the finding of over-expression of MHC class II after diffuse TAI is of interest in relation to the debate regarding trauma and subsequent initiation/exacerbation of multiple sclerosis it is uncertain if this increased expression does predispose to auto-immune myelin destruction.

Other potential functions of MHC class II up-regulation secondary to brain injury are speculative. Microglia are usually activated prior to astrocyte activation and gliosis although both cellular responses are commonly seen in response to brain injury. Microglia may act to control the astrocyte response and to limit the degree of gliosis (Lindholm *et al* 1992) and may be involved in regulating synaptogenesis (Nagata *et al* 1993). Microglia are important to the long-term re-organisation of neuronal synptic connections after TBI. TBI results in primary neuronal loss (ischaemia, excitotoxicity) with substantial re-organisation of the residual tissue including synaptic sprouting and synaptogenesis. Eyupoglu *et al* (2004) studied microglial roles in synaptogenesis in both *in vivo* entorhinal cortex lesion and complex organotypic entorhino-hippocampal slice cultures. Pharmacological blocking of microglial activation protected neurones from microglial induced secondary dendritic modification and promoted useful re-innervation.

Microglia may also be activated to secrete cytokines which may be neurotoxic. Further studies are required to elucidate if the up-regulation of microglia after TBI is a protective response or if is ultimately harmful to the brain.

5.3.1 Genetic factors influencing the neuroinflammatory response

IL-1A genotypes were present in frequencies consistent with those described in Western European control populations (Rebeck 2000). Therefore, there was no over-

representation of *IL-1A* allele 2 in fatal TBI. In addition possession of allele 2, either one or two copies, was not associated with an increased neuroinflammatory response as assessed by CD 68 and MHC class II expression.

As discussed above *APOE* genotypes may influence the neuroinflammatory response with possession of *APOE*ε4 being associated with an over-exuberant response and possession of *APOE*ε2 being associated with a less pronounced response. ApoE can modulate Aβ induced microglial activation. Studies using cultured rat glia demonstrated that exogenous apoE suppressed the Aβ induced production of inducible nitric oxide synthase and cyclo-oxygenase-2 (Guo *et al* 2004). In the absence of Aβ, however, exogenous apoE could induce IL-1β expression, with apoE4 producing a significantly greater response than apoE3. The authors postulate that while Aβ stimulation of glial apoE limits the neuroinflammatory response, overproduction of apoE by glial cells may ultimately be pro-inflammatory.
This study, however, found no *APOE* genotype related differences in the neuroinflammatory response in the TBI cases. It should be noted that the numbers of cases studied were however small and the power of the study was insufficient to exclude a potential role of *APOE* genotypes in modifying the neuroinflammatory response.

6 Conclusions

This study has highlighted a number of potential mechanisms, both cellular and genetic, which may modify the response of a patient to an episode of TBI and may help to explain the possible association between TBI and AD in later life. Possession of *APOE* ε4, although not over-represented in fatal outcome after TBI, results in more significant vascular based pathology (contusions, global cerebral ischaemia) which may account for the association with a more severe outcome after TBI. However, possession of *IL-1A* allele 2 does not appear to be associated with a greater acute neuroinflammatory response after TBI.

There is an increased neuroinflammatory response after TBI which develops within the first week after TBI and persists for many months. The response is particularly pronounced in the white matter in cases with diffuse TAI, a region in which continuing cell death has been demonstrated up to 12 months after an episode of TBI. Studies looking at long-term survivors of TBI (many years) are under way to assess the time scale of the neuroinflammatory response and to assess any influences that *IL-1A* allele 2 may have in the long-term. In addition studies of the long-term survivors will also look for tau-related pathology. Although the present study did not find any neurofibrillary tangles related to acute TBI (death within one month) it is important to see if Alzheimer-type pathology develops at a younger age in survivors of TBI. In addition it will be of interest to follow the glial-related tau pathology identified in the acute group in survivors of many years.

Neurofibrillary pathology

- Neuronal neurofibrillary tangles were not seen after fatal TBI. The cases examined included survival up to 1 month after TBI. A longer survival period may be required.
- Glial tau inclusions were seen in TBI cases but were not a feature of age-matched controls.

Neuroinflammation

- There was no significant increase in neuroinflammation with ageing in control cases.
- Increased phagocytic activity was seen after TBI.
- There was increased expression of MHC class II and increased phagocytic activity in TAI cases.

Genetic

- *APOE* ε4 possession does not make vascular based pathology more likely after TBI, but if present the lesion is likely to be larger.
- *IL-1A* allele 2 possession does not appear to modify the neuroinflammatory response to TBI.
- *APOE* polymorphisms do not appear to modify the neuroinflammatory response to TBI.

7 Future Studies

This study has addressed a number of points, outlined in the conclusions section, relating to potential shared genetic and protein responses in the response to TBI and AD.

Neurofibrillary tangles were not seen in this study. This study assessed cases with a survival of up to one month only. Currently studies are underway to assess cytoskeletal pathology in individuals who have survived many months to years after an episode of TBI. In addition the neuroinflammatory response is being assessed in these cases to investigate the timescale of the inflammatory response after TBI. This inflammatory response will again be correlated with the genetic polymorphisms discussed in this thesis.

A limitation of the interpretation of the genetic polymorphism findings relates to the number of cases studied. While analysis of the pathological features associated with *APOE*ε4 was based on a large number of cases, the analysis of the role of polymorphisms in the neuroinflammatory response was based on a small number of cases. Therefore there is potential for recruitment of greater numbers of cases to fully investigate the role of genetic polymorphisms in modulating the neuroinflammatory response. As discussed in the material and methods section there is increasing difficulty associated with consent for research using human tissues. This will limit the availability of tissue for study in post mortem based research. A potential solution to the problems of long-term recruitment include multi-centre research with fully consented tissue being recruited from a number of centres. Development of a consented prospective archive could allow genotyping of all cases from blood removed at autopsy, the genotype success rate being considerably higher when

fresh tissue is used rather than formalin fixed paraffin-embedded tissue as used in this study. However, the generation of such a prospective multi-centre trauma archive would require willingness and collaboration with the medicolegal profession, and in particular with the Coroners and Procurators Fiscal, to ensure that such an archive was fully consented.

To supplement any pathological data clinical studies could look at long-term outcome after head injury of variable severity correlated with *IL-1A* and *IL-1B* genotypes. As stated previously autopsy based studies look at only the most severe outcome after head injury, death. By incorporating clinical studies, particularly long-term neuropsychological studies, with pathological studies a clearer picture of the significance of genetic polymorphisms to long-term outcome after TBI may begin to emerge. The pathological studies are beginning to suggest mechanisms that may be important in determining long-term outcome and possibly areas of pharmacological intervention which may improve outcome.

Any future studies looking at the neuroinflammatory response will need to define control material carefully. From the studies undertaken in this thesis it has become clear that the inflammatory response within the CNS can be modified by systemic inflammation/ cytokine release. Therefore control cases may have to take into consideration systemic disease such as pneumonia, haematological malignancy, sepsis etc. as well any neurological disease during life. This will considerably reduce the control material available for studies of neuroinflammation. However, careful selection of age-matched controls should allow for greater accuracy in the assessment of genetic factors involved in the neuroinflammatory response to head injury.

8 References

Adle-Biassette H, Duyckaerts C, Wasowicz M, He Y, Fornes P, Foncin JF, Lecomte D, Hauw JJ (1996) Beta AP deposition and head trauma. *Neurobiol Aging* 17: 415-419.

Adams CWM, Bruton CJ (1989) The cerebral vasculature in dementia pugilistica. *J Neurol Neurosurg Psychiat* 52: 600-604.

Adams JH, Graham DI (1976) The relationship between ventricular fluid pressure and the neuropathology of raised intracranial pressure. *Neuropathol Appl Neurobiol* 2: 323-32.

Adams JH, Graham DI, Scott G, Parker LS, Doyle D (1980) Brain damage in fatal non-missile head injury. *J Clin Path* 33: 1132-45.

Adams JH, Doyle D, Graham DI Lawrence AE, McLellan DR, Gennarelli TA, Pastuszko M, Sakamoto T (1985) The contusion index: A reappraisal in human and experimental non-missile head injury. *Neuropath Appl Neurobiol* 11: 299-308.

Adams JH, Doyle D, Ford I, Gennarelli TA, Graham DI, McLellan DR (1989) Diffuse axonal injury in head injury: definition, diagnosis and grading. *Histopathology* 15: 49-59.

Adams JH, Graham, DI, Jennett B (2000) The neuropathology of the vegetative state after an acute brain insult. *Brain* 123: 1327-1338.

Adams JH, Graham, DI, Jennett B (2001) The structural basis of moderate disability after traumatic brain damage. *J Neurol Neurosurg Psychiatry* 71: 521-524.

Alberts MJ, Graffagnino C, McClenny C, DeLong D, Strittmatter W, Saunders AM, Roses AD (1995) ApoE genotype and survival from intracerebral haemorrhage. *Lancet* 346: 575.

Allan SM, Rothwell NJ (2001) Cytokines and acute neurodegeneration. *Nat Rev Neurosci* 2: 734-44.

Amaducci LA, Fratiglioni L, Rocca WA, Fieschi C, Livrea P, Pedone D, Bracco L, Lippi A, Gandolfo C, Bino G, et al (1986) Risk factors for clinically diagnosed Alzheimer's disease: a case-control study of an Italian population. *Neurology* 36: 922-931.

Aronson MK, Ooi WL, Morgenstern H, Hafner A, Masur D, Crystal H, Frishman WH, Fisher D, Katzman R (1990) Women, myocardial infarction, and dementia in the very old. *Neurology* 40: 1102-1106.

Bal-Price A, Brown GC (2001) Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. *J Neurosci* 21: 6480-6491.

Banati RB, Gehrmann J, Czech C, Monning U, Jones LL, Konig G, Beyreuther K, Kreutzberg GW (1993) Early and rapid de novo synthesis of Alzheimer beta A4-amyloid precursor protein (APP) in activated microglia. *Glia* 9: 199-210.

Barger SW, Harmon AD (1997) Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature* 28: 878-881.

Bertram L, Blacker D, Crystal A, Mullin K, Keeney D, Jones J, Basu S, Yhu S, Guenette S, McInnis M, Go R, Tanzi R (2000) Candidate genes showing no evidence for association or linkage with Alzheimer's disease using family-based methodologies. *Exp Gerontol* 35: 1353-1361.

Biasca N, Simmen HP, Trentz O (1993) Head injuries in ice hockey exemplified by the National Hockey League "Hockey Canada" and European teams. *Unfallchirurg.* 96: 259-264.

Bramlett HM, Dietrich WD, Green EJ, Busto R (1997) Chronic histopathological consequences of fluid-percussion brain injury in rats: effects of post-traumatic hypothermia. *Acta Neuropathol* 93: 190-199.

Brandenburg W, Hallervorden J (1954) Dementia pugilistica mit anatomischem Befund. *Virchow's Arch Path Anat Physiol klin Med* 325: 680-709.

Breteler MM, de Groot RR, van Romunde LK, Hofman A (1995) Risk of dementia in patients with Parkinson's disease, epilepsy and severe head trauma: a register-based follow-up study. *Am J Epidemiol* 142: 1300-1305.

Broe GA, Henderson AS, Creasey H, McCusker E, Korten AE, Jorm AF, Longley W, Anthony JC (1990) A case-control study of Alzheimer's disease in Australia. *Neurology* 40: 1698-1707.

Brooks N, Kupshik G, Wilson L, Galbraith S, Ward R (1987) A neuropsychological study of active amateur boxers. *J Neurol Neurosurg Psychiat* 50: 997-1000.

Bruton CJ (1997) Head injury and dementia. In Esiri MM, Morris JH eds. The neuropathology of dementia. 1st ed. Cambridge University Press, Cambridge; 344-356.

Buttini M, Orth M, Bellosta S, Akeefe H, Pitas RE, Wyss-Coray T, Mucke L, Mahley RW (1999) Expression of human apolipoprotein E3 or E4 in the brains of Apoe-/- mice: isoform-specific effects on neurodegeneration. *J Neurosci* 19: 4867-4880.

Buttini M, Akeefe H, Lin C, Mahley RW, Pitas RE, Wyss-Coray T, Mucke L (2000) Dominant negative effects of apolipoprotein E4 revealed in transgenic models of neurodegenerative disease. *Neuroscience* 97: 207-210.

Buxbaum JD, Oishi M, Chen HI, Pinkas-Kramarski R, Jaffe EA, Gandy SE, Greengard P (1992) Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer βA4 amyloid protein precursor. *Proc Natl Acad Sci USA* 89: 10075-10078.

Capruso DX, Levin HS (2000) Neurobehavioural sequelae of head injury. In Cooper PR, Golfinos JG, editors. *Head Injury*. 4th ed. McGraw-Hill: New York: 525-553.

Cardenas DD, McLean A Jr, Farrell-Roberts L, Baker L, Brooke M, Haselkorn J (1994) Oral physostigmine and impaired memory in adults with brain injury. *Brain Inj* 8: 579-587.

Carmel G, Mager EM, Binder LI, Kuret J (1996) The structural basis of monoclonal antibody Alz50's selectivity for Alzheimer's disease pathology. *J Biol Chem* 271: 32789-32795.

Casson IR, Siegel O Sham R, Campbell EA, Tarlan M, DiDomenico A (1984) Brain damage in modern boxers. *J Am* Med *Ass* 251: 2663-2667.

CDC (1999) Traumatic Brain Injury in the United States: A Report to Congress. www.cdc.gov/ncipc/pub-res/tbi_congress/TBI_in_the_US.PDF

Chandra V, Philipose V, Bell PA, Lazaroff A, Schoenberg BS (1987) Case-control study of late onset "probable Alzheimer's disease". *Neurology* 37: 1295-1300.

Clark RSB, Kochanek PM, Chen M, Watkins SC, Marion DW, Chen J, Hamilton RL, Loeffert JE, Graham SE (1999) Increases in Bcl-2 and cleavage of caspase-1 and caspase-3 in human brain after head injury. *FASEB J* 13: 813-821.

Combarros O, Sanchez-Guerra M, Infante J, Llorca J, Berciano J (2002) Gene dose-dependent association of interleukin 1-A [-889] allele 2 polymorphism with Alzheimer's disease. *J Neurol* 249: 1242-1245.

Compston A, Ebers G, Lassmann H, McDonald I, Matthews B, Wekerle H (1998) McAlpines Multiple Sclerosis, 3rd Ed. Churchill Livingstone: London.

Constantinidis J, Tissot R (1967) Lesions neurofibrillaires D'Alzheimer generalisees sans plaques seniles. *Arch Suis Neurol Nurochir Psychiat* 100: 117-130.

Corkin S, Rosen TJ, Sullivan EV, Clegg RA (1989) Penetrating head injury in young adulthood exacerbates cognitive decline in later years. *J Neurosci* 9: 3876-3883.

Corsellis JAN, Bruton CJ, Freeman-Browne D (1973) The aftermath of boxing. *Psychol Med* 3: 270-303.

Das S, Potter H (1995) Expression of the Alzheimer amyloid-promoting factors α1-antichymotrypsin and apolipoprotein E is induced in astrocytes by IL-1. *Neuron* 14: 447-456.

Davies CA, Loddick SA, Toulmond S, Stroemer RP, Hunt J, Rothwell NJ (1999) The progression and topographic distribution of interleukin-1beta expression after permanent middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab*19:87-98.

Davis JB, McMurray HF, Schubert D (1992) The amyloid beta-protein of Alzheimer's disease is chemotactic for mononuclear phagocytes. *Biochem Biophys Res Commun* 189: 1096-100.

Dewar D, Graham DI (1996) Depletion of choline acetyltransferase activity but preservation of M1and M2 muscarinic receptor binding sites in temporal cortex following head injury: a preliminary human postmortem study. *J Neurotrauma* 13: 181-187.

Di Virgilio F. (1995) The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death. *Immunol Today* 16: 524-8.

Dixon CE, Bao J, Johnson KM, Yang K, Whitson J, Clifton GL, Hayes RL (1995) Basal and scopolamine-evoked release of hippocampal acetylcholine following traumatic brain injury in rats. *Neurosci Lett* 198: 111-114.

Dixon CE, Ma X, Marion DW (1997) Reduced evoked release of acetylcholine in the rodent neocortex following traumatic brain injury. *Brain Res* 749: 127-130.

Dong Y, Benveniste EN (2001) Immune function of astrocytes. *Glia.* 36: 180-190.

Egensperger R, Kosel S, von Eitzen U, Graeber MB (1998) Microglial activation in Alzheimer disease: Association with *APOE* genotype. *Brain Pathology* 8, 439-447.

Engel S, Schluesener H, Mittelbronn M, Seid K, Adjodah D, Wehner HD, Meyermann R. (2000) Dynamics of microglial activation after human traumatic brain injury are revealed by delayed expression of macrophage-related proteins MRP8 and MRP14. *Acta Neuropathologica* 100: 313-322.

Everitt BJ, Robbins TW (1997) Central cholinergic systems and cognition. *Annu Rev Psychol* 48: 649-684.

Eyupoglu IY, Bechmann I, Nitsch R (2003) Modification of microglia function protects from lesion-induced neuronal alterations and promotes sprouting in the hippocampus. *FASEB J* 17:1110-1111.

Fazekas F, Strasser-Fuchs S, Kollegger H, Berger T, Kristoferitsch W, Schmidt H, Enzinger C, Schiefermeier M, Schwarz C, Kornek B, Reindl M, Huber K, Grass R, Wimmer G, Vass K, Pfeiffer KH, Hartung HP, Schmidt R (2001) Apolipoprotein E epsilon 4 is associated with rapid progression of multiple sclerosis. *Neurology* 57: 853-857.

Ferguson FR, Mawdsley C (1965) Chronic encephalopathy in boxers. *8th International Congress of Neurology, Vienna.* Wiener Medizinische Akademie, Vienna, Vol. 1: 81-84.

Ferini-Strambi L, Smirne S, Garancicni P, Pinto P, Franceschi M (1990) Clinical and epidemiological aspects of Alzheimer's disease with presenile onset: a case-control study. *Neuroepidemiology* 9: 39-49.

Ferrari D, Chiozzi P, Falzoni S, Hanau S, DiVirgilio (1997) Purinergic modulation of interleukin-1β release from microglial cells stimulated with bacterial endotoxin. *J Exp Med* 185: 579-582.

Fleminger S, Oliver DL, Lovestone S, Rabe-Hesketh S, Giora A (2003) Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication. *J Neurol Neurosurg Psychiatry* 74: 857-862.

Foster JB, Leiguarda R, Tilley PJ (1976) Brain damage in National Hunt jockeys. *Lancet* 1: 981-983.

Frankowski RF, Annegers JF, Whitman S (1985) Epidemiological and descriptive studies. Part 1: The descriptive epidemiology of head trauma in the United States. In: Becker DP, Povlishock JT (eds) Central nervous system trauma status report- 1985. Bethesda, MD: National Institute of Neurological and Communicative Disorders and Stroke, 1985: 33-43.

Fratiglioni L, Ahlbom A, Viitanen M, Winblad B (1993) Risk factors for late-onset Alzheimer;s disease: a population-based, case-control study. *Ann Neurol* 33: 258-266.

French LR, Schuman LM, Mortimer JA, Hutton JT, Boatman RA, Christians B (1985) A case-control study of dementia of the Alzheimer type. *Am J Epidemiol* 121: 414-421.

Ganter S, Northoff H, Mannel D, Gebicke-Harter PJ (1992) Growth control of cultured microglia. *J Neurosci Res* 33: 218-230.

Geddes JF, Vowles GH, Nicoll JAR, Revesz T (1999) Neuronal cytoskeletal changes are an early consequence of repetitive head injury. *Acta Neuropathol* 98: 171-178.

Geddes JF, Whitwell HL, Graham DI (2000) Traumatic axonal injury: practical issues for diagnosis in medicolegal cases. *Neuropathol Appl Neurobiol* 26: 105-16.

Gennarelli TA (1993) Cerebral concussion and diffuse brain injuries. In Cooper PR (ed) *Head Injury*, 3rd ed. Williams and Wilkins, Baltimore, USA; 137-158.

Gentleman SM, Roberts GW, Gennarelli TA, Maxwell WL, Adams JH, Kerr S, Graham DI (1995) Axonal injury: a universal consequence of fatal closed head injury? *Acta Neuropathol* 89: 537-543.

Gentleman SM, Greenberg BD, Savage MJ, Noori M, Newman SJ, Roberts GW, Griffin WS, Graham DI (1997) A beta 42 is the predominant form of amyloid beta-protein in the brains of short-term survivors of head injury. *Neuroreport* 8: 1519-1522.

Geula C, Mesulam MM (1994) Cholinergic systems and related neuropathological predilection patterns in Alzheimer's disease. In Terry RD, Katzman R, Bick KL eds. *Alzheimer's disease*. New York: Raven Press; 263- 294.

Giraud V, Naveau S, Betoulle D, Abella A, Bardou M, Borotto E, Fumeron F, Chaput JC (1998) Influence of apolipoprotein E polymorphism in alcoholic cirrhosis *Gastroenterol Clin Biol* 22: 571-575.

Goldgaber D, Harris HW, Hla T, Maciag T, Donnelly RG, Jacobsen JS, Vitek MP, Gajdusek DC (1989) Interleukin-1 regulates synthesis of amyloid beta-protein precursor mRNA in human endothelial cells. *Proc Natl Acad Sci USA* 86: 7606-7610.

Graeber MB, Bise K, Mehraein P (1994) CR3/43, a marker for activated human microglia: application to diagnostic neuropathology. *Neuropathol Appl Neurobiol* 20: 406-408.

Graham DI, Lawrence AE, Adams JH, Doyle D, McLellan DR (1988) Brain damage in fatal non-missile head injury without high intracranial pressure. *J Clin Pathol* 41: 34-7.

Graham DI, Ford I, Adams JH, Doyle D, Teasdale GM, Lawrence AE, McLellan DR (1989) Ischaemic brain damage is still common in fatal non-missile head injury. *J Neurol Neurosurg Psychiatry* 52: 346-50.

Graham DI, Adams JH, Nicoll JAR, Maxwell WL, Gennarelli TA, (1995a) The nature, distribution and cause of traumatic brain injury. *Brain Pathology* 5: 397-406.

Graham DI, Gentleman SM, Lynch A, Roberts GW (1995b) Distribution of beta-amyloid protein in the brain following severe head injury. *Neuropathol Appl Neurobiol* 21: 27-34.

Graham DI, McIntosh TK, Maxwell WL, Nicoll JAR (2000) Recent advances in neurotrauma. *J Neuropath Exp Neurol* 59: 641-651.

Grahmann H, Ule G (1957) Beitrag zur Kenntnis der chronischen cerebralen Krankheitsbilder bei Boxern. *Psychiat Neurol* 134: 261-283.

Graves AB, White E, Koepsell TD, Reifler BV, van Belle G, Larson EB, Raskind M (1990) The association between head trauma and Alzheimer's disease. *Am J Epidemiol* 131: 491-501.

Green EK, Harris JM, Lemmon H, Lambert JC, Chartier-Harlin MC, St. Clair D, Mann DMA, Iwatsubo T, Lendon CL (2002) Are interleukin-1 gene polymorphisms risk factors or disease modifiers in AD? *Neurology* 58: 1566-1568.

Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G (1995) Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J Biol Chem* 270: 13757-65.

Griffin WST, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, White CD III, Araoz C (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer's disease. *Proc Natl Acad Sci USA* 86: 7611-7615.

Griffin WST, Sheng JG, Gentleman SM, Graham DI, Mrak RE, Roberts GW (1994) Microglial interleukin-1α expression in human head injury: correlations with neuronal and neuritic β-amyloid precursor protein expression. *Neurosci Lett* 176: 133-136.

Griffin WST, Sheng JG, Royston MC, Gentleman SM, McKenzie JE, Graham DI, Roberts GW, Mrak RE (1998) Glial-neuronal interactions in Alzheimer's disease: the potential role of a "cytokine cycle" in disease progression. *Brain Pathology* 8: 65-72.

Grimaldi LM, Casadei VM, Ferri C, Veglia F, Licastro F, Annoni G, Biunno I, De Bellis G, Sorbi S, Mariani C, Canal N, Griffin WS, Franceschi M (2000) Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism. *Ann Neurol* 47: 361-365.

Guo L, LaDu MJ, Van Eldik LJ (2004) A dual role for apolipoprotein e in neuroinflammation: anti- and pro-inflammatory activity. *J Mol Neurosci* 23:205-212.

Guo Z, Cupples LA, Kurz A, Auerbach SH, Volicer L, Chui H, Green RC, Sadovnick AD, Duara R, DeCarli C, Johnson K, Go RC, Growdon JH, Haines JL, Kukull WA, Farrer LA (2000) Head injury and the risk of Alzheimer's disease in the MIRAGE study. *Neurology* 54: 1316-1323.

Haga S, Akai K, Ishii T (1989) Demonstration of microglial cells in and around senile (neuritic) plaques in the Alzheimer brain. An immunohistochemical study using a novel monoclonal antibody. *Acta Neuropathol* 77: 569-575.

Heales SJ, Bolanos JP, Stewart VC, Brookes PS, Land JM, Clark JB (1999) Nitric oxide, mitochondria and neurological disease. *Biochim Biophys Acta* 1410: 215-228.

Heilbronner RL, Henry GK, Carson-Brewer M (1991) Neuropsychologic test performance in amateur boxers. *Am J Sports Med* 19: 376-380.

Holmes C, El-Okl M, Williams AL, Cunningham C, Wilcockson D, Perry VH (2003) Systemic infection, interleukin 1beta, and cognitive decline in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 74; 788-789.

Holness CL, Simmons DL (1993) Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. *Blood* 81: 1607-13.

Horsburgh K, Graham DI, Stewart J, Nicoll JA (1999a) Influence of apolipoprotein E genotype on neuronal damage and apoE immunoreactivity in human hippocampus following global ischemia. *J Neuropathol Exp Neurol*; 58: 227-34.

Horsburgh K, Kelly S, McCulloch J, Higgins GA, Roses AD, Nicoll JA (1999b) Increased neuronal damage in apolipoprotein E-deficient mice following global ischaemia. *Neuroreport* 10: 837-841.

Horsburgh K, Cole GM, Yang F, Savage MJ, Greenberg BD,Gentleman SM, Graham DI, Nicoll JA (2000a) beta-amyloid (Abeta)42(43), abeta42, abeta40 and apoE immunostaining of plaques in fatal head injury. *Neuropathol Appl Neurobiol* 26: 124-132.

Horsburgh K, McCarron MO, White F, Nicoll JA (2000b) The role of apolipoprotein E in Alzheimer's disease, acute brain injury and cerebrovascular disease: evidence of common mechanisms and utility of animal models. *Neurobiol Aging*; 21: 245-55.

Horsburgh K, McCulloch J, Nilsen M, McCracken E, Large C, Roses AD, Nicoll JA (2000c) Intraventricular infusion of apolipoprotein E ameliorates acute neuronal damage after global cerebral ischemia in mice. *J Cereb Blood Flow Metab* 20: 458-462.

Horsburgh K, McCulloch J, Nilsen M, Roses AD, Nicoll JA (2000d) Increased neuronal damage and apoE immunoreactivity in human apolipoprotein E, E4 isoform-specific, transgenic mice after global cerebral ischaemia. *Eur J Neurosci* 12: 4309-4317.

Jennett B, Bond M (1975) Assessment of outcome after severe brain damage. *Lancet* 1: 480-487.

Jennett B, MacMillan R (1981) Epidemiology of head injury. *BMJ* 282:101-104.

Jennett B, Snoek J, Bond MR (1981) Disability after severe head injury: Observations on the use of the Glasgow Outcome Scale. *J Neurol Neurosurg Psychiatry* 44: 285-293.

Jennett B, Adams JH, Murray LS, Graham DI (2001) Neuropathology in vegetative and severely disabled patients after head injury. *Neurology* 56: 486-490.

Jordan BD, Relkin NR, Ravdin LD, Jacobs AR, Bennett A, Gandy S (1997) Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. *JAMA* 278: 136-140.

Kantarci OH, Hebrink DD, Achenbach SJ, Pittock SJ, Altintas A, Schaefer-Klein JL, Atkinson EJ, De Andrade M, McMurray CT, Rodriguez M, Weinshenker BG (2004) Association of APOE polymorphisms with disease severity in MS is limited to women. *Neurology* 62: 811-814.

Katzman R, Aronson M, Fuld P, Kawas C, Brown T, Morgenstern H, Frishman W, Gidez L, Eder H, Ooi WL (1989) Development of dementing illnesses in an 80 year old volunteer cohort. *Ann Neurol* 25: 317-324.

Kay AD, Petzold A, Kerr M, Keir G, Thompson EJ, Nicoll JA (2003) Cerebrospinal fluid apolipoprotein E concentration decreases after traumatic brain injury. *J Neurotrauma* 20:243-250.

Kennedy I (2001) The Report of the Public Inquiry into children's heart surgery at the Bristol Royal Infirmary 1984- 1995. (www.bristol-inquiry.org.uk/final_report/rpt_print.htm)

Kirkendall DT, Jordan SE, Garrett WE (2001) Heading and head injuries in soccer. *Sports Med* 31: 369-386.

Klegeris A, Bissonnette CJ, McGeer PL (2003) Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. *Br J Pharmacol* 139: 775-786.

Kotapka MJ, Graham DI, Adams JH, Gennarelli TA (1992) Hippocampal pathology in fatal non-missile human head injury. *Acta Neuropathol.* 83: 530-534.

Kushi H, Saito T, Makino K, Hayashi N (2003) IL-8 is a key mediator of neuroinflammation in severe traumatic brain injuries. *Acta Neurochir Suppl* 86: 347-350.

Laskowitz DT, Sheng H, Bart RD, Joyner KA, Roses AD, Warner DS (1997) Apolipoprotein E-deficient mice have increased susceptibility to focal cerebral ischemia. *J Cereb Blood Flow Metab* 17: 753-758.

Launer LJ, Anderson K, Dewey ME, Letenneur L, Ott A, Amaducci LA, Brayne C, Copeland JR, Dartigues JF, Kragh-Sorensen P, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A (1999) Rates and risk factors for dementia and Alzheimer's disease: results from EURODEM incidence research group and work groups. European studies of dementia. *Neurology* 52: 78-84.

Leclercq PD, Murray LS, Graham DI, Smith C, Nicoll JAR, Gentleman SM (2002) Cerebral amyloid angiopathy and traumatic brain injury: an association with apolipoprotein E genotype. *Neurobiol Aging* 23: S407.

Li G, Shen YC, Li YT, Chen CH, Zhau YW, Silverman JM (1992) A case-control study of Alzheimer's disease in China. *Neurology* 42: 1481-1488.

Li Y, Liu L, Barger SW, Griffin WS (2003) Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-MAPK pathway. *J Neurosci* 23: 1605-1611.

Liaquat I, Dunn LT, Nicoll JA, Teasdale GM, Norrie JD (2002) Effect of apolipoprotein E genotype on hematoma volume after trauma. *J Neurosurg* 96: 90-96.

Lindholm D, Castren E, Kiefer R, Zafra F, Thoenen H (1992) Transforming growth factor-beta 1 in the rat brain: increase after injury and inhibition of astrocyte proliferation. *J Cell Biol* 117: 395-400.

Luce T (2003) Death certification and investigation in England, Wales and Northern Ireland. The report of a fundemental review 2003. (www.official-documents.co.uk/document/cm58/5831/5831.pdf)

Lynch JR, Tang W, Wang H, Vitek MP, Bennett ER, Sullivan PM, Warner DS, Laskowitz DT (2003) APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* 278: 48529-48533.

McCarron MO, Muir KW, Weir CJ, Dyker AG, Bone I, Nicoll JAR, Lees KR (1998). The apolipoprotein E ε4 allele and outcome in cerebral vascular disease. *Stroke* 29, 1882-1887.

McCarron MO, Hoffmann KL, DeLong DM, Gray L, Saunders AM, Alberts MJ (1999) Intracerebral haemorrhage outcome: apolipoprotein E genotype, hematoma, and edema volumes. *Neurology* 53: 2176-2179.

McCarron MO, Weir CJ, Muir KW, Hoffmann KL, Graffagnino C, Nicoll JA, Lees KR, Alberts MJ (2003) Effect of apolipoprotein E genotype on in-hospital mortality following intracerebral haemorrhage. *Acta Neurol Scand* 107: 106-109.

MacFarlane DP, Nicoll JAR, Smith C, Graham DI (1999) APOE epsilon 4 allele and amyloid beta-protein deposition in long term survivors of head injury. *Neuroreport* 10: 3945-3948.

McGeer PL, Itagaki S, Boyes BE, McGeer EG (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* 38: 1285-1291.

McGeer PL, McGeer E, Rogers J, Sibley J (1990) Anti-inflammatory drugs and Alzheimer disease. *Lancet* 335: 1037.

McGeer PL, Schulzer M, McGeer EG (1996) Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology* 47: 425-432.

McIntosh AS, McCrory P, Comerford J (2000) The dynamics of concussive head impacts in rugby and Australian rules football. *Med Sci Sports Exerc* 32: 1980-1984.

McLatchie G, Brooks N, Galbraith S, Hutchison JSF, Wilson L, Melville I, Teasdale E (1987) Clinical neurological examination, neuropsychology, electroencephalography and computed tomographic head scanning in active amateur boxers. *J Neurol Neurosurg Psychiat* 50: 96-99.

McLean S (2001) Independent review group on retention of organs at post mortem. Final Report. (www.show.scot.nhs.uk/sehd/scotorgrev/Final%20Report/ropm-09.htm)

McMillan R, Strang I, Jennett B (1979) Head injuries in primary surgical wards in Scottish hospitals. Scottish head injury management study. *Health Bull* 37: 75-81.

Maroon JC, Lovell MR, Norwig J, Podell K, Powell JW, Hartl R (2000) Cerebral concussion in athletes: evaluation and neuropsychological testing. *Neurosurgery* 47: 659-669.

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutierrez SO, van der Stelt M, Lopez-Rodriguez ML, Casanova E, Schutz G, Zieglgansberger W, Di Marzo V, Behl C, Lutz B (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302: 84-88.

Martland HS (1928) Punch drunk. *J Am Med Ass* 91: 1103-1107.

Matser EJ, Kessels AG, Lezak MD, Jordan BD, Troost J (1999) Neuropsychological impairment in amateur soccer players. *JAMA* 282: 971-973.

Mawdsley C, Ferguson FR (1963) Neurological disease in boxers. *Lancet* 2 : 795-801.

Mayeux R, Ottman R, Tang MX, Noboa-Bauza L, Marder K, Gurland B, Stern Y (1993) Genetic susceptability and head injury as risk factors for Alzheimer's disease among community-dwelling elderly persons and their first degree relatives. *Ann Neurol* 33: 494-501.

Mehta KM, Ott A, Kalmijn S, Slooter AJ, van Duijn CM, Hofman A, Breteler MM (1999) Head trauma and the risk of dementia and Alzheimer's disease: The Rotterdam study. *Neurology* 53: 1959-1962.

Mendez MF, Underwood KL, Zander BA, Mastri AR, Sung JH, Frey WH (1992) Risk factors in Alzheimer's disease: a clinicopathological study. *Neurology* 42: 770-775.

Menge T, Jander S, Stoll G (2001) Induction of the proinflammatory cytokine interleukin-18 by axonal injury. *J Neurosci Res* 65: 332-339.

Metters JS (2003) Isaacs Report. The investigation of events that followed the death of Cyril Mark Isaacs. (www.doh.gov.uk/cmo/isaacsreport/index.htm)

Millar K, Nicoll JA, Thornhill S, Murray GD, Teasdale GM (2003) Long term neuropsychological outcome after head injury: relation to APOE genotype. *J Neurol Neurosurg Psychiatry* 74: 1047-1052.

Millspaugh JA (1937) Dementia pugilistica. *US Nav Med Bull* 35: 297-303.

Morioka T, Kalehua AN, Streit WJ (1991) The microglial reaction in the rat dorsal hippocampus following transient forebrain ischemia. *J Cereb Blood Flow Metab* 11: 966-73.

Mortimer JA, French LR, Hutton JT, Schuman LM (1985) Head injury as a risk factor for Alzheimer's disease. *Neurology* 35: 264-267.

Mortimer JA, van Duijn CM, Chandra V, Fratiglioni L, Graves AB, Heyman A, Jorm AF, Kokmen E, Kondo K, Rocca WA (1991) Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM risk factors research group. *Int J Epidemiol* 20 Suppl 2: S28-35.

Mrak RE, Sheng JG, Griffin WST (1996) Correlation of astrocytic S100β expression with dystrophic neurites in amyloid plaques of Alzheimer's disease. *J Neuropathol Exp Neurol* 55: 273-279.

Mrak RE, Griffin WST (2001) Interleukin-1, neuroinflammation, and Alzheimer's disease. *Neurobiol Aging* 22: 903-908.

Murdoch I, Perry EK, Court JA, Graham DI, Dewar D (1998) Cortical cholinergic dysfunction after human head injury. *J Neurotrauma* 15: 295-305.

Murdoch I, Nicoll JAR, Graham DI, Dewar D (2002) Nucleus basalis of Meynert pathology in the human brain after fatal head injury. *J Neurotrauma* 19: 279-284.

Murelius O, Haglund Y (1991) Does Swedish amateur boxing lead to chronic brain damage? A retrospective neuropsychological study. *Acta Neurol Scand* 83: 9-13.

Murray GD, Teardale GM, Braakman R, Cohadon F, Dearden M, Iannotti F, Karimi A, Lapierre F, Maas A, Ohman J, Persson L, Servadei F, Stocchetti N, Trojanowski T, Unterberg A (1999) The European Brain Injury Consortium Survey of Head Injuries. *Acta Neurochirg.* 141: 223-236.

Nagata K, Takei N, Nakajima K, Saito H, Kohsaka S (1993) Microglial conditioned medium promotes survival and development of cultured mesencephalic neurons from embryonic rat brain. *J Neurosci Res* 34 : 357-363.

Nemetz PN, Leibson C, Naessens JM, Beard M, Kokmen E, Annegers JF, Kurland LT (1999) Traumatic brain injury and time to onset of Alzheimer's disease: a population-based study. *Am J Epidemiol* 149: 32-40.

Newman SJ, Gentleman SM, Graham DI, Brown F, Roberts GW (1995) Tissue distribution and cellular localisation of hyperphosphorylated tau in human head injury and age-matched controls. *In Research Advances in Alzheimer's Disease and Related Disorders* Eds. K Iqbal, JA Mortimer, B Winblad, HM Wisniewski. Wiley, Chichester. 397-403.

Nicoll JA, Roberts GW, Graham DI (1995) Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury. *Nat Med* 1: 135-137.

Nicoll JA, Mrak RE, Graham DI, Stewart J, Wilcock G, MacGowan S, Esiri MM, Murray LS, Dewar D, Love S, Moss T, Griffin WS. (2000) Associations of Interleukin-1 gene polymorphisms with Alzheimer`s disease. *Ann Neurol* 47: 365-368.

O'Meara ES, Kukull WA, Sheppard L, Bowen JD, McCormick WC, Teri L, Pfanschmidt M, Thompson JD, Schellenberg GD, Larson EB (1997) Head injury and the risk of Alzheimer's disease by apolipoprotein E genotype. *Am J Epidemiol* 146: 373-384.

Palmer MS (1995) Ploymerase chain reaction. In, Roberts GW, Polak JM eds, *Molecular Neuropathology*. Cambridge University Press, Cambridge. 22-37.

Parmentier-Batteur S, Jin K, Mao XO, Xie L, Greenberg DA (2002) Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. *J Neurosci* 22: 9771-9775.

Perry VH, Newman TA, Cunningham C (2003) The impact of systemic infection on th eprogression of neurodegenerative disease. *Nat rev Neurosci* 4; 103-112.

Pierce JE, Smith DH, Trojanowski JQ, McIntosh TK (1998) Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience* 87:359-369.

Plassman BL, Havlik RJ, Steffens DC, Helms MJ, Newman TN, Drosdick D, Phillips C, Gau BA, Welsh-Bohmer KA, Burke JR, Guralnik JM, Breitner JCS (2000) Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology* 55: 1158-1166.

Prinjha R, Moore SE, Vinson M, Blake S, Morrow R, Christie G, Michalovich D, Simmons DL, Walsh FS (2000) Inhibitor of neurite outgrowth in humans. *Nature* 403: 383-384.

Raghupathi R, Graham DI, McIntosh TK (2000) Apoptosis after traumatic brain injury. *J Neurotrauma* 17: 927-938.

Rasmusson DX, Brandt J, Martin DB, Folstein MF (1995) Head injury as a risk factor in Alzheimer's disease. *Brain Inj* 9: 213-219.

Rebeck GW (2000) Confirmation of the genetic association of interleukin-!A with early sporadic Alzheimer's disease. *Neuroscience Letters* 293: 75-77.

Redfern M (2003) The Royal Liverpool Children's Inquiry Report. (www.rlcinquiry.org.uk/download/index.htm)

Rezaie P, Male D (2002) Differentiation, ramification and distribution of microglia within the central nervous system examined. *Neuro-embryology* 1: 29-43.

Rink AD, Fung KM, Trojanowski JQ, Lee VM-Y, Neugebauer E, McIntosh TK (1995) Evidence of apoptotic cell death after experimental traumatic brain injury in the rat. *Am J Path* 147: 1575-1583.

Roberts AH (1969) *Brain Damage in Boxers. A study of prevalence of traumatic encephalopathy among ex-professional boxers.* Pitman, London.

Roberts GW, Gentleman SM, Lynch A, Graham DI (1991) β-A4 amyloid protein deposition in brain after head trauma. *Lancet*, 338: 1422-1423.

Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI (1994) β- amyloid protein deposition in the brain following severe head injury: implications for the pathogenesis of Alzheimer's disease. *J Neurol Neurosurg Psychiat* 57: 419-425.

Roe SY, McGowan EM, Rothwell NJ (1998) Evidence for the involvement of corticotrophin-releasing hormone in the pathogenesis of traumatic brain injury. *Eur J Neurosci* 10: 553-9.

Roses AD, Saunders A (1995) Head injury, amyloid beta and Alzheimer's disease. *Nat Med* 1:603-604.

Ross DT, Graham DI, Adams JH (1993) Selective loss of neurons from the thalamic reticular nucleus following severe human head injury. *J Neurotrauma* 10: 151-165.

Rothwell NJ, Luheshi GN (2000) Interleukin 1 in the brain: biology, pathology and therapeutic target. *Trends Neurosci* 23: 618-25.

Royo NC, Schouten JW, Fulp CT, Shimizu S, Marklund N, Graham DI, McIntosh TK (2003) From cell death to neuronal regeneration: building a new brain after traumatic brain injury. *J Neuropathol Exp Neurol* 62: 801-811.

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230: 1350-1354.

Salib E, Hillier V (1997) Head injury and the risk of Alzheimer's disease: a case-control study. *Int J Geriatr Psychiatry* 12: 363-368.

Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 43, 1467-72.

Savettieri G, Andreoli V, Bonavita S, Cittadella R, Caltagirone C, Fazio MC, Girlanda P, Le Pira F, Liguori M, Logroscino G, Lugaresi A, Nocentini U, Reggio A, Salemi G, Serra P, Tedeschi G, Toma L, Trojano M, Valentino P, Quattrone A (2003) Apolipoprotein E genotype does not influence the progression of multiple sclerosis. *J Neurol* 250: 1094-1098.

Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak Vance MA, Goldgaber D, Roses AD (1993) Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci* 90: 9649-53.

Schmidt ML, Zhukareva V, Newell KL, Lee, VM-Y, Trojanowski JQ (2001) Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. *Acta Neuropathol* 101: 518-524.

Schmidt RH, Grady MS (1995) Loss of forebrain cholinergic neurons following fluid-percussion injury: implications for cognitive impairment in closed head injury. *J Neurosurg* 83: 496-502.

Schmidt S, Barcellos LF, DeSombre K, Rimmler JB, Lincoln RR, Bucher P, Saunders AM, Lai E, Martin ER, Vance JM, Oksenberg JR, Hauser SL, Pericak-Vance MA, Haines JL; Multiple Sclerosis Genetics Group (2002) Association of polymorphisms in the apolipoprotein E region with susceptibility to and progression of multiple sclerosis. *Am J Hum Genet* 70: 708-717.

Schofield PW, Tang M, Marder K, Bell K, Dooneief G, Chun M, Sano M, Stern Y, Mayeux R (1997) Alzheimer's disease after remote head injury: an incidence study. *J Neurol Neurosurg Psychiat* 62: 119-124.

Serou MJ, DeCoster MA, Bazan NG (1999) Interleukin-1 beta activates expression of cyclooxygenase-2 and inducible nitric oxide synthase in primary hippocampal neuronal culture: platelet-activating factor as a preferential mediator of cyclooxygenase-2 expression. *J Neurosci Res* 58: 593-598.

Shaked I, Porat Z, Gersner R, Kipnis J, Schwartz M (2004) Early activation of microglia as antigen-presenting cells correlates with T cell-mediated protection and repair of the injured central nervous system. *J Neuroimmunol* 146: 84-93.

Shaw K, MacKinnon M-A, Raghupathi R, Saatman KE, McIntosh TK, Graham DI (2001) TUNEL-positive staining in white and grey matter after fatal head injury in man. *Clin Neuropathol* 20: 106-112.

Shearer MJ (1995) Vitamin K. *Lancet* 345: 229-234.

Sheng JG, Mrak RE, Griffin WST (1997) Glial-neuronal interactions in Alzheimer's disease: progressive association of IL-1α+ microglia and S100β+ astrocytes with neurofibrillary tangle stages. *J Neuropathol Exp Neurol* 56: 285-290.

Sheng JG, Zhu SG, Griffin WST, Mrak RE (2000) Interleuki-1 promotes expression and phosphorylation of tau protein in vivo. *Exp Neurol* 163: 388-391.

Shore VG, Shore B (1973) Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry*; 12: 502-507.

Shytle RD, Mori T, Townsend K, Vendrame M, Sun N, Zeng J, Ehrhart J, Silver AA, Sanberg PR, Tan J (2004) Cholinergic modulation of microglial activation by alpha 7 nicotinic receptors. *J Neurochem* 89: 337-343.

Smith DH, Chen XH, Pierce JE, Wolf JA, Trojanowski JQ, Graham DI, McIntosh TK (1997) Progressive atrophy and neuron death for one year following brain trauma in the rat. *J Neurotrauma* 14: 715-727.

Smith DH, Chen XH, Nonaka M, Trojanowski JQ, Lee VM, Saatman KE, Leoni MJ, Xu BN, Wolf JA, Meaney DF (1999) Accumulation of amyloid beta and tau and the formation of neurofilament inclusions following diffuse brain injury in the pig. *J Neuropath Exp Neurol* 58: 982-992.

Smith DH, Chen XH, Iwata A, Graham DI (2003) Amyloid beta accumulation in axons after traumatic brain injury in humans. *J Neurosurg* 98: 1072-1077.

Smith FM, Raghupathi R, MacKinnon M-A,Saatman KE, McIntosh TK, Meaney DF, Graham DI (2000) TUNEL-positive staining of surface contusions after fatal head injury in man. *Acta Neuropathol* 100: 537-545.

Sorbi S, Nacmias N, Piacentini S, Repice A, Latorraca S, Forleo P, Amaducci L (1995) ApoE as a prognostic factor for post-traumatic coma. *Nat Med* 1:852.

Streit WJ, Kreutzberg GW (1988) Response of endogenous glial cells to motor neuron degeneration induced by toxic ricin. *J Comp Neurol* 268: 248-63.

Streit WJ, Graeber MB, Kreutzberg GW (1989) Peripheral nerve lesion produces increased levels of major histocompatibility complex antigens in the central nervous system. *J Neuroimmunol* 21: 117-23.

Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D, Roses AD (1993) Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci* 90: 8098-102.

Teasdale G, Jennett B (1974) Assessment of coma and impaired consciousness: A practical scale. *Lancet* 2: 81-84.

Teasdale G, Jennett B (1976) Assessment and prognosis of coma after head injury. *Acta Neurochir* 34: 45-55.

Teasdale GM, Nicoll JA, Murray G, Fiddes M (1997) Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* 350, 1069-1071.

Teasdale GM, Pettigrew LE, Wilson JT, Murray G, Jennett B (1998) Analyzing outcome of treatment of severe head injury: a review and update on advancing the use of the Glasgow Outcome Scale. *J Neurotrauma* 15: 587-597.

Thornhill S, Teasdale GM, Murray GD, McEwen J, Roy CW, Penny, KI (2000) Disability in young people and adults one year after head injury: prospective cohort study. *BMJ* 320:1631-1635.

Uchihara T, Duyckaerts C, He Y, Kobayashi K, Seilhean D, Amouyel P, Hauw JJ (1995) ApoE immunoreactivity and microglial cells in Alzheimer's disease brain. *Neurosci Lett* 195: 5-8.

van Duijn CM, Tanja TA, Haaxma R, Schulte W, Saan RJ, Lameris AJ, Antonides-Hendriks G, Hofman A (1992) Head trauma and the risk of Alzheimer's disease. *Am J Epidemiol* 135: 775-782.

Vollmer DG (1993) Prognosis and outcome of severe head injury. . In, Cooper PR ed, *Head Injury 3rd Edition*. Williams and Wilkins, Baltimore. 553-581.

Wainwright MS, Craft JM, Griffin WS, Marks A, Pineda J, Padgett KR, Van Eldik LJ (2004) Increased susceptibility of S100B transgenic mice to perinatal hypoxia-ischemia. *Ann Neurol* 56: 61-67.

Wang KC, Kim JA, Sivasankaran R, Segal R, He Z (2002) p75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature* 420: 74-78.

Weir CJ, McCarron MO, Muir KW, Dyker AG, Bone I, Lees KR, Nicoll JA (2001) Apolipoprotein E genotype, coagulation, and survival following acute stroke. *Neurology* 57: 1097-1100.

Weller RO, Nicoll JA (2003) Cerebral amyloid angiopathy: pathogenesis and effects on the ageing and Alzheimer brain. *Neurol Res* 25: 611-616.

Williams DB, Annegers JF, Kokmen E, O'Brien PC, Kurland LT (1991) Brain injury and neurologic sequelae: a cohort study of dementia, parkinsonism, and amyotrophic lateral sclerosis. *Neurology* 41: 1554-1557.

Williams S, Raghupathi R, MacKinnon M-A, McIntosh TK, Saatman KE, Graham DI (2001) In-situ DNA fragmentation occurs in white matter up to 12 months after head injury in man. *Acta Neuropathol* 102: 581-590.

Wilson S, Raghupathi R, Saatman KE, MacKinnon MA, McIntosh TK, Graham DI (2004) Continued in situ DNA fragmentation of microglia/macrophages in white matter weeks and months after traumatic brain injury. *J Neurotrauma* 21: 239-250.

Witting A, Walter L, Wacker J, Moller T, Stella N (2004) P2X7 receptors control 2-arachidonoylglycerol production by microglial cells. *Proc Natl Acad Sci U S A* 101: 3214-3219.

Wyatt J, Beard D, Gray A, Busuttil A, Robertson C (1995) The time of death after trauma. *BMJ* 310: 1502

Yoles E, Hauben E, Palgi O, Agranov E, Gothilf A, Cohen A, Kuchroo V, Cohen IR, Weiner H, Schwartz M (2001) Protective autoimmunity is a physiological response to CNS trauma. *J Neurosci* 21: 3740-3748.

Zemlan FP, Rosenberg WS, Luebbe PA, Campbell TA, Dean GE, Weiner NE, Cohen JA, Rudick RA, Woo D (1999) Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins. *J Neurochem* 72: 741-750.

Zhang J, Dawson VL, Dawson TM, Snyder SH (1994) Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science* 263: 687-689.

9 Appendices

9.1 Appendix 1

Study Proforma

Study No. ☐☐☐☐

BRAIN DAMAGE IN NON-MISSILE HEAD INJURY

University Department of Neuropathology,
Institute of Neurological Sciences,
Southern General Hospital,
Glasgow, G51 4TF.

Age _____

1.Retrospective Study Number

☐☐☐☐☐

Not applicable : 99999

2.PM Number ☐☐☐☐☐☐

3.Institute

No : 1
Yes : 2 ☐

4.Institute Number

Not applicable : 9999999 ☐☐☐☐☐☐☐

5.Histological study

No : 1
Limited : 2
Comprehensive : 3 ☐

6.Pathologist (If not PM then brain cut)

DIG : 1
JARN : 2
Other : 3 ☐

7.Survival ☐☐

≤24 hours : 1
≤48 hours : 2
≤72 hours : 3
≤7 days : 4
≤14 days : 5
≤28 days : 6
≤3 months : 7
≤6 months : 8
≤12 months : 9
>12 months : 10 (specify)
Not known : 99

8.If survival <24 hours, specify hours

Instantaneous death : 00

not known/not applicable : 99

9.Fracture of skull

No : 1
Yes : 2
Not known : 9

10.Contusion Index

None : 1
Mild (<20) : 2
Moderate (20-37) : 3
Severe (>37) : 4
Not known : 9

11.Total Contusion Index (TCI)

None : 00
Not known : 99

12.Diffuse hypoxic brain damage

Absent : 1
Present : 2
Not known: 9

(global hypoxia/ischaemia)

	L	R
Cerebral hemisphere		
Cerebellum		

13.Intracranial Haematoma

None : 1
≤2 cm diameter : 2
>2 cm diameter : 3
Present, size unknown: 4
Removed at operation : 5
Not known : 9

	L	R
Supratentorial-Extradural		
Subdural		

	Subarachnoid	☐	☐
Intracerebral	- Frontal	☐	☐
	- Temporal	☐	☐
	- Parietal	☐	☐
	- Occipital	☐	☐
	- Basal gangli	☐	☐
Burst lobe	-Frontal	☐	☐
	-Temporal	☐	☐
	-Parietal	☐	☐
	-Occipital	☐	☐
Infratentorial-	Extradural	☐	☐
	Subdural	☐	☐
	Intracerebellar	☐	☐
	Burst lobe	☐	☐

14.Raised Intracranial Pressure

Absent : 1
Macroscopic : 2
Macro plus micro: 3
Microscopic only: 4
Not known : 9

	L	R
Supracallosal hernia	☐	☐
Tentorial hernia	☐	☐
Tonsillar hernia	☐	☐
Oculomotor nerve lesion	☐	☐
Kernohan lesion	☐	☐
ACA infarction	☐	☐
MCA infarction	☐	☐
PCA infarction	☐	☐
PICA infarction	☐	☐
SCA infarction	☐	☐
Other infarction (specify)	☐	☐
Secondary brainstem haemorrhage/ infarction	☐	☐

15.Infarction in the absence of raised intracranial pressure

Absent : 1
Arterial : 2
Venous : 3

	L	R
Cerebral hemisphere	☐	☐

Cerebellum ☐ ☐

Brainstem ☐ ☐

16.Boundary zone infarction

Absent : 1 Not known : 9
Present : 2

	L	R
Cerebral hemisphere	☐	☐
Cerebellum	☐	☐

17.Other hypoxic damage

Absent : 1
Sulcal : 2
Other : 3
Not known : 9

	L	R
Cerebral hemisphere	☐	☐
Cerebellum	☐	☐

18.Hypoxic brain damage in other sites

Absent : 1
Focal : 2
Diffuse : 3
Not known : 9

	L	R
Hippocampus	☐	☐
Thalamus	☐	☐

19.Severity of hypoxic brain damage

Absent : 1

Mild(<5 small lesions): 2

Moderate(5-10 small : 3

subcortical lesions, BZ, some

AT, and BZ)

Severe(diffuse, multifocal,

AT, AT and BZ) : 4

Not known : 9

☐

AT = arterial territory BZ = boundary zone

20.Diffuse axonal injury

Absent : 1

DAI grade 1 : 2

DAI grade 2m : 3

DAI grade 2M : 4

DAI grade 3m : 5

DAI grade 3M : 6

21.Acute vascular injury

Absent : 1
Present : 2
Not known : 9

22.Brain swelling

L R

Absent :1

Related to contusions : 2

Related to intracranial haematoma : 3

Related to hypoxic brain damage : 4

Related to combination : 5

Other : 6

23.Internal Carotid Arteries

Normal : 1

Stenosis<50% : 2

Stenosis>50% : 3

Thrombosis : 4

Dissection : 5

Other(specify) : 6

Not known : 9

24.Cerebral fat embolism

No : 1 Yes : 2 Not known : 9

25.Intracranial infection

No : 1 Yes : 2 Not known : 9

26.Pathologists assessment of the Cause of Death

Primary brain damage ☐

Expanding intracranial lesion ☐

Other intracranial complications ☐

Extracranial complications ☐

Allocate a total of 5 points between causes

9.2 Appendix 2

Database

1987

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
870006	Yes	2,4	1,2	1,1	45	M	13 days	Fall	-	BSDH	-	-	Severe	L	+
870070	Yes	4,4	2,2	1,2	46	M	10 days	RTA	+	RSDH	Moderate	1	-	-	+
870182	Yes	3,3	1,1	1,2	48	M	3 weeks	Fall	-	LSDH	Mild	-	Severe	Bilateral	+
870257	Yes	3,4	1,2	1,1	18	M	3 days	Ass.	-	RSDH	Mild	-	Severe	R	+
870258	Yes	3,3	2,2	1,2	16	F	10 hours	RTA	+	-	Mild	-	Moderate	Bilateral	+
870288	Yes	3,3	1,1	1,1	18	M	4 days	RTA	-	LICH	Mild	3m	Mild	-	+
870296	Yes	3,4	1,1	1,1	24	M	4 days	Fall	+	-	Mild	-	Mild	Bilateral	+
870333	Yes	2,4	1,2		31	M	36 hours	RTA	+	BSDH BICH	Mild	3M	Severe	Bilateral	+
870391	Yes	3,4		1,2	20	M	5 days	Ass.	+	LICH	Moderate	-	Severe	Bilateral	+
870417	Yes	2,3	1,2	1,2	47	M	12 hours	Fall	+	-	Moderate	-	Moderate	Bilateral	+
870427	Yes	3,4	1,1	1,1	0.58	M	6 hours	Fall	+	-	-	-	-	-	-
870439	Yes	2,2	1,1	1,1	5	M	13 hours	RTA	+	-	Mild	-	Moderate	Bilateral	+
870440	Yes	3,3	1,2	1,2	48	M	11 days	Fall	+	LSDH	Moderate	-	Severe	-	+
870443	Yes	2,3	1,1	1,1	47	F	2 days	RTA	+	RICH	Moderate	-	Mild	Bilateral	+
870444	Yes	3,3	1,2	1,2	69	F	20 hours	RTA	+	BSDH	Mild	-	Mild	-	+
870478	Yes	3,3	1,2	1,1	56	F	3 days	Fall	+	RSDH RICH	Mild	-	Moderate	Bilateral	+
870485	Yes	3,4	1,1	1,1	20	M	31 hours	Ass.	+	LSDH	Mild	-	Severe	-	+
870526	Yes	2,2	1,1	1,1	26	M	50 hours	Fall	+	RSDH	Moderate	-	-	Bilateral	+
870688	Yes	3,4	1,1	1,1	0.15	F	4 hours	RTA	-	RICH LICH	-	-	-	-	-
870694	Yes	3,4	1,1	1,2	25	M	24 hours	RTA	+	RSDH RICH	Mild	-	Severe	R	+
870696	Yes	3,3	1,1	1,1	52	F	4 days	RTA	+	RSDH	Mild	-	Moderate	Bilateral	+
870762	Yes	2,3	1,2	1,1	18	M	54 hours	Ass.	-	LSDH	-	-	Severe	-	+
870778	Yes	3,3	1,2	1,1	22	F	13 days	RTA	-	RICH LICH	-	-	Severe	Bilateral	+

870788	Yes	3,4	1,1	1,2	47	M	36 hours	RTA	+	RSDH	Mild	3M	Mild	-	-
870814	Yes	3,4	1,1	1,1	75	M	16 hours	Fall	+	BSDH LICH	Mild	-	-	L	+
870825	Yes	3,3	1,1	1,1	51	F	3 days	Fall	+	BEDH BSDH	Mild	-	Severe	-	+

1988

PM No.	INS Case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
880054	Yes	4,4	1,2	1,2	65	M	27 hours	Fall	+	BSDH	Moderate	-	Severe	Bilateral	+
880364	Yes	3,3	2,2	2,2	20	M	5 days	RTA	+	RICH	Mild	3M	Moderate	R	+
880413	Yes	3,3	1,1	2,2	11	M	24 hours	RTA	+	BSDH LICH	Moderate	-	Mild	-	-
880415	Yes	2,4	1,1	1,1	46	F	10 days	Fall	+	BSDH	Mild	-	Severe	Bilateral	+
880431	No	3,3	1,2	1,2	33	F	12 hours	RTA	-	BSDH LICH	-	-	Severe	L	+
880433	Yes	3,4	1,2	1,1	42	M	60 hours	RTA	-	LSDH	Mild	-	Severe	Bilateral	+
880489	Yes	3,3	1,2		23	M	8 hours	RTA	+	LICH	Mild	3M	Mild	-	+
880531	Yes	3,3	1,2	1,1	17	M	7 days	RTA	+	BICH	Moderate	3M	Moderate	Bilateral	+
880592	Yes	3,3	1,1	2,2	29	M	12 days	Ass.	-	-	-	-	Severe	-	+
880719	Yes	3,4	2,2	1,1	46	M	19 hours	Fall	+	REDH LSDH LICH	Moderate	-	Severe	-	+

1989

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
890098	Yes	3,4	1,1	1,2	73	M	30 hours	Fall	+	LSDH BICH	Moderate	-	Mild	Bilateral	+
890152	Yes	3,3	1,2	1,1	26	M	24 hours	Fall	+	REDH	Mild	-	Severe	Bilateral	+
890215	Yes	3,3	2,2	1,1	36	F	6 hours	RTA	+	RSDH RICH	Moderate	3M	-	Bilateral	-
890243	Yes	2,3	2,2	1,1	37	M	48 hours	Fall	-	LICH	Mild	-	Mild	Bilateral	+
890262	Yes	3,3	1,1	1,1	1.4	M	24 hours	Fall	+	BICH	Moderate	-	Mild	Bilateral	-
890311	Yes	3,3	1,1	1,1	21	M	29 hours	RTA	+	LSDH	Moderate	2m	Severe	-	+
890326	Yes	3,3			74	M	3 days	RTA	+	RICH	Mild	2M	Mild	-	+
890329	Yes	3,3	1,2	1,1	41	M	24 hours	RTA	+	LSDH	Moderate	1	Mild	Bilateral	+
890390	Yes	3,3	1,2	1,2	16	M	60 hours	RTA	+	REDH	Mild	-	Moderate	Bilateral	+
890521	Yes	3,4	1,1	1,1	26	M	30 hours	RTA	-	RSDH BICH	Mild	-	Mild	Bilateral	-
890537	Yes	3,3	1,2	1,2	9	F	21 hours	RTA	+	LSDH	Mild	-	Moderate	Bilateral	+
890538	Yes	3,3	1,2	1,2	27	M	36 hours	Fall	+	BSDH RICH	Moderate	-	Severe	Bilateral	+
890553	Yes	3,3	1,1	1,1	3	M	21 hours	RTA	+	-	Mild	-	-	Bilateral	-
890595	Yes	3,3	1,1	1,1	9	F	5 days	Fall	+	LSDH	Moderate	1	Severe	Bilateral	+
890630	Yes	3,3	1,2	1,2	25	M	17 hours	RTA	+	REDH LSDH RICH	Mild	2m	Moderate	Bilateral	+
890660	Yes	3,4	1,2	1,1	62	M	24 hours	RTA	+	BSDH LICH	Mild	-	Severe	-	+
890697	Yes	3,4	1,1	1,2	53	M	12 hours	Fall	+	LSDH BICH	Severe	-	Severe	L	+
890713	Yes	3,3	1,1	1,1	10	M	6 days	RTA	+	BEDH LSDH	Moderate	2M	Mild	-	+

890755	Yes	3,4	1,2	1,2	4	M	28 hours	RTA	+	-	Moderate	-	-	Bilateral	+
890800	Yes	3,3	1,2	1,2	32	M	3 days	Fall	+	BEDH LSDH	Mild	-	Severe	L	+
890869	Yes	3,4	1,1	1,1	67	M	17 hours	RTA	+	LICH	-	-	Severe	L	+

1990

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
900092	Yes	3,4	1,1	1,1	60	F	24 hours	RTA	+	BSDH	Moderate	1	Moderate	-	+
900128	Yes	3,4	1,1	1,1	28	M	18 hours	RTA	+	-	Mild	-	Severe	Bilateral	+
900163	Yes	3,3	1,2	1,1	16	M	2 days	RTA	+	RSDH	Mild	1	Severe	Bilateral	+
900254	Yes	2,4	1,2		14	M	7 hours	RTA	+	-	Mild	-	-	-	-
900338	Yes	3,3	1,1	1,1	3	F	2 days	RTA	+	-	Mild	-	-	Bilateral	-
900357	Yes	3,4	1,2	1,1	25	M	12 hours	RTA	-	BICH	Moderate	-	-	-	-
900384	Yes	3,4	1,1	1,1	16	M	2 days	Ass.	-	-	-	-	Severe	Bilateral	+
900432	Yes	4,4	1,1		71	M	24 hours	Fall	+	BSDH LICH	Moderate	-	Mild	-	+
900444	Yes	4,4			59	F	5 days	Fall	+	LSDH	Moderate	-	Moderate	-	+
900452	Yes	3,3	1,2	1,2	5	F	9 days	RTA	+	BSDH	Moderate	3m	Severe	-	+
900514	Yes	3,4			6	M	24 hours	RTA	-	-	-	-	Severe	Bilateral	-
900532	No	3,3			63	F	17 hours	RTA	+	-	Mild	3m	Moderate	-	+
900536	Yes	2,3	1,2	1,2	62	M	11 days	Fall	+	BSDH LICH RICH	Moderate	-	Mild	-	+
900661	Yes	3,4	1,1	1,1	14	M	4 days	RTA	+	REDH RSDH RICH	Severe	-	Severe	Bilateral	+
900702	Yes	3,3	1,1	1,1	21	M	2 days	RTA	+	Rcer.H	Moderate	-	Mild	Bilateral	-
900750	Yes	2,3	1,2	1,2	32	M	5 days	Fall	+	RSDH	Mild	-	Severe	Bilateral	+
900829	Yes	4,4	1,1	1,1	51	M	24 hours	RTA	+	BSDH RICH	Severe (54)	-	Severe	Bilateral	+

1991

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
910013	Yes	3,4	1,2	1,2	10	F	3 days	RTA	+	-	Moderate	-	Severe	Bilateral	+
910085	Yes	3,4	1,2	1,2	31	M	29 hours	RTA	+	-	Moderate	3M	Mild	-	-
910086	Yes	3,3	1,2	1,2	22	F	28 hours	RTA	+	-	Moderate	-	Severe	Bilateral	+
910096	Yes	3,4	1,1	1,1	30	M	16 hours	RTA	+	LSDH LICH	Mild	3M	Severe	Bilateral	+
910097	Yes	3,3	1,2		17	M	4 days	RTA	+	RSDH	Mild	1	Severe	Bilateral	+
910143	Yes	3,4	1,1	1,1	21	M	8 days	RTA	+	BEDH Lcer.H	Moderate	2m	Mild	Bilateral	+
910264	Yes	3,3	2,2	2,2	75	M	3 days	Fall	+	RSDH RICH	Mild	1	Mild	-	+
910295	Yes	4,4	1,2	1,1	57	M	24 hours	Fall	+	LSDH LICH	Mild	1	Severe	L	+
910296	Yes	2,3	1,2	1,1	79	M	2 days	RTA	+	LSDH BICH	Mild	3m	Mild	-	-
910487	Yes	3,4	1,2	1,2	56	F	4 days	Fall	+	RSDH RICH	Moderate	1	Mild	R	+
910514	Yes	3,4	2,2	1,1	30	F	2 days	Fall	-	LSDH	-	-	-	-	-
910516	Yes	3,3	1,1	1,1	43	M	11 days	RTA	+	LEDH	Moderate	1	Severe	-	+
910781	Yes	3,3	1,2	1,1	59	M	4 days	Fall	+	LEDH	Mild	-	-	-	+

1992

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
920054	No	3,3	1,2	1,1	66	M	14 days	Fall	+	RSDH	Mild	-	-	-	+
920248	Yes	3,3	2,2	2,2	59	F	24 hours	RTA	-	-	Mild	-	Mild	-	-
920250	Yes	3,3	1,1	1,1	49	M	2 days	Fall	+	LSDH BICH	Moderate	-	Severe	Bilateral	+

920255	Yes	3,3	2,2	1,1	84	F	<24 hour	RTA	+	LSDH	Moderate	1	Severe	-	+
920320	Yes	3,4	1,2		38	M	4 days	RTA	-	LICH	Mild	3M	Severe	Bilateral	+
920340	Yes	2,4	1,1	1,1	56	F	24 hours	RTA	+	LSDH LICH	Moderate	1	Severe	L	+
920343	Yes	4,4	1,1		73	F	26 days	Ass.	-	-	Mild	3m	-	-	-
920446	Yes	3,3	2,2	2,2	64	M	3 days	?	-	BSDH	-	-	Severe	-	+
920449	Yes	3,3	1,1	1,1	50	M	30 hours	Fall	+	BEDH LSDH RICH	Moderate	-	Mild	R	+
920483	Yes	2,3	1,1	1,1	59	F	7 days	RTA	-	RSDH LICH Bcer.H	-	3M	Mild	-	+
920484	Yes	3,3	1,1	1,1	43	M	2 days	Ass.	+	LSDH LICH	Mild	-	Severe	L	+
920580	Yes	4,4	2,2	1,2	74	M	14 days	Fall	-	BSDH	-	1	-	-	+
920631	Yes	3,3	1,1	2,2	74	F	2 days	?	+	LSDH LICH	Mild	1	Mild	L	+
920633	Yes	3,3	1,2	1,1	41	M	6 days	RTA	-	RICH	Mild	-	Severe	Bilateral	+
920775	Yes	3,3	1,2	1,1	27	M	7 days	?	+	LEDH RSDH	Mild	3M	Mild	-	+

1993

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
930209	Yes	3,3	1,1		73	F	7 days	RTA	+	-	Mild	1	Mild	Bilateral	+
930213	Yes	3,3	1,1	1,1	59	M	3 days	Fall	+	BEDH BICH	Mild	1	Mild	Bilateral	+
930215	Yes	3,3	1,1	1,1	53	M	11 days	Fall	+	LSDH	Mild	1	Mild	-	+
930432	Yes	3,3	1,2	1,2	59	F	24 hours	Fall	-	BSDH	Mild	-	Severe	-	+
930534	Yes	3,4	1,1	1,1	6	M	17 hours	RTA	+	REDH	Moderate	-	Severe	Bilateral	-
930535	Yes	3,3	1,1	1,1	19	M	57 hours	RTA	+	BICH	Moderate	-	-	R	-
930588	Yes	3,3	1,2	1,1	44	M	48 hours	RTA	+	BEDH	Mild	3m	Severe	Bilateral	+

										BSDH					
930649	Yes	3,4	1,1	1,1	7	F	26 hours	RTA	+	-	Mild	-	Mild	Bilateral	+
930763	No	3,3	1,2	1,2	69	F	1 hour	RTA	-	BSDH	Mild	-	-	Bilateral	-
930765	No	3,3	2,2	2,2	41	M	3 hours	Fall	+	BSDH	Mild	2M	Mild	-	-
930791	Yes	3,3	1,2	1,2	41	M	<24 hours	Fall	+	RSDH	Moderate	-	-	-	+
930875	Yes	2,3	1,1	1,1	25	M	24 hours	RTA	-	BSDH	Mild	-	Severe	Bilateral	+

1994

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
940010	Yes	3,4	1,1	1,1	20	F	4 days	RTA	+	-	Moderate	3M	Severe	Bilateral	+
940016	Yes	3,3	1,1	1,1	28	F	10 hours	Fall	+	-	Mild	-	Severe	Bilateral	+
940060	Yes	3,3	1,2	1,2	52	M	4 days	Fall	+	LSDH	-	-	Moderate	Bilateral	+
940227	Yes	3,4	1,2	1,1	70	M	24 hours	RTA	+	BSDH	Mild	1	Moderate	Bilateral	+
940294	Yes	3,3	1,2	1,2	47	M	72 hours	Fall	-	LSDH LICH	Mild	-	Severe	Bilateral	+
940419	Yes	4,4	1,1	1,1	37	M	4 hours	Fall	+	BEDH RSDH Lcer.H	Moderate	-	Severe	-	-
940717	Yes	3,3	1,2	1,2	27	M	11 days	Fall	+	REDH RSDH BICH	Moderate	1	Mild	Bilateral	+
940726	Yes	3,3	1,2	1,1	69	F	5 days	Fall	+	LEDH BSDH LICH	Severe	3m	Severe	L	+
940791	Yes	3,3	1,2		71	M	5 days	RTA	-	-	-	-	Severe	L	+
940807	Yes	3,4		1,1	61	F	12 hours	Fall	+	LEDH BSDH LICH	Severe	-	Severe	-	+

1995

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
950040	Yes	3,3	1,1	2,2	37	M	24 hr	Fall	+	BSDH	+	Min	Min	R	+
950106	Yes	3,3		1,1	18	M	10 hr	Fall	+	LSDH	+	Min	-	-	+
950212	Yes	3,3		1,1	63	M	36 hr	? Fall	+	LSDF	+	1	Min	-	+
950280	Yes	3,3	1,2	1,2	73	M	3 hr	RTA	+	LSDH	+	Min	-	-	+
950336	Yes	3,4	1,2	1,2	51	M	2 months	Fall	+	L Burst lobe	+	-	Severe	-	+
950390	Yes	3,3	1,1	1,1	21	M	9 days	RTA	-	L SDH	+	-	Severe	+	+
950415	Yes	3,4	1,2	1,2	62	M	24 hours	Fall	+	L SDH	+	1	Mod	R	+
950513	Yes	3,3	1,1		45	M	20 days	RTA	+	-	+	1	Mod	-	+
950675	Yes	3,3	1,2	1,1	17	M	30 hr	Ass	+	LSDH	+	Min	Severe	-	+
950716	Yes	3,3	1,1	1,1	76	M	6 wk	Ass	+	RSDH	+	1	R.MCA	-	+

1996

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
960001	Yes	3,3	1,1	1,1	46	M	24 hr	Fall	+	RSDH	+	Min	Severe	Bilateral	+
960008	Yes	2,3	1,1	1,1	79	M	4 mths	RTA	-	LSDH	+	Min	Mod	-	-
960017	Yes	3,3	1,1	1,1	19	M	21 days	RTA	+	-	+	1	Mod	-	-
960023	No	3,3	1,1	1,1	47	M	2 wks	Ass	-	BSDH	+	Min	Severe	R	+
960105	Yes	3,3	1,1	1,1	39	M	12 hr	Ass	-	BSDH	+	Min	Mod	R	+
960175	Yes	3,3	1,1	1,1	50	M	7 wks	NK	-	RSDH	+	Min	Mod	-	+
960198	Yes	3,4	1,1	1,1	61	F	16 hr	Fall	+	LSDH	+	1	Severe	L	+
960228	Yes	3,4	1,1	1,1	55	F	10 days	Fall	-	RSDH	+	1	-	-	+
960256	No	3,3	1,2		73	F	24 hr	Fall		RSDH	+	3	Severe	R	+
960312	No	3,3	1,1		38	M	?6 hr	NK	+	-	+	-	-	-	-
960339	No	3,3	1,1		54	F	54 hr	Fall	-	RSDH	-	Min	Severe	-	+
960340	No	3,3	1,1		88	F	7 hr	Fall	-	LSDH	-	Min	Min	-	+
960341	No	3,3	1,2		41	M	41 hr	Fall	+	RSDH	+	Min	Severe	-	+

960342	No	2,3	1,2	1,1	40	F	3.5 hr	Fall	+	LSDH	+	Min	-	-	?+
960351	Yes	3,3	1,2	1,1	60	M	9 days	Fall	+	RF LF	+	Min	-	-	+
960440	Yes	3,3	1,2	1,1	44	M	32 hr	Fall	+	RT	+	1	Severe	-	+
960449	No	3,3	1,2	1,1	55	M	4 days	NK	+	B.SDH	+	Min	-	-	+
960462	Yes	3,3	1,1	1,1	66	M	21 hr	RTA	+	LSDH	+	Min	Mod	-	+
960495	No	3,4	1,2		70	F	7 wks	Fall	-	LSDH	+	1	Mild	-	-
960510	No	2,3	1,2	1,2	58	M	6 hr	Fall	+	SDH	+	-	-	-	-
960518	No	3,3	1,1	1,1	76	M	5 wks	Fall	+	RP	+	1	-	-	-
960523	Yes	3,3	1,2	1,2	17	M	2 wks	RTA	-	-	+	3	-	-	-
960534	Yes	3,3			49	M	12 mths	Ass	-	RP	+	-	-	-	+
960640	Yes	3,3	1,1	1,1	14	M	2 days	RTA	+	-	+	2	Severe	B	+
960662	No	3,3	1,1		89	F	4 days	Fall	-	-	-	1	-	-	-
960696	Yes	2,3	1,2	1,1	54	M	48 hr	Ass	-	-	+	Min	Severe	L	+
960754	Yes	3,3	2,2	2,2	62	M	12 hr	Fall	+	LSDH	19	1	Severe	L	+
960813	No	3,4	1,2	1,1	80	F	9 days	Fall	+	RSDH	+	Min	-	-	+
960824	Yes	3,3	1,1	1,1	61	F	20 hr	RTA	+	REDH	23	Min	Severe	R	+
960919	Yes	2,3	1,1	1,1	54	M	1.5 days	Fall	+	BSDH LF	22	1	Severe	B	+
960920	Yes	3,4	1,2	1,2	64	M	1 hr	Fall	+	BSDH	0	1	Severe	-	-
960970	No	3,3	1,2	1,1	41	M	?4 hr	Ass	-	BSDH	+	3	-	?R	+
960997	Yes	3,3	1,2	1,1	18	M	3 mths	RTA	+	-	+	3	-	-	+
961000	No	2,3	1,2	1,2	23	M	4 weeks	Ass.	-	-	-	2	-	-	-
961002	Yes	2,3	1,1	1,1	15	F	8 days	RTA	+	RF	1	1	Severe	B	+

1997

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
970055	No	3,3	1,1	1,1	68	M	10 days	Fall	+	RSDH RT	+	Min	Min	R	+
970056	No	3,3	1,1	1,1	46	M	48 hr	Fall	+	-	+	1	Min	-	-
970057	No	3,4	1,2	1,1	47	M	3 hr	Fall	+	RSDH	+	Min	Min	R	+
970062	Yes	3,4	1,1	1,1	18	M	3 days	Ass	+	REDH	-	Min	Extensive	R	+
970112	Yes	2,3	1,1	1,1	52	M	6 days	RTA	+	LSDH (e)	+	1	Min	L	+
970202	Yes	3,3	1,2	1,1	40	M	5 days	Ass	-	LSDH (e)	0	Min	Severe	L	+
970368	Yes	3,4	1,1		73	M	8 days	RTA	+	REDH	12	1	Severe	B	+
970372	Yes	2,3	1,1	1,1	17	M	3 days	RTA	-	-	+	3	Severe	B	+
970442	Yes	3,3	1,2		67	M	8 months	Fall	+	RSDH (e)	+	Min	Mod	-	+
970475	Yes	2,3	2,2		31	F	9 days	RTA	-	-	+	3	Min	-	+
970512	Yes	3,4	1,1	1,1	67	M	2 wks	Fall	+	RSDH (e)	27	1	Mod	-	+
970552	Yes	3,3	1,1	1,1	11	M	4 days	RTA	+	-	15	2	Min	-	-
970640	Yes	3,3	1,1	1,1	82	M	< 24 hrs	Fall	+	RSDH (e)	18	1	Min	-	+
970696	Yes	3,3	1,1	1,1	37	M	24 hrs	Fall	+	RSDH	+	-	Mod	R	+
970716	Yes	3,3	1,1	1,1	66	M	8 days	Fall	-	RSDH	0	-	Severe	R	+
970717	Yes	3,4	1,1	1,1	16	M	2 days	Cycle	+	RF	9	1	Min	-	+
970758	Yes	3,4	1,1	1,1	33	F	5 days	RTA	+	LSDH (e)	21	2	Min	L	+
970759	Yes	3,3	1,1		17	M	3 days	RTA	+	-	19	3	Min	-	+
970786	Yes	3,3	1,1	1,1	50	F	6 days	Fall	-	LSDH (e)	6	-	Severe	L	+
970787	Yes	3,3	1,1	1,1	60	M	10 days	Fall	+	LSDH	+	1	Min	-	-
970801	Yes	3,3	1,2	1,2	18	F	24 hrs	RTA	+	LSDH	+	3	Mod	-	+

1998

PM No.	INS case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
980067	No	3,4	2,2	1,2	79	F	13 hrs	RTA	+	LSDH	-	-	Mod	L	+
980072	No	3,4	1,2	1,2	75	F	1 yr	Ass	-	ICH	-	-	-	-	-
980104	Yes	2,3	1,1	1,1	59	M	17 days	Fall	-	LSDH (e)	0	-	Severe	-	+
980145	No	3,4	1,1	1,1	64	M	3 days	Fall	-	LSDH	+	-	Mod	-	+
980193	Yes	3,4	1,1		44	M	4 days	?Fall	-	EDH(e)	+	-	Severe	R	+
980228	Yes	3,3	1,1	1,1	70	F	6 months	Ass	+	EDH(e) ICH	+	-	Min	-	-
980230	No	2,3	2,2	1,1	65	M	5 months	NK	+	-	-	-	-	-	-
980234	Yes	3,3	1,2	1,1	18	M	4 wks	Ass	+	-	+	2	Min	-	-
980288	Yes	3,3	1,1	1,1	19	M	23 hrs	RTA	+	-	+	2	Min	R	+
980290	Yes	3,3	2,2	1,1	28	M	9 months	RTA	-	-	-	3	Min	-	-
980326	No	2,2			16	M	7 days	RTA	+	-	+	3	Min	R	+
980328	Yes	3,4	2,2	2,2	9	F	10 hrs	RTA	+	-	+	3	Min	-	?
980331	Yes	3,4	1,1	1,1	6	F	18 hrs	RTA	-	RSDH (e)	Min	1	Severe	R	+
980345	Yes	3,4	1,1	1,1	28	F	10 days	nk	+	LSDH (e)	13	-	Severe	L	+
980348	Yes	3,3	1,1	1,1	42	M	26 hrs	Fall	-	LSDH	14	-	Severe	-	+
980380	Yes	3,3	1,2	1,2	43	F	26 hrs	Ass	-	RSDH (e)	4	-	Mod	R	+
980389	Yes	2,3	1,2	1,2	5	F	5 days	RTA	-	-	6	3	Severe	-	+
980433	Yes	3,3	1,1	1,1	62	F	23 hrs	Fall	+	Rceb.H	12	2	-	-	+
980452	Yes	3,4	1,1	1,1	72	M	24 hrs	Fall	+	RSDH	10	-	Severe	-	+
980465	Yes	3,4	1,2	1,2	23	M	3 days	Ass	+	-	-	-	-	-	-

980470	Yes	3,3	1,2	1,2	65	M	27 hrs	Fall	-	LSDH	0	1	Severe	-	+
980472	Yes	3,3	1,1	1,1	43	M	4 days	Ass	+	SDH	+	-	Mod	R	+
980515	Yes	3,3	1,1	1,1	58	M	12 days	Fall	-	SDH Post fossa	+	-	Mod	-	+
980522	Yes	3,4	1,2	1,2	31	M	27 days	Fall	+	LSDH (e)	20	1		Min	-
980526	Yes	3,3	1,2	1,1	37	F	nk	Ass	-	-	-	-	Mod	L	+
980543	Yes	2,3	1,1	1,1	68	M	3 days	RTA	+	-	16	1	Min	L	+
980552	Yes	3,3	1,1	1,2	51	M	2-4 weeks	Fall	-	RSDH	0	-	Mod	-	+
980564	Yes	3,4	1,2	1,2	37	M	22 hrs	RTA	-	-	8	3	Severe	Bilateral	+
980589	Yes	2,3	1,1	1,1	58	M	24 hrs	Fall	+	LF	6	-	Mod	Bilateral	+
980590	Yes	4,4	1,2	1,1	50	M	11 days	Fall	+	RSDH (e)	10	-	Severe	Bilateral	+

1999

PM No.	INS Case	APO E	IL-1A	IL-1B	Age	Sex	Survival	Type of injury	Fracture of skull	ICH	Contusions	DAI	HBD	Swelling	Raised ICP
990051	Yes	3,4	1,1	1,1	34	M	22 hours	Fall	+	SDH	+	-	Severe	-	+
990072	Yes	3,3	1,1	1,1	26	M	24 hours	Ass	+	SDH	+	-	Mild	Bilateral	+
990143	Yes	3,3	1,2	1.1	12	F	7 days	RTA	-	ICH	+	2	Severe	Bilateral	+
990149	Yes	3,3		1,1	66	M	7 days	RTA	-	SDH	-	-	Mild	-	+
990202	Yes	3,3	1,2		73	M	7 days	Fall	-	ICH	-	-	Mild	R	+
990224	Yes	3,4	1,1	1,1	17	M	31 hours	RTA	+		+	2	Severe	Bilateral	+
990233	Yes	3,3	1,2		75	F	8 days	RTA	-	-	+	3	Severe	Bilateral	+
990234	Yes	3,4	1,1		16	F	7 days	RTA	-	-	-	3	Mod	Bilateral	+
990303	Yes	3,3			58	M	24 hours	Fall	+	SDH	+		Mod	-	+
990304	Yes	2,4	1,1	1,1	54	F	48 hours	Fall	-	SDH(e)	-	-	Severe	-	+

990329	Yes	3,4	1,2	1,2	22	M	8 days	RTA	+	SDH	+	2	Mild	-	+
990339	Yes	3,3	1,2	1,1	78	M	4 months	RTA	-	SDH	+	1	-	-	-
990347	Yes	3,4	1,2	1,2	17	F	45 hours	Fall	+	SDH(e)	+	-	-	Bilateral	+
990414	Yes	3,4	1,1	1,1	29	F	48 hours	ass	-	SDH	+	-	Severe	Bilateral	+
990451	Yes	3,3	2,2	1,2	35	M	11 days	Fall	_	SDH	-	-	-	-	+
990493	Yes	3,3	1,1	1,1	65	M	12 hours	Fall	+	SDH ICH	+	2	Severe	Bilateral	+
990545	No	2,4	1,1	1,1	51	F	20 hours	Fall	+	SDH	+	-	Mod	-	+
990601	Yes	3,3	1,1		46	M	8 days	Ass	+	SDH	+	_	Severe	Bilateral	+
990602	Yes	3,4	2,2	1,1	60	M	24 hours	Fall	+	SDH(e)	+	_	Mod	R	+

9.3 Appendix 3

PCR protocols

9.3.1 DNA extraction

[1] Microtomy-

1/ Clean the knife with analar xylene followed by ethanol before cutting and between blocks.

2/ Use sterile forceps for each sample.

3/ Wear gloves and change them every few samples or if you feel they have become contaminated.

4/ Cut paraffin sections 10-20 μm thick.

5/ Place section a sterile pre-labelled eppendorf.

[2] De-waxing-

1/ Wear gloves and change them every few samples or if you feel they have become contaminated.

2/ Use dedicated pipettes and autoclaved tips.

3/ Add 1ml of analar xylene to each tube and vortex for 1 minute.

4/ Centrifuge at 13000 rpm for 5 minutes.

5/ Decant xylene, add 1ml of ethanol and vortex for 1 minute.

6/ Centrifuge at 13000 rpm for 5 minutes.

7/ Decant ethanol, add 1ml fresh ethanol and vortex for 1 minute.

8/ Centrifuge at 13000 rpm for 3 minutes.

9/ Decant the ethanol, cover with parafilm (pierced) and dry on a heat block at 55-60°C for approximately 1 hour.

[3] Proteinase K digestion-

1/ Resuspend dried pellet of de-waxed tissue in Proteinase K mix to a final concentration of 200 μg/ml.

Mix (per sample); Water 176 μl

Buffer	20 µl
Proteinase K (10mg/ml)	4 µl

2/ Pipette a layer of mineral oil (5-6 drops) into eppendorf.

3/ Incubate at 56°C overnight.

4/ Inactivate Proteinase K at 95°C for 10 minutes.

9.3.2 Common steps in PCR process

When setting up a PCR the reagents are made up as a master mix. A master mix includes the appropriate volumes of PCR reagents times the number (n) of target samples being amplified. When making up a master mix always allow extra to ensure that there is sufficient for all your samples i.e. if you are amplifying 9 samples make the master mix (x10).

For a 15µl reaction the standard volume of target DNA added is 0.8µl making the total volume up to 15µl.

Adjust the water content of the reaction to allow for any variation which may have to be made to target sample volumes, primer concentrations, or any other constituent of the reaction you may wish to alter. All racks used for handling eppendorfs are stored at -20°C. It is also helpful if the PCR is set up on ice or chill blocks. This procedure will help to minimize the amount of primer artefact produced.

Procedure; Wear gloves and change them if you feel they have become contaminated.

All tips and tubes used in setting up a PCR must be autoclaved prior to use.

[1] Label a 1.5ml Eppendorf (autoclaved), pre- chilled (see above).

[2] Remove the PCR reagents (listed above) from the PCR freezer, allow to thaw.

Add in order.

When pipetting the amplitaq gold polymerase use a plugged sterile tip.

[3] Pipette 14.2µl master mix into each 0.5ml or 0.2ml Eppendorf.

[4] Overlay each reaction volume with one drop of mineral oil.

(NB This step is required only when the genieE thermal cycler is being used).

[5] Pipette 0.8µl target DNA into each. Use dedicated pipettemans and plugged tips, a unique tip for each sample.

Change gloves every few samples. Ensure the target DNA is taken from beneath the mineral oil layer. For no target sample use aliquoted water (the same aliquot that was used for the master mix).

[6] Transfer to thermal cycler APOE programmes. The AmpliTaq Gold Polymerase is provided in an inactive state. Heat activates the enzyme and therefore it is necessary to include an additional heat step at the beginning of the thermal cycle programmes of 7-12 mins. This can be included in the first denaturing step of the thermal cycle programmes.

[7] Store the PCR product in the PCR product freezer in the gel room or proceed to the restriction enzyme digestion.

9.3.3 APOE reactions

APOE PCR mastermix; 15µl reaction

Analar Water		9.025µl
Amplitaq Buffer	(x10 conc)	1.5µl
dNTPs	(2mM conc)	1.5µl
Primer L3,	(10µm conc)	0.3µl
Primer R3+	(10µm conc)	0.3µl
Amplitaq Gold Polymerase		0.075µl
Total		14.2µl

Restriction enzyme digestion (Hha1); 15µl reaction

[1] To the 15µl PCR product add 1.2µl of Hha1.

[2] Incubate samples at 37°C for a minimum of 3hrs up to overnight.

[3] Store Hha1 digested samples in the PCR product freezer (-20°C) or proceed to gel electrophoresis.

Thermal cycle; Techne Genius (with heated lid)

APOE Thermal Cycling Programmes -

Prog 1 - 35.5°C - to preheat lid

Prog 2 - 94.0°C - 12 mins to denature target/activate Hha1

Prog 3 - 94.0°C - 1min

65.0°C - 1min x 40 cycles

72.0°C - 2mins

Prog 4 - 4°C Hold

9.3.4 IL-1A reactions

IL-1A PCR mastermix; 15µl reaction

Analar Water		8.7µl
Amplitaq Buffer	(x10 conc)	1.5µl
dNTPs	(2mM conc)	1.5µl
Primer IL-1AR	(10µm conc)	1.2µl
Primer IL-1AL	(10µm conc)	1.2µl
Amplitaq Gold Polymerase		0.1µl
Total		14.2µl

Restriction enzyme digestion (Nco1); 15µl reaction

[1] To the 15µl PCR product add 3 units of Nco1.

[2] Incubate samples at 37°C for a minimum of 3hrs up to overnight.

[3] Store Nco1 digested samples in the PCR product freezer (-20°C) or proceed to gel electrophoresis.

Reagents Sigma Molecular Biology Grade Water

Sigma dNTPs

Perkin Elmer Amplitaq Gold and Buffer

Primer Sequences IL-1A (-899) IL-1AR TTACATATGAGCCTTCCATG

IL-1AL AAGCTTGTTCTACCACCTGAACTAGGC

Supplier Sigma Genosys

Restriction Endonuclease: - Nco 1

Supplier - Promega

Fragment Length (after digestion) :- Allele 1 88bp, 16bp

Allele 2 104bp

Thermal cycle; Techne GENIUS (with heated lid)

IL-1A Thermal Cycling Programmes - Prog 10- 35.5°C - to preheat lid

Prog 11- 94.0°C - 10 mins to denature target/activate Taq

Prog 7 - 95.0°C - 1min

65.0°C - 1min x 35 cycles

74.0°C - 2mins

Prog 8 - 95.0°C - 1min

65.0°C - 1min x 3 cycles

74.0°C - 2mins

Prog 9 - 4°C Hold

9.3.5 IL-1B reactions

IL-1B PCR mastermix; 15µl reaction

AnalarWater		9.3µl
Amplitaq Buffer	(x10 conc)	1.5µl
dNTPs	(2mM conc)	1.5µl
Primer L3,	(10µm conc)	0.9µl

Primer R3+	(10μm conc)	0.9μl
Amplitaq Gold Polymerase		0.1μl
Total		14.2μl

Restriction enzyme digestion (Taq1); 15μl reaction

[1] To the 15μl PCR product add 4.5units of Taq1.

[2] Incubate samples at 65°C for a minimum of 3hrs up to overnight.

[3] Store Taq1 digested samples in the PCR product freezer (-20°C) or proceed to gel electrophoresis.

Reagents Sigma Molecular Biology Grade Water

Sigma dNTPs

Sigma DMSO

Perkin Elmer Amplitaq Gold and Buffer

Primer Sequences IL-1B (+3953) IL-1BR GCTTTTTTGCTGTGAGTCCCG

IL-1BL CTCAGGTGTCCTCGAAGAAATCAAA

Supplier Sigma Genosys

Restriction Endonuclease: - Taq 1

Supplier - Promega

Fragment Length (after digestion) :- Allele 1 97bp, 85bp,12bp

Allele 2 182bp,12bp

Thermal cycle; Techne GENIUS (with heated lid)

IL-1B Thermal Cycling Programmes - Prog 5 - 35.5°C - to preheat lid

Prog 6 - 94.0°C - 10 mins to denature target/activate Taq

Prog 7 - 95.0°C - 1min

65.0°C - 1min x 35 cycles

74.0°C - 2mins

Prog 8 - 95.0°C - 1min

65.0°C - 1min x 3 cycles

74.0°C - 2mins

Prog 9 - 4°C Hold

9.3.6 Polyacrylamide gel electrophoresis

[1] Preparing polyacrylamide gel solution

For a 10% gel add the following in order;

17ml distilled water

6ml 5x Tris borate EDTA buffer

7ml 19:1 acrylamide/bis acrylamide (40%)

230 µl ammonium persulphate 0.5g/5ml

23 µl TEMED

This is sufficient for six 0.75 mm thickness gels.

[2] Pouring polyacrylamide gels

1/ Glass plates are cleaned with ethanol and dried.

2/ Spacers (15 well) are placed between plates which are then screwed into the clamp assembly and then into the gel stand.

3/ Gel solution is poured between the glass plates and allowed to polymerise for 30 minutes.

4/ Assembly is clamped into gel running apparatus which is then filled with approximately 500ml EDTA buffer.

5/ The spacers are removed from the gels and the wells are rinsed with buffer.

[3] Loading of DNA samples

1/ 3µl of gel loading solution (Sigma) is added to each 15µl sample of PCR product.

2/ Samples are loaded into wells using round gel loading tips, a unique tip being used for each sample.

3/ A DNA ladder is prepared by adding 0.5µl HinfI to 24µl distilled water and 3µl of gel loading solution.

4/ Gels are run at 200 volts/0.06 amps per gel.

5/ When completed gels are removed and stained with ethidium bromide solution (1.25µg/ml). Gloves must be worn and changed immediately after being in staining solution.

6/ Gels are viewed on ultraviolet (UV) viewing box using protective glasses and photographed.

9.4 Appendix 4

Statistical Methods

9.4.1 Assessment of age-related increase in neuroinflammation in the control group

CR 3/43

Variable	conage	N	N*	Mean	Median	TrMean
avcc-hip	11	11	4	1.641	1.406	1.549
	12	9	1	2.908	2.065	2.908

Variable	conage	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	11	0.983	0.296	0.557	3.555	0.890
	12	2.867	0.956	0.958	10.170	1.183

Variable	conage	Q3
avcc-hip	11	2.157
	12	3.232

Mann-Whitney Confidence Interval and Test
C28 N = 11 Median = 1.406
C29 N = 9 Median = 2.065
Point estimate for ETA1-ETA2 is -0.473
95.2 Percent CI for ETA1-ETA2 is (-1.874,0.377)
W = 101.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2875

CD68

Variable	conage	N	N*	Mean	Median	TrMean
avcc-hip	11	11	4	0.834	0.363	0.569
	12	9	1	0.375	0.185	0.375

Variable	conage	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	11	1.083	0.327	0.243	3.810	0.288
	12	0.361	0.120	0.110	1.040	0.162

Variable	conage	Q3
avcc-hip	11	0.930
	12	0.632

Mann-Whitney Confidence Interval and Test
C28 N = 11 Median = 0.363
C29 N = 9 Median = 0.185
Point estimate for ETA1-ETA2 is 0.142
95.2 Percent CI for ETA1-ETA2 is (0.005,0.647)
W = 142.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0482

9.4.2 Assessment of the difference of the neuroinflammatory response between TBI and control groups

CR3/43

Variable	1con2TBI	N	N*	Mean	Median	TrMean
avcc-hip	1	20	5	2.211	1.512	1.861
	2	55	8	3.390	2.223	3.050

Variable	1con2TBI	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	2.095	0.468	0.557	10.170	1.052
	2	3.435	0.463	0.010	13.043	0.368

Variable	1con2TBI	Q3
avcc-hip	1	2.954
	2	4.879

Mann-Whitney Confidence Interval and Test
avh-ccco N = 20 Median = 1.512
avh-cctb N = 55 Median = 2.223
Point estimate for ETA1-ETA2 is -0.541
95.1 Percent CI for ETA1-ETA2 is (-1.985,0.630)
W = 692.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4187

CD68

Variable	1con2TBI	N	N*	Mean	Median	TrMean
avcc-hip	1	20	5	0.627	0.300	0.479
	2	55	8	0.8887	0.6871	0.8169

Variable	1con2TBI	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.853	0.191	0.110	3.810	0.199
	2	0.7231	0.0975	0.0286	3.6929	0.4000

Variable	1con2TBI	Q3
avcc-hip	1	0.808
	2	1.3714

Mann-Whitney Confidence Interval and Test
C23 N = 20 Median = 0.3000
C24 N = 55 Median = 0.6871
Point estimate for ETA1-ETA2 is -0.2643
95.1 Percent CI for ETA1-ETA2 is (-0.5614,-0.0299)
W = 572.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0251

9.4.3 CR3/43 staining in TBI cases, comparing TAI cases with non-TAI cases.

For TAI; 1 = no, 2 = yes

Corpus callosum

Mann-Whitney Test and CI: cc_1, cc_2
cc_1 N = 19 Median = 1.000
cc_2 N = 28 Median = 4.755
Point estimate for ETA1-ETA2 is -3.810
95.0 Percent CI for ETA1-ETA2 is (-7.501,-0.770)
W = 336.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0096
The test is significant at 0.0094 (adjusted for ties)

Cingulate grey matter

Mann-Whitney Test and CI: cg_1, cg_2
cg_1 N = 18 Median = 0.095
cg_2 N = 26 Median = 1.105
Point estimate for ETA1-ETA2 is -0.570
95.1 Percent CI for ETA1-ETA2 is (-2.410,-0.090)
W = 308.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0213
The test is significant at 0.0210 (adjusted for ties)

Cingulate white matter

Mann-Whitney Test and CI: cw_1, cw_2
cw_1 N = 18 Median = 0.240
cw_2 N = 26 Median = 3.375
Point estimate for ETA1-ETA2 is -1.560
95.1 Percent CI for ETA1-ETA2 is (-4.810,-0.470)
W = 303.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0159
The test is significant at 0.0157 (adjusted for ties)

Temporal grey matter

Mann-Whitney Test and CI: tg_1, tg_2
tg_1 N = 23 Median = 0.130
tg_2 N = 29 Median = 0.340
Point estimate for ETA1-ETA2 is -0.080
95.1 Percent CI for ETA1-ETA2 is (-0.350,0.030)
W = 521.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1069
The test is significant at 0.1066 (adjusted for ties)

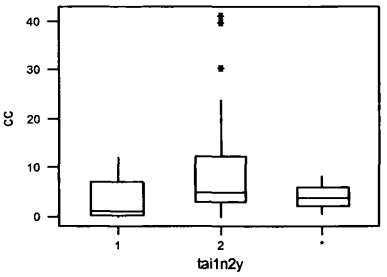
Temporal white matter

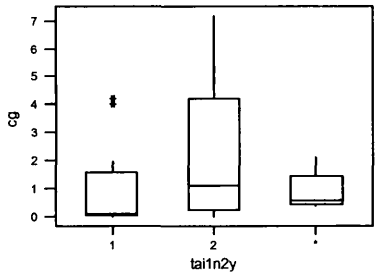
Mann-Whitney Test and CI: tw_1, tw_2
tw_1 N = 22 Median = 0.840
tw_2 N = 29 Median = 2.350

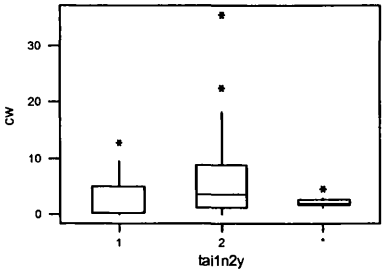
Point estimate for ETA1-ETA2 is -0.625
95.1 Percent CI for ETA1-ETA2 is (-2.321,0.170)
W = 487.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1101
The test is significant at 0.1101 (adjusted for ties)

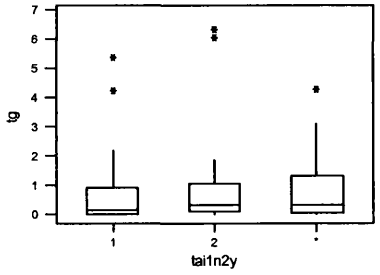
Alveus
Mann-Whitney Test and CI: alveus_1, alveus_2
alveus_1 N = 22 Median = 1.150
alveus_2 N = 28 Median = 2.155
Point estimate for ETA1-ETA2 is -0.290
95.0 Percent CI for ETA1-ETA2 is (-1.959,0.921)
W = 522.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4518
The test is significant at 0.4516 (adjusted for ties)

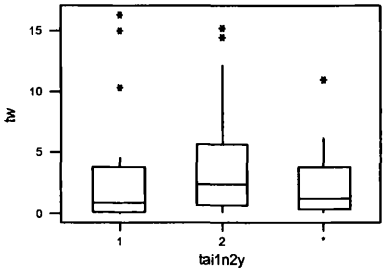
Hippocampus
Mann-Whitney Test and CI: hippo_1, hippo_2
hippo_1 N = 22 Median = 0.300
hippo_2 N = 28 Median = 0.700
Point estimate for ETA1-ETA2 is -0.075
95.0 Percent CI for ETA1-ETA2 is (-0.750,0.221)
W = 526.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5001

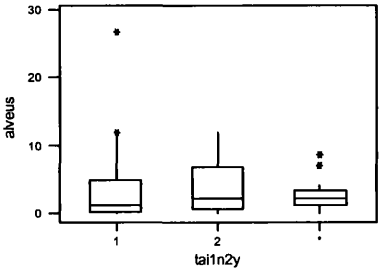


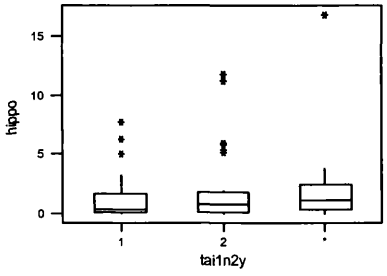












1 = no TAI
2 = yes TAI
* = missing (i.e. controls)

9.4.4 CD 68 staining in TBI cases, comparing TAI cases with non-TAI cases.

For TAI; 1 = no, 2 = yes

Corpus callosum

Mann-Whitney Test and CI: cc_1, cc_2
cc_1 N = 19 Median = 0.930
cc_2 N = 28 Median = 1.455
Point estimate for ETA1-ETA2 is -0.675
95.0 Percent CI for ETA1-ETA2 is (-1.430,0.041)
W = 375.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0810

Cingulate grey matter

Mann-Whitney Test and CI: cg_1, cg_2
cg_1 N = 18 Median = 0.2150
cg_2 N = 26 Median = 0.5200
Point estimate for ETA1-ETA2 is -0.1900
95.1 Percent CI for ETA1-ETA2 is (-0.4397,0.0099)
W = 323.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0517

Cingulate white matter

Mann-Whitney Test and CI: cw_1, cw_2
cw_1 N = 18 Median = 0.575
cw_2 N = 26 Median = 0.930
Point estimate for ETA1-ETA2 is -0.400
95.1 Percent CI for ETA1-ETA2 is (-0.770,-0.030)
W = 317.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0378

Temporal grey matter

Mann-Whitney Test and CI: tg_1, tg_2
tg_1 N = 23 Median = 0.1000
tg_2 N = 30 Median = 0.2350
Point estimate for ETA1-ETA2 is -0.0700
95.1 Percent CI for ETA1-ETA2 is (-0.1702,0.0398)
W = 553.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2292

Temporal white matter

Mann-Whitney Test and CI: tw_1, tw_2
tw_1 N = 23 Median = 0.2700
tw_2 N = 30 Median = 0.7400
Point estimate for ETA1-ETA2 is -0.3600
95.1 Percent CI for ETA1-ETA2 is (-0.5899,0.0500)
W = 526.5

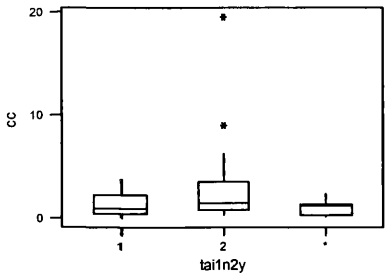
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0916

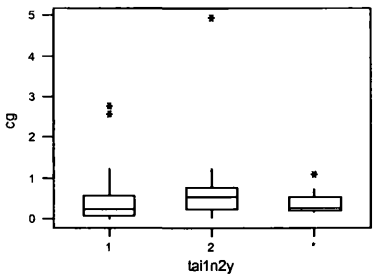
Alveus

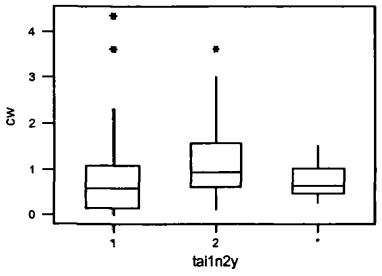
Mann-Whitney Test and CI: alveus_1, alveus_2
alveus_1 N = 22 Median = 0.4450
alveus_2 N = 29 Median = 0.6100
Point estimate for ETA1-ETA2 is -0.1450
95.1 Percent CI for ETA1-ETA2 is (-0.7703,0.1201)
W = 515.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2869

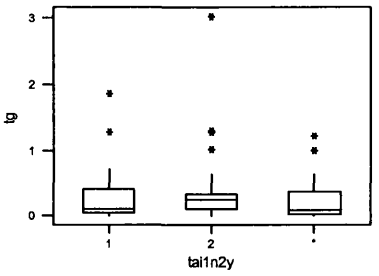
Hippocampus

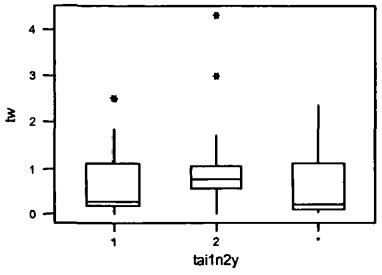
Mann-Whitney Test and CI: hippo_1, hippo_2
hippo_1 N = 22 Median = 0.2050
hippo_2 N = 29 Median = 0.2100
Point estimate for ETA1-ETA2 is -0.0600
95.1 Percent CI for ETA1-ETA2 is (-0.2798,0.0799)
W = 533.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4699

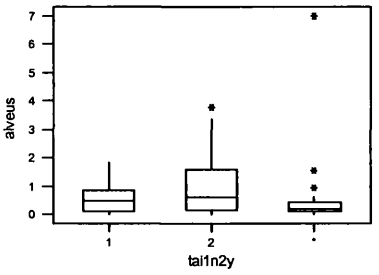

cc
tai1n2y

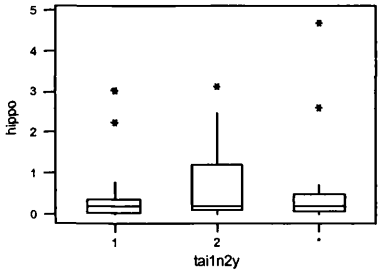

cg
tai1n2y


cw
tai1n2y


tg
tai1n2y


tw
tai1n2y


alveus
tai1n2y


hippo
tai1n2y

9.4..5 Distribution of *IL-1A* and *IL-1B* alleles between TBI and control cases

Assessment of *IL-1A*

Chii-Square Test

	C24	C25	Total
11	9	30	39
	10.01	28.99	
22	10	25	35
	8.99	26.01	
Tottal	19	55	74

C24= controls, C25= TBI
1= 1,1; 2= any 2

Chii-Sq = 0.103 + 0.035 +
0.114 + 0.039 = 0.292
DF = 1, **P-Value = 0.589**

Assessment of *IL-1B*

Chii-Square Test

	C28	C29	Total
11	10	30	40
	10.34	29.66	
22	5	13	18
	4.66	13.34	
Tottal	15	43	58

C28= controls, C29= TBI
1= 1,1; 2= any 2

Chii-Sq = 0.011 + 0.004 +
0.026 + 0.009 = 0.050
DF = 1, **P-Value = 0.823**

9.4.6 Assessment of the possession of any copy of *IL-1A* or *IL-1B* allele 2 and the neuroinflammatory response.

CR3/43

IL-1A

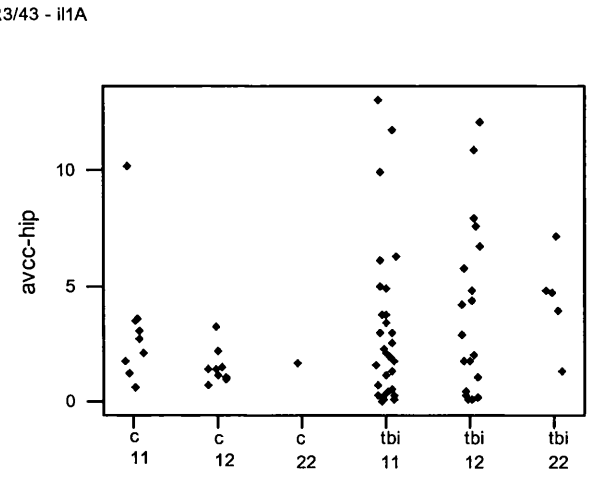
All cases (TBI and control cases)

Variable	il1aany2	N	N*	Mean	Median	TrMean
avcc-hip	1	39	3	3.050	2.065	2.689
	2	34	0	3.258	1.828	2.923
	*	2	10	0.472	0.472	0.472

Variable	il1aany2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.274	0.524	0.010	13.043	0.557
	2	3.108	0.533	0.017	12.161	1.040
	*	0.592	0.418	0.053	0.890	*

Variable	il1aany2	Q3
avcc-hip	1	3.694
	2	4.744
	*	

Mann-Whitney Confidence Interval and Test
C50 N = 39 Median = 2.065
C51 N = 34 Median = 1.828
Point estimate for ETA1-ETA2 is -0.181
95.0 Percent CI for ETA1-ETA2 is (-1.237,0.833)
W = 1410.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7193



TBI cases only

Variable	tbi1a any	N	N*	Mean	Median	TrMean
avcc-hip	1	30	3	3.018	1.982	2.527
	2	24	0	3.995	4.022	3.804

Variable	tbi1a any	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.442	0.628	0.010	13.043	0.337
	2	3.424	0.699	0.017	12.161	1.106

Variable	tbi1a any	Q3
avcc-hip	1	4.012
	2	6.468

Mann-Whitney Confidence Interval and Test
C33 N = 30 Median = 1.982
C34 N = 24 Median = 4.022
Point estimate for ETA1-ETA2 is -1.018
95.2 Percent CI for ETA1-ETA2 is (-2.793,0.446)
W = 757.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2400

IL-1B

All cases (TBI and controls)

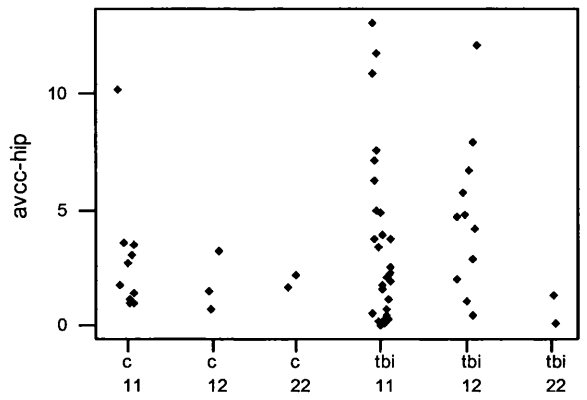
Variable	il1bany2	N	N*	Mean	Median	TrMean
avcc-hip	1	39	2	3.215	2.047	2.873
	2	19	0	3.551	2.838	3.250
	*	17	11	2.225	1.335	1.855

Variable	il1bany2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.445	0.552	0.010	13.043	0.652
	2	3.054	0.701	0.053	12.161	1.294
	*	2.560	0.621	0.033	9.966	0.380

Variable	il1bany2	Q3
avcc-hip	1	3.861
	2	4.753
	*	2.982

Mann-Whitney Confidence Interval and Test
C50 N = 39 Median = 2.047
C51 N = 19 Median = 2.838
Point estimate for ETA1-ETA2 is -0.540
95.1 Percent CI for ETA1-ETA2 is (-1.902,0.792)
W = 1099.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3981

cr3/43 - IL1B



TBI cases only

Variable	tbi1b any	N	N*	Mean	Median	TrMean
avcc-hip	1	29	2	3.323	2.047	3.086
	2	14	0	4.172	4.447	3.849

Variable	tbi1b any	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.691	0.685	0.010	13.043	0.309
	2	3.325	0.889	0.053	12.161	1.231

Variable	tbi1b any	Q3
avcc-hip	1	4.914
	2	5.958

Mann-Whitney Confidence Interval and Test
C33 N = 29 Median = 2.047
C34 N = 14 Median = 4.447
Point estimate for ETA1-ETA2 is -1.012
95.3 Percent CI for ETA1-ETA2 is (-3.164,0.841)
W = 593.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2488
The test is significant at 0.2487 (adjusted for ties)

CD68

IL-1A

All cases (TBI and control cases)

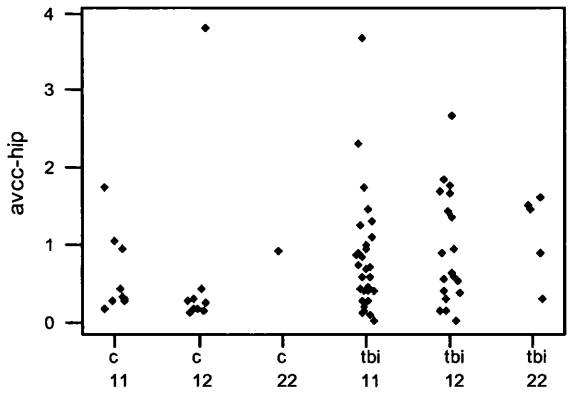
Variable	il1aany2	N	N*	Mean	Median	TrMean
avcc-hip	1	39	3	0.770	0.581	0.682
	2	34	0	0.900	0.579	0.799
	*	2	10	0.4000	0.4000	0.4000

Variable	il1aany2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.706	0.113	0.030	3.693	0.288
	2	0.843	0.145	0.029	3.810	0.276
	*	0.0525	0.0371	0.3629	0.4371	*

Variable	il1aany2	Q3
avcc-hip	1	0.995
	2	1.487

C50 N = 39 Median = 0.5814
C51 N = 34 Median = 0.5793
Point estimate for ETA1-ETA2 is -0.0300
95.0 Percent CI for ETA1-ETA2 is (-0.3315,0.1717)
W = 1414.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7526

CD68 - IL1A



TBI cases only

Variable	tbi1a any	N	N*	Mean	Median	TrMean
avcc-hip	1	30	3	0.816	0.641	0.705
	2	24	0	0.999	0.899	0.967

Variable	tbi1a any	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.753	0.138	0.030	3.693	0.372
	2	0.695	0.142	0.029	2.671	0.379

Variable	tbi1a any	Q3
avcc-hip	1	1.021
	2	1.607

Mann-Whitney Confidence Interval and Test
C33 N = 30 Median = 0.6414
C34 N = 24 Median = 0.8986
Point estimate for ETA1-ETA2 is -0.1914
95.2 Percent CI for ETA1-ETA2 is (-0.6013,0.1216)

W = 759.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2542

IL-1B

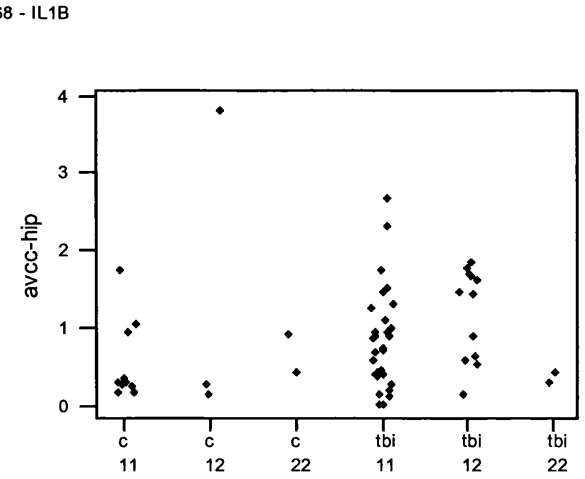
All cases (TBI and control cases)

Variable	il1bany2	N	N*	Mean	Median	TrMean
avcc-hip	1	39	2	0.774	0.687	0.718
	2	19	0	1.093	0.891	0.988
	*	17	11	0.617	0.407	0.446

Variable	il1bany2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.626	0.100	0.029	2.671	0.288
	2	0.895	0.205	0.152	3.810	0.437
	*	0.850	0.206	0.104	3.693	0.219

Variable	il1bany2	Q3
avcc-hip	1	1.040
	2	1.674
	*	0.581

C50 N = 39 Median = 0.6871
C51 N = 19 Median = 0.8914
Point estimate for ETA1-ETA2 is -0.2143
95.1 Percent CI for ETA1-ETA2 is (-0.6341,0.0944)
W = 1071.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1906



TBI cases only

Variable	tbi1b any	N	N*	Mean	Median	TrMean
avcc-hip	1	29	2	0.848	0.754	0.810
	2	14	0	1.081	1.166	1.093

Variable	tbi1b any	StDev	SE Mean	Minimum	Maximum	Q1

avcc-hip	1	0.649	0.121	0.029	2.671	0.385
	2	0.624	0.167	0.152	1.864	0.521

Variable	tbi1b any	Q3
avcc-hip	1	1.181
	2	1.682

Mann-Whitney Confidence Interval and Test
C33 N = 29 Median = 0.7543
C34 N = 14 Median = 1.1657
Point estimate for ETA1-ETA2 is -0.2654
95.3 Percent CI for ETA1-ETA2 is (-0.7472,0.1615)
W = 590.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2183

9.4.7 Assessment of the possession of *IL-1A* 2,2 and the neuroinflammatory response.

CR3/43
IL-1A
All cases (TBI and control cases)

Variable	a22n1y2	N	N*	Mean	Median	TrMean
avcc-hip	1	67	3	3.081	1.940	2.777
	2	6	0	3.883	4.286	3.883
	*	2	10	0.472	0.472	0.472

Variable	a22n1y2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.256	0.398	0.010	13.043	0.667
	2	2.177	0.889	1.294	7.096	1.518
	*	0.592	0.418	0.053	0.890	*

Variable	a22n1y2	Q3
avcc-hip	1	4.183
	2	5.330

C32 N = 67 Median = 1.940
C33 N = 6 Median = 4.286
Point estimate for ETA1-ETA2 is -1.278
95.2 Percent CI for ETA1-ETA2 is (-3.583,0.930)
W = 2416.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2094

TBI cases only

Variable	tbia22	N	N*	Mean	Median	TrMean

avcc-hip	1	49	3	3.361	2.047	3.099
	2	5	0	4.341	4.711	4.341
	*	21	10	2.108	1.430	1.792

Variable	tbia22	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.548	0.507	0.010	13.043	0.367
	2	2.086	0.933	1.294	7.096	2.578
	*	2.095	0.457	0.053	10.170	0.994

Variable	tbia22	Q3
avcc-hip	1	4.914
	2	5.919
	*	2.876

C36 N = 49 Median = 2.047
C37 N = 5 Median = 4.711
Point estimate for ETA1-ETA2 is -1.727
95.1 Percent CI for ETA1-ETA2 is (-4.115,1.344)
W = 1308.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2445

IL-1B

All cases (TBI and controls)

Variable	b22n1y2	N	N*	Mean	Median	TrMean
avcc-hip	1	54	2	3.477	2.622	3.140
	2	4	0	1.275	1.444	1.275
	*	17	11	2.225	1.335	1.855

Variable	b22n1y2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.364	0.458	0.010	13.043	0.941
	2	0.889	0.445	0.053	2.157	0.364
	*	2.560	0.621	0.033	9.966	0.380

Variable	b22n1y2	Q3
avcc-hip	1	4.784
	2	2.016
	*	2.982

C32 N = 54 Median = 2.622
C33 N = 4 Median = 1.444
Point estimate for ETA1-ETA2 is 1.387
95.2 Percent CI for ETA1-ETA2 is (-0.635,4.646)
W = 1636.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1922

TBI cases only

Variable	tbib22	N	N*	Mean	Median	TrMean
avcc-hip	1	41	2	3.742	2.838	3.465
	2	2	0	0.674	0.674	0.674
	*	32	11	2.372	1.655	1.985

Variable	tbib22	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.582	0.559	0.010	13.043	0.565
	2	0.877	0.620	0.053	1.294	*
	*	2.419	0.428	0.033	10.170	0.976

Variable	tbib22	Q3
avcc-hip	1	5.326
	2	*
	*	3.020

C36 N = 41 Median = 2.838
C37 N = 2 Median = 0.674
Point estimate for ETA1-ETA2 is 2.285
95.3 Percent CI for ETA1-ETA2 is (-1.043,10.463)
W = 927.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1577

CD68

IL-1A

All cases (TBI and control cases)

Variable	a22n1y2	N	N*	Mean	Median	TrMean
avcc-hip	1	67	3	0.8037	0.5483	0.7133
	2	6	0	1.130	1.201	1.130
	*	2	10	0.4000	0.4000	0.4000

Variable	a22n1y2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.7863	0.0961	0.0286	3.8100	0.2871
	2	0.510	0.208	0.306	1.633	0.756
	*	0.0525	0.0371	0.3629	0.4371	*

Variable	a22n1y2	Q3
avcc-hip	1	1.0400
	2	1.556

C32 N = 67 Median = 0.5483
C33 N = 6 Median = 1.2014
Point estimate for ETA1-ETA2 is -0.5195
95.2 Percent CI for ETA1-ETA2 is (-1.0303,0.1029)
W = 2396.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0975

TBI cases only

Variable	tbia22	N	N*	Mean	Median	TrMean
avcc-hip	1	49	3	0.869	0.640	0.804
	2	5	0	1.169	1.473	1.169
	*	21	10	0.618	0.303	0.477

Variable	tbia22	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.741	0.106	0.029	3.693	0.385
	2	0.560	0.250	0.306	1.633	0.606
	*	0.832	0.182	0.110	3.810	0.214

Variable	tbia22	Q3
avcc-hip	1	1.287
	2	1.581
	*	0.686

C36 N = 49 Median = 0.6400
C37 N = 5 Median = 1.4729
Point estimate for ETA1-ETA2 is -0.4778
95.1 Percent CI for ETA1-ETA2 is (-1.0512,0.2426)
W = 1305.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2101

IL-1B

All cases (TBI and control cases)

Variable	b22n1y2	N	N*	Mean	Median	TrMean
avcc-hip	1	54	2	0.904	0.740	0.830
	2	4	0	0.529	0.440	0.529
	*	17	11	0.617	0.407	0.446

Variable	b22n1y2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.750	0.102	0.029	3.810	0.295
	2	0.275	0.137	0.306	0.930	0.339
	*	0.850	0.206	0.104	3.693	0.219

Variable	b22n1y2	Q3
avcc-hip	1	1.446
	2	0.808
	0.581	

C32 N = 54 Median = 0.7400
C33 N = 4 Median = 0.4398
Point estimate for ETA1-ETA2 is 0.2002
95.2 Percent CI for ETA1-ETA2 is (-0.2445,1.0062)
W = 1614.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5293

TBI cases only

Variable	tbib22	N	N*	Mean	Median	TrMean
avcc-hip	1	41	2	0.950	0.891	0.917
	2	2	0	0.3714	0.3714	0.3714
	*	32	11	0.678	0.350	0.500

Variable	tbib22	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.647	0.101	0.029	2.671	0.417
	2	0.0929	0.0657	0.3057	0.4371	*
	*	0.891	0.157	0.104	3.810	0.250

Variable	tbib22	Q3
avcc-hip	1	1.469
	2	*
	*	0.774

C36 N = 41 Median = 0.8914
C37 N = 2 Median = 0.3714
Point estimate for ETA1-ETA2 is 0.4647
95.3 Percent CI for ETA1-ETA2 is (-0.2756,1.4328)
W = 925.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1944

9.4.8 Assessment of the possession of any copy of *APOE*ε4 and the neuroinflammatory response.

CD68; Control cases

Variable	con e4-4	N	N*	Mean	Median	TrMean
avcc-hip	1	15	0	0.752	0.363	0.567
	2	5	1	0.2520	0.2825	0.2520

Variable	con e4-4	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.958	0.247	0.110	3.810	0.243
	2	0.0764	0.0342	0.1586	0.3367	0.1718

Variable	con e4-4	Q3
avcc-hip	1	0.961
	2	0.3169

Mann-Whitney Confidence Interval and Test
C40 N = 15 Median = 0.3629
C41 N = 5 Median = 0.2825
Point estimate for ETA1-ETA2 is 0.1272
95.5 Percent CI for ETA1-ETA2 is (-0.0487,0.7716)
W = 174.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1625

CR343; Control cases

Variable	con e4-4	N	N*	Mean	Median	TrMean
avcc-hip	1	15	0	2.540	1.737	2.097
	2	5	1	1.226	1.119	1.226

Variable	con e4-4	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	2.324	0.600	0.667	10.170	1.157
	2	0.565	0.253	0.557	2.065	0.757

Variable	con e4-4	Q3
avcc-hip	1	3.210
	2	1.747

Mann-Whitney Confidence Interval and Test
C42 N = 15 Median = 1.737
C43 N = 5 Median = 1.119
Point estimate for ETA1-ETA2 is 0.727
95.5 Percent CI for ETA1-ETA2 is (-0.228,2.165)
W = 176.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1161

CD68; TBI cases

Variable	tbie4-4	N	N*	Mean	Median	TrMean
avcc-hip	1	42	3	0.926	0.740	0.854
	2	13	2	0.770	0.437	0.739

Variable	tbie4-4	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.752	0.116	0.029	3.693	0.405
	2	0.633	0.176	0.104	1.777	0.252

Variable	tbie4-4	Q3
avcc-hip	1	1.327
	2	1.536

Mann-Whitney Confidence Interval and Test
C40 N = 42 Median = 0.7400
C41 N = 13 Median = 0.4371
Point estimate for ETA1-ETA2 is 0.1302
95.1 Percent CI for ETA1-ETA2 is (-0.2535,0.4891)
W = 1213.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4696

CR343; TBI cases

Variable	tbie4-4	N	N*	Mean	Median	TrMean
avcc-hip	1	42	3	3.657	2.909	3.389
	2	13	2	2.53	0.48	1.88

Variable	tbie4-4	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.377	0.521	0.010	13.043	1.107
	2	3.62	1.00	0.03	12.16	0.13

Variable	tbie4-4	Q3
avcc-hip	1	5.138

2 4.46

Mann-Whitney Confidence Interval and Test
C42 N = 42 Median = 2.909
C43 N = 13 Median = 0.479
Point estimate for ETA1-ETA2 is 1.221
95.1 Percent CI for ETA1-ETA2 is (-0.228,2.948)
W = 1251.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1400

CD68; TAI cases

Variable	tai e4-4	N	N*	Mean	Median	TrMean
avcc-hip	11	17	0	0.669	0.596	0.602
	12	8	0	0.618	0.431	0.618
	21	25	0	1.100	0.891	1.023
	22	5	0	1.013	0.898	1.013

Variable	tai e4-4	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	11	0.589	0.143	0.029	2.320	0.154
	12	0.590	0.208	0.104	1.633	0.166
	21	0.810	0.162	0.272	3.693	0.448
	22	0.689	0.308	0.306	1.777	0.357

Variable	tai e4-4	Q3
avcc-hip	11	0.927
	12	1.217
	21	1.498
	22	1.726

Mann-Whitney Confidence Interval and Test
C43 N = 8 Median = 0.431
C45 N = 5 Median = 0.898
Point estimate for ETA1-ETA2 is -0.279
95.2 Percent CI for ETA1-ETA2 is (-1.351,0.243)
W = 48.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.2723

CR343; TAI cases

Variable	tai e4-4	N	N*	Mean	Median	TrMean
avcc-hip	11	17	0	2.773	2.523	2.273
	12	8	0	1.621	0.367	1.621
	21	25	0	4.258	3.694	4.111
	22	5	0	3.98	2.22	3.98

Variable	tai e4-4	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	11	3.200	0.776	0.010	13.043	0.167
	12	2.597	0.918	0.033	6.723	0.090
	21	3.424	0.685	0.147	11.757	1.449
	22	4.82	2.15	0.04	12.16	0.67

Variable	tai e4-4	Q3
avcc-hip	11	4.286
	12	3.676
	21	6.668
	22	8.17

Mann-Whitney Confidence Interval and Test
C43 N = 8 Median = 0.367
C45 N = 5 Median = 2.223
Point estimate for ETA1-ETA2 is -1.503
95.2 Percent CI for ETA1-ETA2 is (-7.419,2.522)
W = 49.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3413

9.4.9 Assessment of the possession of any copy of *APOE*ε2 and the neuroinflammatory response.

CD68; Control cases

Variable	con e4-2	N	N*	Mean	Median	TrMean
avcc-hip	1	18	0	0.469	0.300	0.412
	2	2	1	2.05	2.05	2.05

Variable	con e4-2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.429	0.101	0.110	1.753	0.181
	2	2.49	1.76	0.29	3.81	*

Variable	con e4-2	Q3
avcc-hip	1	0.564
	2	*

Mann-Whitney Confidence Interval and Test
C40 N = 18 Median = 0.300
C41 N = 2 Median = 2.049
Point estimate for ETA1-ETA2 is -1.118
96.2 Percent CI for ETA1-ETA2 is (-3.652,0.751)
W = 181.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3447

CR343;Control cases

Variable	con e4-2	N	N*	Mean	Median	TrMean
avcc-hip	1	18	0	2.323	1.512	1.943
	2	2	1	1.202	1.202	1.202

Variable	con e4-2	StDev	SE Mean	Minimum	Maximum	Q1

avcc-hip	1	2.177	0.513	0.557	10.170	1.096
	2	0.757	0.535	0.667	1.737	*

Variable	con e4-2	Q3
avcc-hip	1	3.076
	2	*

Mann-Whitney Confidence Interval and Test
C42 N = 18 Median = 1.512
C43 N = 2 Median = 1.202
Point estimate for ETA1-ETA2 is 0.579
96.2 Percent CI for ETA1-ETA2 is (-0.847,8.433)
W = 196.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4128

CD68; TBI cases

Variable	tbi e4-2	N	N*	Mean	Median	TrMean
avcc-hip	1	45	5	0.853	0.596	0.779
	2	10	0	1.050	0.950	1.008

Variable	tbi e4-2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	0.722	0.108	0.029	3.693	0.389
	2	0.745	0.236	0.116	2.320	0.339

Variable	tbi e4-2	Q3
avcc-hip	1	1.317
	2	1.744

Mann-Whitney Confidence Interval and Test
C40 N = 45 Median = 0.5957
C41 N = 10 Median = 0.9504
Point estimate for ETA1-ETA2 is -0.2314
95.2 Percent CI for ETA1-ETA2 is (-0.7464,0.2676)
W = 1218.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3651

CR343; TBI cases

Variable	tbi e4-2	N	N*	Mean	Median	TrMean
avcc-hip	1	45	5	3.309	2.223	3.048
	2	10	0	3.76	2.65	3.06

Variable	tbi e4-2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	1	3.327	0.496	0.010	12.161	0.367
	2	4.07	1.29	0.03	13.04	0.55

Variable	tbi e4-2	Q3
avcc-hip	1	4.816
	2	5.69

Mann-Whitney Confidence Interval and Test

C42 N = 45 Median = 2.223
C43 N = 10 Median = 2.648
Point estimate for ETA1-ETA2 is -0.198
95.2 Percent CI for ETA1-ETA2 is (-2.173,1.797)
W = 1245.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7517

CD68; TAI cases

Variable	tai e4-2	N	N*	Mean	Median	TrMean
avcc-hip	11	20	0	0.592	0.493	0.565
	12	5	0	0.898	0.906	0.898
	21	25	0	1.062	0.891	0.982
	22	5	0	1.201	1.312	1.201

Variable	tai e4-2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	11	0.483	0.108	0.029	1.633	0.166
	12	0.894	0.400	0.116	2.320	0.136
	21	0.817	0.163	0.272	3.693	0.424
	22	0.627	0.280	0.400	1.864	0.563

Variable	tai e4-2	Q3
avcc-hip	11	0.854
	12	1.657
	21	1.498
	22	1.784

Mann-Whitney Confidence Interval and Test
C43 N = 5 Median = 0.906
C45 N = 5 Median = 1.312
Point estimate for ETA1-ETA2 is -0.406
96.3 Percent CI for ETA1-ETA2 is (-1.588,1.008)
W = 24.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.5309

CR343; TAI cases

Variable	tai e4-2	N	N*	Mean	Median	TrMean
avcc-hip	11	20	0	1.898	1.118	1.735
	12	5	0	4.43	3.86	4.43
	21	25	0	4.437	3.723	4.293
	22	5	0	3.08	1.94	3.08

Variable	tai e4-2	StDev	SE Mean	Minimum	Maximum	Q1
avcc-hip	11	2.067	0.462	0.010	6.723	0.153
	12	5.28	2.36	0.03	13.04	0.14
	21	3.731	0.746	0.037	12.161	1.307

	22	2.86	1.28	0.65	7.89	1.12

Variable	tai e4-2	Q3
avcc-hip	11	2.983
	12	9.00
	21	6.668
	22	5.62

Mann-Whitney Confidence Interval and Test
C43 N = 5 Median = 3.861
C45 N = 5 Median = 1.940
Point estimate for ETA1-ETA2 is 0.506
96.3 Percent CI for ETA1-ETA2 is (-4.031,11.105)
W = 28.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 1.0000

9.5 Appendix 5

Tau immunostaining and Image analysis data

Tau data

Semi-quantitative analysis of tau perikaryal immunoreactivity.
Control cases

Age	CA 1	CA 2	CA 3	CA 4	Sub	EC	Neo
33							8
50						1	
59					2		1
69	1				1	4	
71	2		1	1	3	16	

Survival <24 hours

Age	CA 1	CA 2	CA 3	CA 4	Sub	EC	Neo
66					5	6	2
71	1	2			2	1	3
88						15	35

Survival 24 hours- 1 week

Age	CA 1	CA 2	CA 3	CA 4	Sub	EC	Neo
37						1	
73	1				2	5	3
60		1					

Survival 1 week- 1 month

Age	CA 1	CA 2	CA 3	CA 4	Sub	EC	Neo
53						3	1
79	2				6	3	1
83					4	15	4

Legend: CA1-CA4= regions of hippocampus
Sub= subiculum
EC= entorhinnal cortex
Neo= neocortex

Neuroinflammation data

0-19 years **CD 68**

Controls

	cc	cg	cw	tg	tw	alveus	hippo
418/84	x	x	x	1.22	2.38	6.98	4.66
960608	0.08	0.24	0.46	0.03	0.09	0.61	0.50
960051							
980309							
960934							

<24 hours

	cc	cg	cw	tg	tw	alveus	hippo
688/87							
262/89	x	x	x	0.13	0.18	x	x
553/89	x	x	x	0.23	0.34	0.22	0.36
254/90	0.34	0.09	0.23	0.08	0.19	0.08	0.08
980328	0.71	0.34	0.52	0.03	0.12	0.24	0.18

1 day- 1 week

	cc	cg	cw	tg	tw	alveus	hippo
338/90	1.40	0.38	0.60	0.45	0.81	0.66	0.51
649/93	0.40	x	x	0.21	0.17	0.17	0.04
960640							
980389	2.47	0.80	1.05	3.02	0.83	1.50	2.26
990143	2.14	4.94	3.02	0.28	4.33	1.53	2.46

1 week- 1 month

	cc	cg	cw	tg	tw	alveus	hippo
452/90	0.76	0.55	1.00	0.17	0.61	0.61	0.45
960017	3.77	1.10	2.77	0.25	1.02	0.98	0.37
960523	1.45	0.47	0.67	0.17	0.44	x	x
961002	0.18	0.78	1.00	1.28	1.36	0.36	0.12
990234	1.24	0.74	0.72	0.07	0.06	0.01	0.01

20-49 years CD68

Controls

	cc	cg	cw	tg	tw	alveus	hippo
469/90	0.88	0.15	0.50	0.03	0.12	0.01	0.01
55/92	1.10	0.71	1.00	0.01	0.06	0.07	0.02
165/94	1.2	0.16	0.39	0.06	0.18	0.05	0.04
960280	1.2	x	0.63	0.02	0.05	0.11	0.01
960545							

<24 hours

	cc	cg	cw	tg	tw	alveus	hippo
489/88	x	x	x	0.25	0.72	0.18	0.10
96/91	0.24	0.04	0.12	0.44	1.10	0.60	0.32
875/93	0.03	0.06	0.02	0.02	0.02	0.40	0.26
990051	0.33	0.15	0.36	0.55	1.10	0.31	0.18
990219							

1 day- 1 week

	cc	cg	cw	tg	tw	alveus	hippo
980465	x	0.63	1.25	0.06	0.23	0.73	0.39
152/89	0.02	0.01	0.01	0.01	0.01	0.09	0.05
960228	0.48	x	x	0.31	0.26	1.40	2.04
970202	1.96	0.59	1.39	0.15	0.57	3.04	1.90
970696	1.50	0.28	1.00	0.40	1.80	0.84	0.21

1 week- 1 month

	cc	cg	cw	tg	tw	alveus	hippo
717/94	1.71	0.70	0.72	1.02	1.70	0.31	0.08
390/95	x	x	x	0.38	1.74	1.32	0.35
960118							
961000	5.60	1.22	2.94	0.31	0.91	1.13	0.94
990451	2.86	1.21	2.32	0.05	0.16	0.68	3.03

50+ years **CD68**

Controls

	cc	cg	cw	tg	tw	alveus	hippo
335/84							
488/86	x	x	x	0.10	0.19	0.08	0.07
675/87	x	x	x	0.06	0.29	0.41	0.37
08/94	0.18	0.23	0.53	0.02	0.02	0.04	0.09
288/95	0.17	0.21	0.63	0.22	0.58	0.11	0.20

<24 hours

	cc	cg	cw	tg	tw	alveus	hippo
825/87	0.04	0.01	0.06	0.02	0.06	0.01	0.01
432/90	0.02	0.03	0.03	0.05	0.40	0.02	0.18
432/93	0.60	x	x	0.02	0.09	0.03	0.02
960340	1.46	0.42	0.62	x	x	x	x
960462	x	x	x	0.17	0.69	0.49	0.30

1 day- 1 week

	cc	cg	cw	tg	tw	alveus	hippo
487/91	0.57	0.50	0.60	1.30	3.00	3.35	3.12
209/93	x	x	x	0.22	0.60	0.14	0.13
960339	1.45	0.11	0.43	0.07	0.96	0.05	0.01
960351							
980543	0.44	x	x	0.62	1.23	2.34	1.93

1 week- 1 month

	cc	cg	cw	tg	tw	alveus	hippo
215/93	1.46	0.52	0.93	0.01	0.01	0.05	0.21
741/95							
970787	1.37	0.58	1.51	0.19	1.00	2.70	0.34
980067	0.71	2.76	4.34	1.27	1.55	0.61	0.19
980104	1.6	2.57	3.64	1.86	2.52	1.80	2.25

0-19 years **CD68**

Controls

1-3 months

	cc	cg	cw	tg	tw	alveus	hippo
960997	2.22	0.80	2.84	0.03	0.63	0.10	0.03

3-12 months

20-49 years **CD68**

Controls

	cc	cg	cw	tg	tw	alveus	hippo
860477	x	x	x	0.36	1.09	0.20	0.12
860640	x	x	x	0.98	1.89	1.55	2.59
940083	1.02	0.33	0.81	0.05	0.18	0.03	0.12
940433	x	x	x	0.25	0.40	0.20	0.30
940634	2.27	0.27	0.25	x	x	x	x

1-3 months

	cc	cg	cw	tg	tw	alveus	hippo
950513	19.45	0.50	3.64	0.16	0.76	1.14	0.20

3-12 months

	cc	cg	cw	tg	tw	alveus	hippo
880532	x	x	x	0.71	1.86	1.15	0.26
960312	0.93	0.14	0.55	0.05	0.19	1.54	0.77
960534							
980290	5.88	0.21	1.25	0.07	1.08	1.59	0.63

50 + years **CD68**

Controls

	cc	cg	cw	tg	tw	alveus	hippo
850595	1.18	1.09	1.52	0.46	1.36	0.94	0.73
860617	x	x	x	0.12	0.37	0.09	0.08
880377	x	x	x	0.09	0.19	0.19	0.27
900041	x	x	x	0.03	0.09	0.37	0.18
950597	2.00	0.45	1.34	0.63	1.32	0.30	0.69

1-3 months

	cc	cg	cw	tg	tw	alveus	hippo
960175	4.04	0.27	0.93	0.01	0.01	0.01	0.01
950336	3.14	0.54	0.64	x	x	x	x
950716	6.26	0.65	1.75	0.37	0.79	1.72	0.63
960518	1.37	0.52	0.72	0.26	0.69	3.79	1.49

3-12 months

	cc	cg	cw	tg	tw	alveus	hippo
980230	3.78	0.36	0.82	0.10	0.26	0.82	0.20
980512	2.18	0.06	0.32	0.08	0.35	0.03	0.04
980228	3.20	0.06	0.16	0.07	0.27	0.27	0.04
990339	1.18	0.11	0.27	0.28	0.60	0.09	0.06
960008	0.68	0.11	0.56	0.25	0.96	0.09	0.15
970442	2.39	0.19	0.59	0.12	0.46	0.11	0.10
980072	8.97	0.25	0.83	0.18	0.69	0.61	0.19

0-19 years CR343

Controls

	cc	cg	cw	tg	tw	alveus	hippo
418/84	x	x	x	0.46	1.00	0.69	0.52
960608	5.86	1.07	2.70	x	x	x	x
960051							
980309							
960934							

<24 hours

	cc	cg	cw	tg	tw	alveus	hippo
688/87							
262/89	x	x	x	0.06	0.33	x	x
553/89	x	x	x	0.01	0.06	0.20	0.08
254/90	0.01	0.08	0.10	0.01	0.01	0.01	0.01
980328	4.10	x	x	0.09	0.78	1.24	0.26

1 day- 1 week

	cc	cg	cw	tg	tw	alveus	hippo
338/90	0.11	0.03	0.04	0.01	0.02	0.62	0.14
649/93	1.00	x	x	0.07	0.13	0.50	0.13
960640							
980389	10.93	0.68	1.57	0.20	0.14	0.05	0.01
990143	4.96	4.15	6.02	1.88	15.3	9.45	11.18

1 week- 1 month

	cc	cg	cw	tg	tw	alveus	hippo
452/90	4.21	1.10	3.25	6.33	10.66	1.87	5.85
960017	12.65	6.94	11.13	1.47	6.67	3.79	1.03
960523	2.99	0.18	1.56	0.06	0.43	x	x
961002	4.54	1.27	4.08	0.12	0.72	0.23	0.09
990234	3.05	0.20	1.20	1.02	2.22	2.61	1.71

20-49 years **CR343**

Controls

	cc	cg	cw	tg	tw	alveus	hippo
469/90	3.60	0.44	1.14	0.44	1.30	2.32	0.60
55/92	5.60	0.52	1.93	0.01	0.02	0.01	0.01
165/94	2.50	0.38	1.70	0.31	1.20	1.40	0.34
960280	1.10	x	1.60	0.12	0.18	0.18	0.16
960545							

<24 hours

	cc	cg	cw	tg	tw	alveus	hippo
489/88	x	x	x	0.07	0.10	0.49	0.15
96/91	0.01	0.01	0.01	0.02	0.06	0.09	0.06
875/93	0.35	0.04	0.15	0.12	0.16	0.62	0.32
990051	1.01	0.04	0.18	0.13	0.89	0.92	0.18
990219							

1 day- 1 week

	cc	cg	cw	tg	tw	alveus	hippo
980465	x	0.11	0.25	0.13	0.29	1.15	0.28
152/89	0.02	0.01	0.01	0.01	0.01	0.01	0.05
960228	0.78	0.60	0.47	0.68	0.69	11.80	0.54
970202	0.01	0.14	0.63	0.03	0.17	0.02	0.03
970696	4.80	1.10	4.04	1.93	3.21	1.15	1.43

1 week- 1 month

	cc	cg	cw	tg	tw	alveus	hippo
717/94	4.55	7.17	9.15	1.12	4.62	8.12	5.19
390/95	x	x	x	0.16	1.06	5.05	1.40
960118							
961000	15.83	3.30	8.47	0.95	12.25	9.07	5.38
990451	7.42	1.48	5.35	0.90	2.73	11.87	3.23

50+ years **CR343**

Controls

	cc	cg	cw	tg	tw	alveus	Hippo
335/84							
488/86	x	x	x	0.04	0.25	3.27	0.56
675/87	x	x	x	x	x	1.95	2.18
08/94	3.75	0.42	1.17	0.03	0.44	1.46	2.74
288/95	4.87	0.55	1.62	1.15	6.23	4.13	2.67

<24 hours

	cc	cg	cw	tg	tw	alveus	hippo
825/87	0.01	0.01	0.01	0.01	0.01	0.01	0.01
432/90	0.05	0.04	0.23	0.03	0.79	0.22	0.05
432/93	0.01	x	x	0.45	3.69	5.06	4.98
960340	2.48	1.15	1.64	x	x	x	x
960462	x	x	x	2.20	4.58	2.76	2.38

1 day- 1 week

	cc	cg	cw	tg	tw	alveus	hippo
487/91	0.47	0.54	0.74	1.14	3.58	11.00	11.81
209/93	x	x	x	6.06	14.47	2.80	0.89
960339	3.43	0.08	0.74	0.14	3.74	1.06	0.05
960351							
980543	0.89	x	x	0.49	1.28	0.30	0.30

1 week- 1 month

	cc	cg	cw	tg	tw	alveus	hippo
215/93	4.18	0.32	1.24	0.36	0.92	0.65	0.23
741/95							
970787	9.55	4.45	7.84	0.09	2.91	0.97	0.05
980067	9.89	4.20	12.80	0.06	1.53	3.62	1.09
980104	7.11	1.78	1.55	0.98	4.17	11.34	7.72

0-19 years **CR343**

Controls

1-3 months

	cc	cg	cw	tg	tw	alveus	hippo
960997	41.07	6.42	22.45	0.05	0.63	5.38	0.12

3-12 months

20-49 years **CR343**

Controls

	cc	cg	cw	tg	tw	alveus	hippo
860477	x	x	x	0.03	0.14	7.04	1.42
860640	x	x	x	3.08	5.47	1.92	3.75
940083	0.40	x	x	0.06	0.36	1.76	1.87
940433	x	x	x	0.21	0.84	3.62	2.28
940634	2.20	0.58	2.00	x	x	x	x

1-3 months

	cc	cg	cw	tg	tw	alveus	hippo
950513	39.84	6.79	18.28	0.23	2.18	1.97	0.47

3-12 months

	cc	cg	cw	tg	tw	alveus	hippo
880532	x	x	x	4.25	15.05	26.63	6.24
960312	7.28	1.97	6.60	0.14	0.23	4.53	0.14
960534							
980290	30.56	0.26	5.82	0.05	4.67	7.17	1.14

50 + years **CR343**

Controls

	cc	cg	cw	tg	tw	alveus	hippo
850595	5.83	1.75	2.48	1.95	4.18	2.17	0.69
860617	x	x	x	0.11	1.99	2.94	0.30
880377	x	x	x	0.87	2.49	0.28	0.19
900041	x	x	x	4.26	11.05	8.56	16.81
950597	8.14	2.12	4.40	1.45	3.38	2.19	2.35

1-3 months

	cc	cg	cw	tg	tw	alveus	hippo
960175	2.74	0.22	1.09	0.57	6.72	2.13	0.86
950336	6.71	4.01	9.45	x	x	x	x
950716	16.69	3.64	14.94	x	x	x	x
960518	7.55	1.69	3.25	0.16	0.38	11.39	1.64

3-12 months

	cc	cg	cw	tg	tw	alveus	hippo
980230	0.01	0.05	0.01	5.36	16.31	4.31	0.98
980512	0.18	0.02	0.09	0.01	0,04	0.01	0.01
980228	11.97	1.17	4.82	0.63	10.30	4.71	0.55
990339	5.72	1.00	2.20	0.30	2.35	0.14	0.12
960008	10.90	1.11	3.50	0.79	3.01	2.36	1.82
970442	11.08	3.27	5.04	0.34	2.38	2.18	5.94
980072	23.95	7.17	35.4	1.04	10.56	6.03	0.98

9.6 **Appendix 6**

Publications

CPD Cellular Pathology 2001; 3(3):148-151

The Pathology of Head Injury

Colin Smith & David Graham

Colin Smith
MRCPath.
Specialist Registrar in Neuropathology
University of Glasgow

David Graham
FRCPath, PhD
Professor of Neuropathology
University of Glasgow

Correspondence:
Professor DI Graham
University Department of Neuropathology
Institute of Neurological Sciences, South Glasgow University Hospitals NHS Trust
Glasgow, G51 4TF
UK
Tel: 0141 201 2046
Fax: 0141 201 2998

Abstract

Head injury is commonly encountered in autopsies related to trauma. While many of the macroscopic features of head injury do not provide difficulty in interpretation, the microscopical pathology has generated a confusing literature. The interpretation of the microscopical pathology can be of importance to the forensic pathologist. This article aims to offer a guide to the pathologist who may undertake an autopsy with a clinical history of, and/or macroscopic evidence of a head injury. Further details of the autopsy examination may be found in the guideline section of the British Neuropathological Society website (www.bns.org.uk)

Keywords

Trauma, diffuse brain injury.

Introduction

Pathologists undertaking coronial/ procurator fiscal autopsies frequently encounter non-missile traumatic head injury, the neuropathology of which can be divided into two principal categories: 1) focal; and 2) diffuse. Focal injuries include lacerations of the scalp, fracture of the skull, lacerations and contusions of the brain, raised intracranial pressure lesions, and intracranial haemorrhages. Diffuse injuries are ischaemia, brain swelling and diffuse traumatic axonal injury.

In this article we shall try to illustrate the components of adult traumatic brain injury, and relate these to the clinical history.

Scalp lacerations and skull fractures should be accurately documented. This should be routine practise in any autopsy examination and will not be detailed further.

Haematomas

Intracranial haemorrhage is the most common cause of clinical deterioration and death in patients who experience a lucid interval after head injury.[1] Haematomas may act as a mass lesion and produce secondary effects (as detailed below). Extradural, subdural, subarachnoid, and intracerebral haematomas can all be associated with traumatic brain injury.

Extradural haematomas (Figure 1) are seen in some 10% of severely head injured patients, 80% being associated with skull fractures. The majority involve the middle meningeal artery with fractures of the temporal bone, but 20-30% occur at other sites.[2]

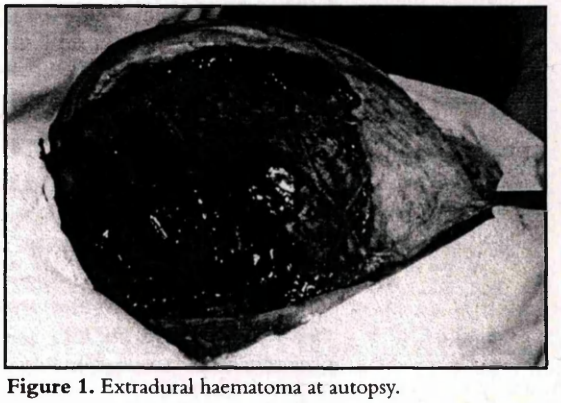


Figure 1. Extradural haematoma at autopsy.

Subdural haematomas (Figure 2) tend to be more extensive than extradural lesions as blood can spread more freely within the subdural space. The majority are due to disruption of parasagittal bridging veins and these can be detected by careful reflection of the dura at autopsy. The volume of the subdural haematoma should be estimated; this can be done by pouring the haematoma into a measuring cylinder. Ageing of subdural haematomas can be difficult both macroscopically and microscopically, although some attempt has been made.[3] In cases of unilateral subdural haematoma there is often accentuation of the gyral pattern ipsilateral to the haematoma, with flattening of the contralateral gyri.

Subarachnoid blood is commonly associated with contusions, but is rarely significant.

Intracerebral haemorrhages (Figure 3) may be superficial, usually associated with contusions, or they may be more deeply seated, usually within the basal ganglia (Table 1). When an intracerebral lesion is in continuity with a subdural haematoma the term "burst lobe" is used.

Contusions

Contusions are superficial lesions in which the pia is intact, whereas in lacerations the pia is

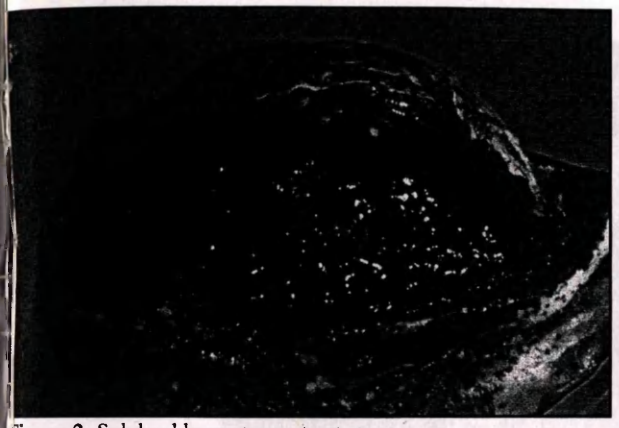


Figure 2. Subdural haematoma at autopsy.

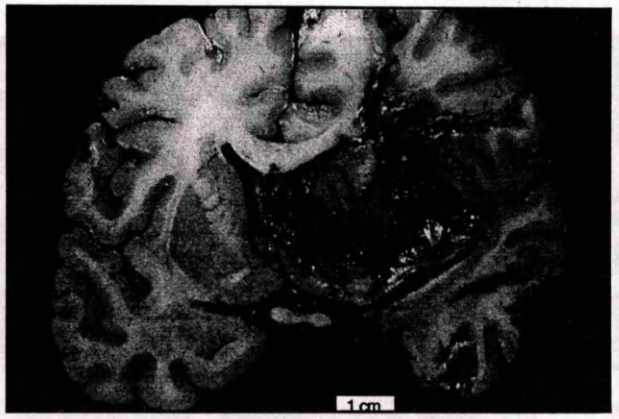


Figure 3. Traumatic intracerebral haemorrhage.

Aetiology	Site
Hypertension	Usually putamen, thalamus, cerebellum, or pons.
Trauma	Deep-basal ganglia and thalamus. Superficial-generally related to overlying contusions.
Cerebral amyloid angiopathy	Superficial lobar haemorrhage,associated with Alzheimer's disease.
Saccular aneurysms	MCA aneurysm- Sylvian fissure IntCA- medial temporal lobe AcomA aneurysm- frontal lobe.
Vascular malformation	May occur at any site.
Neoplasms	Particularly seen with oligodendrogliomas and metastatic tumours.

Other causes such as iatrogenic bleeding (eg warfarin therapy), vasculitis, infection, recreational drugs.
MCA= middle cerbral artery. AcomA= anterior communicating artery. IntCA= internal carotid artery.

Table 1. Causes of intracerebral haematoma.

ruptured. These are seen in approximately 90% of fatal cases of traumatic brain injury, although they may be absent in some 6% of fatal cases.[4] They are more commonly seen at the crests of the frontal and temporal gyri than within sulci and occur principally at sites where the brain comes in contact with the uneven bony surfaces of the base of the skull. When contusions occur on the lateral aspect of the cerebral hemispheres they are usually in association with localised impact injury with or without skull fracture. They are initially haemorrhagic and swollen, but with time they become brown and shrunken as a result of haemosiderin pigmentation and gliosis respectively.

Complications of mass lesions

A supracallosal hernia may obstuct flow within the pericallosal artery (anterior cerebral circulation) resulting in infarction within the corpus callosum and cingulate gyrus (Figure 4). A tentorial hernia may obstruct flow within the posterior cerebral artery resulting in medial occipital cortical infarction, and caudal displacement and elongation of the rostral brainstem may result in brainstem haemorrhage and infarction, a common terminal event in raised intracranial pressure. Clinically a tentorial hernia may produce an ipsilateral fixed dilated pupil, due to ipsilateral oculomotor nerve damage, and ipsilateral weakness due to contralateral cerebral peduncle compression (Kernohan lesion -false localising sign). A wedge of necrosis in the parahippocampal gyrus provides good evidence of raised intracranial pressure during life.[5]

Diffuse injury

Three forms of diffuse brain injury are seen as a consequence of trauma; diffuse ischaemic injury, which involves grey matter, diffuse traumatic axonal injury (TAI), which involves white matter, and brain swelling which may be cytotoxic (grey matter) or vasogenic (principally white matter).

In the absence of a mass lesion, such as a subdural haematoma, persistent coma due to structural damage must be due to diffuse TAI, global hypoxia/ischaemia, or a combination of both.

While focal infarcts are commonly seen after fatal traumatic head injury (91% in one study[6]), usually as a consequence of raised intracranial pressure (ICP) as detailed above, global cerebral ischaemia is less common. Global cerebral ischaemia may be related to hypotension, e.g. after multiple injuries, or secondary to raised ICP resulting in reduced cerebral blood flow. Ischaemic neurons are widely distributed, initially following a pattern of selective vulnerability (Table 2).

Diffuse traumatic axonal injury (TAI) is a histological diagnosis, which requires examination of multiple brain areas[7] (Table 3). A pictorial representation of the recommended blocks is provided (Figure 5). Three grades of diffuse TAI are recognised (Table 4).

Damaged axons are best identified using immunocytochemistry for amyloid precursor protein (Aβ-PP)[8] (Figure 6a); using this technique damaged axons can be identified 2 hours after traumatic head injury,[9] and are seen in all cases who survived 3 hours or more. Aβ-PP acts purely as a marker for disrupted axonal flow and is not a specific indicator of trauma. Aβ-PP positive axons are seen in a variety of conditions that include infarction, hypoglycaemia, and HIV infection.[7] In the presence of an appropriate history of trauma, immunoreactve axons scattered throughout white matter tracts allow a confident diagnosis of diffuse TAI. Aβ-PP positive axons can be

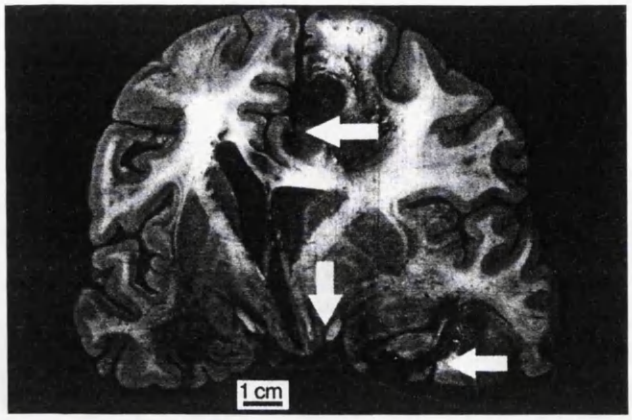


Figure 4a. Brain section demonstrating the complications of raised intracranial pressure. There is a supracallosal hernia with infarction of the parasagittal tissue, and a tentorial hernia with associated infarction. In addition there is downward displacement of the midline structures.

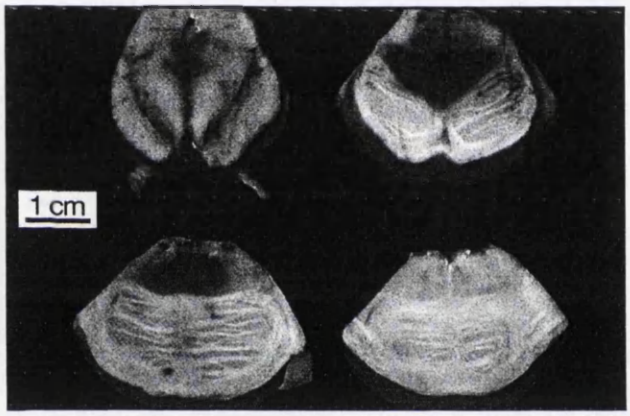


Figure 4c. Brain stem haemorrhage is a common terminal event when the intracranial pressure is high.

identified up to between 1 and 3 months after traumatic brain injury. With longer survival CD 68 immunoreactivity (Figure 6b) can be useful in assessing the extent of axonal pathology, microglial activation mirroring the distribution of axonal pathology.[10]

Traumatic axonal injury is not a static process. A small proportion of axons may be damaged at the time of head injury (primary axotomy), but animal experiments suggest this is not the case for most of the damaged axons, which degenerate over a period of time after the head injury (secondary axotomy).[11] Current evidence indicates that this process may proceed at different rates in different brain areas and that axon size influences the rate of damage. As a consequence, the size and morphology of axonal swellings after trauma do not convey information relating to the timing or severity of the head injury.

Brain swelling can develop either locally, such as in relation to contusions, or can be diffuse involving one or both hemispheres. In diffuse brain swelling hypoxia/ischaemia is the most common underlying pathology. As a consequence of brain swelling there is raised ICP producing ischaemic lesions in the distribution described above. The vascular complications of raised ICP can produce a pattern of Aβ-PP immunoreactivity that is difficult to distinguish from diffuse TAI, and in some cases the interpretation of the underlying pathology is impossible.

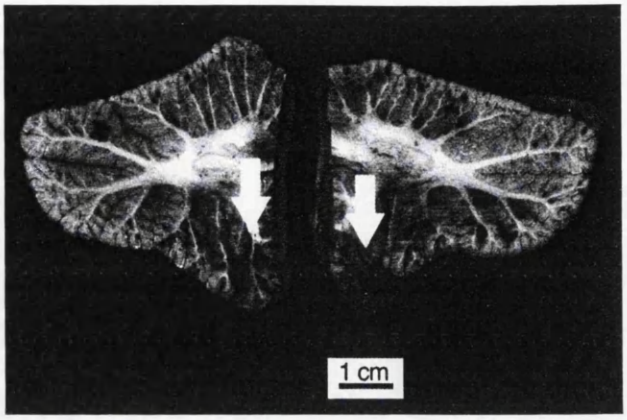


Figure 4b. Tonsillar hernia secondary to raised intracranial pressure. The tonsils are haemorrhagic and necrotic.

- Hippocampus- sector CA1 is most vulnerable, sector CA2 least so.
- Cerebral cortex- neurons of layers 3, 5 and 6 are most vulnerable. Damage is most pronounced within the depths of sulci and is initially most severe posteriorly within the cerebral hemispheres (triple watershed zone).
- Basal ganglia (including thalamus)- variable.
- Cerebellum- Purkinje cells.
- Brainstem- brainstem nuclei tend to be relatively preserved in adults, but when they are affected sensory nuclei are more susceptible than motor nuclei.
- Ischaemic neurons have a time course and incontrovertible evidence of irreversible damage is the presence of incrustations.

Table 2. Selective vulnerability of neurons after cerebral ischaemia.

The following survey is recommended as a minimum to make the diagnosis of diffuse traumatic axonal injury.
Bilateral blocks from a coronal brain slice at the level of the lateral geniculate bodies:

- Corpus callosum with adjacent parasagittal cortex and white matter.
- Deep grey matter including posterior limb of internal capsule
- Temporal lobe including hippocampus.

In addition the following areas require to be sampled:

- Genu (anterior sections)of corpus callosum.
- Cerebellar hemisphere including the dentate nucleus.
- Midbrain at the level of the decussation of the superior cerebellar peduncles.
- Pons at the level of the middle cerebellar peduncles.
- Medulla.

Table 3. Recommened block sampling for diagnosis of diffuse TAI.

Grade 1-	abnormalities limited to histological evidence of axonal damage throughout the white matter.
Grade 2-	in addition to widely distributed axonal injury, there is a focal lesion in the corpus callosum.
Grade 3-	represents the worst injuries within the spectrum, characterised by diffuse axonal damage in the presence of focal lesions in both corpus callosum and brainstem.

Table 4. Grades of diffuse traumatic axonal injury

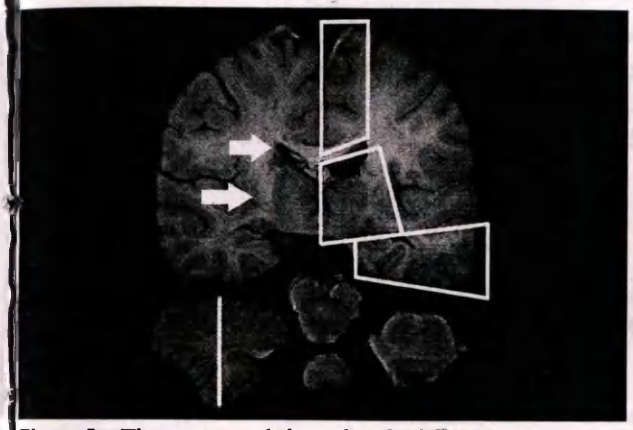


Figure 5a. The recommended sampling for diffuse traumatic axonal injury. The brain is sampled at the level of the lateral geniculate body, and samples should include the corpus callosum and the internal capsule (arrows). Both hemispheres should be sampled.

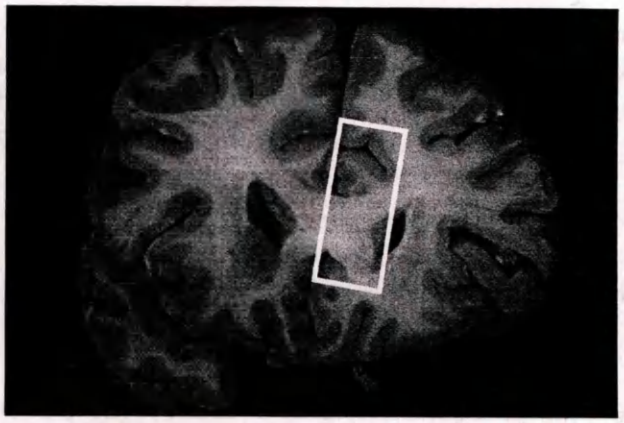


Figure 5b. The rostral corpus callosum should be sampled at the level of the genu.

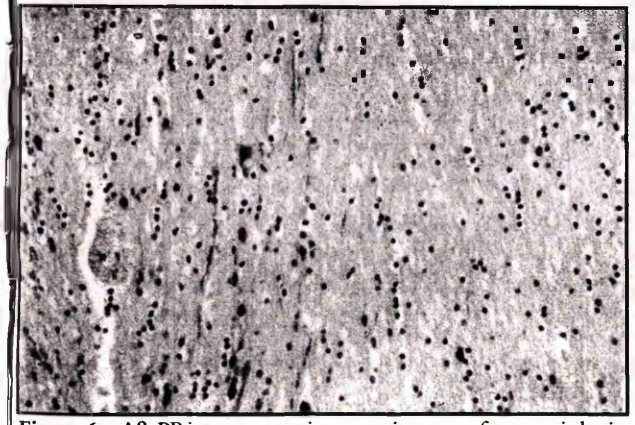


Figure 6a. Aβ-PP immunoreactive axons in a case of traumatic brain injury.

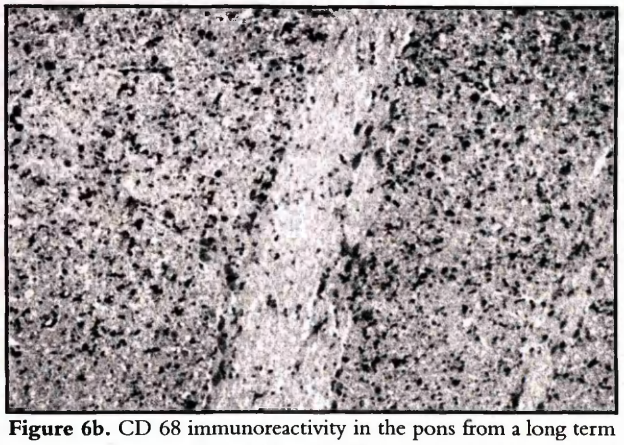


Figure 6b. CD 68 immunoreactivity in the pons from a long term survivor of traumatic brain injury. The macrophages are found predominantly within the corticospinal pathways, and are indicative of Wallerian degeneration within these tracts. There is a relative paucity of immunoreactivity within the transverse pontine fibres.

References

1. Bullock R, Teasdale G: Surgical management of traumatic intracranial haematomas; Braakman R, Editor: Handbook of Clinical Neurology Vol. 15 Head Injury. 1990 Elsevier: Amsterdam; 249-298.
2. McKissock W, Taylor JC, Bloom W *et al.* Extradural haematoma: observations on 125 cases. *Lancet* 1960; **ii**; 167-172.
3. Hardman JM. The pathology of traumatic brain injuries; Thompson RA, Green JR, editors: Advances in Neurology Vol. 22 Complications of Nervous System Trauma. 1979 Raven Press New York; 30-34.
4. Adams JH, Doyle D, Graham DI *et al.* The contusion index: a reappraisal in man and experimental non-missile head injury. *Neuropathology and Applied Neurobiology* 1985; **11**; 299-308.
5. Adams JH, Graham DI. The relationship between ventricular fluid pressure and the neuropathology of raised intracranial pressure. *Neuropathology and Applied Neurobiology* 1976; **2**; 323-332.
6. Graham DI, Adams JH, Doyle D. Ischaemic brain damage in fatal non-missile head injuries. *Journal of Neurological Sciences* 1978; **39**; 213-234.
7. Geddes JF, Whitwell HL, Graham DI. Traumatic axonal injury: practical issues for diagnosis in medicolegal cases. *Neuropathology and Applied Neurobiology* 2000; **26**; 105-116.
8. Sherriff FE, Bridges LR, Gentleman SM *et al.* Markers of axonal injury in post mortem human brain. *Acta Neuropathologica* 1994; **88**; 433-439.
9. McKenzie KJ, McLellan DR, Gentleman SM *et al.* Is beta-APP a marker of axonal damage in short-surviving head injury? *Acta Neuropathologica* 1996; **92**; 608-613.
10. Geddes JF, Vowles GH, Beer TW *et al.* The diagnosis of diffuse axonal injury: implications for forensic practise. *Neuropathology and Applied Neurobiology* 1997; **23**; 339-347.
11. Maxwell WL, Povlishock JT, Graham DI. A mechanistic analysis of non-disruptive axonal injury. *Journal of Neurotrauma* 1997; **14**; 419-440.

Neuropathology and Applied Neurobiology (2003), **29**, 496–502

Tau immunohistochemistry in acute brain injury

C. Smith*†, D. I. Graham*, L. S. Murray‡ and J. A. R. Nicoll*§

**Department of Neuropathology, University of Glasgow, Institute of Neurological Sciences, Southern General Hospital, Glasgow, †Neuropathology Laboratory, Department of Pathology, University of Edinburgh, Western General Hospital, Edinburgh, ‡Department of Medicine and Therapeutics, University of Glasgow, Western Infirmary, Glasgow, and §Division of Clinical Neurosciences, University of Southampton, Southampton General Hospital, Southampton, UK*

C. Smith, D. I. Graham, L. S. Murray and J. A. R. Nicoll (2003) *Neuropathology and Applied Neurobiology* **29**, 496–502
Tau immunohistochemistry in acute brain injury

Epidemiological studies have identified a history of head injury as a risk factor for Alzheimer's disease. However, the neuropathological mechanism underlying this relationship is as yet unclear. Neuronal cytoskeletal changes in the form of neurofibrillary tangles and neuropil threads have recently been demonstrated in young men who had sustained repetitive head injury and subsequently died in their 20s. In addition, recent experimental studies have found accumulation of tau within neuronal somata and damaged axons following diffuse brain injury. We hypothesized that tau-immunoreactive tangles may be present in the brains of patients who died after a single acute blunt head injury. A total of 45 cases of fatal head injury were immunostained for tau. They comprised nine groups ($n = 5$ for each group) separated by age (0–19 years, 20–50 years, 50+years) and survival time (<24 h, 24 h–1 week, 1 week–1 month) and were compared with age-matched controls. Subtle alterations in tau immunoreactivity, for example, in oligodendrocytes, were present in some head injury cases but not controls. However, neurofibrillary tangles did not appear more prevalent after traumatic brain injury (TBI) when compared with age-matched controls. Although alterations in tau immunoreactivity may occur which warrant further study, neurofibrillary tangles were not more prevalent after a single fatal episode of TBI.

Keywords: Alzheimer's disease, tau, traumatic brain injury

Introduction

Alzheimer's disease (AD) is a major cause of morbidity and mortality in the community. A number of risk factors have been identified, one of which is a previous history of head injury. There is a considerable epidemiological literature examining the relationship between traumatic brain injury (TBI) and the development of AD in later life. Many of these studies take the form of retrospective case–control studies and are therefore subject to recall bias. However, an association between TBI and AD has been reported by some [9,14,17,18] but not all authors [5,16,27]. Guo *et al.* [9] studied 2233 individuals who met the criteria for probable or definite AD, and compared them with 14 668 controls (first-degree relatives or spouses) as part of the MIRAGE (Multi-Institutional Research in Alzheimer Genetic Epidemiology) project. They reported that TBI was a risk factor for AD and that the risk was proportional to the severity of the injury. For example, comparison of probands with unaffected spouses yielded odds ratios for AD of 9.9 for head injury with loss of consciousness and 3.1 for head injury without loss of consciousness. Comparison of probands with their parents and sibs yielded odds ratios of 4.0 for head injury with loss of consciousness and 2.0 for head injury without loss of consciousness. At age

Correspondence: C. Smith, Neuropathology Laboratory, Department of Pathology, University of Edinburgh, Western General Hospital, Edinburgh, EH4 2XU, UK. Tel: 0131–537 1975; Fax: 0131–537 1013: E-mail: col.smith@ed.ac.uk

93 years the lifetime risk of developing AD was 77.2% for those with and 40.1% for those without a history of head injury. A number of prospective studies have been designed to try and address the problems of recall bias inherent in case–control studies. Again, however, there are conflicting data. Some studies [3,20,24] have reported between a 3 × and 4 × increased incidence of AD with a history of head injury. Against these data, however, there are a number of prospective studies which have failed to demonstrate an association between TBI and AD [13,15].

The mechanisms underlying a possible association between TBI and AD have not been determined. One of the principal features of AD is pathology in the form of neurofibrillary tangles and neuropil threads within which abnormal forms of the microtubule-associated protein tau are found. Cytoskeletal pathology after TBI has been examined experimentally using a pig model with injury induced via controlled head rotational acceleration [25]. Head-injured pigs were examined at days 1, 3, 7 and 10 after injury, and compared to control animals without head injury. Within the experimental group tau and neurofilament accumulations were identified immunohistochemically in the white matter, colocalized with damaged axons (Aβ-PP immunoreactive), and within neuronal perikarya in the cerebral cortex.

Very little data exist on tau antibodies in man after a single episode of TBI, although cleaved forms of tau protein are markedly elevated in the cerebrospinal fluid of brain-injured patients [28]. Newman *et al.* [19] assessed hyperphosphorylated tau in man after fatal TBI using the Alz50 antibody. They studied 27 cases of fatal TBI with 23 age-matched, nonhead-injured controls. A number of brain regions were examined including frontal and cingulate cortex, hippocampus, thalamus and pons. While eight trauma cases had tau-immunoreactive neurofibrillary pathology, the majority were older individuals (only one case was under the age of 51) and the distribution was comparable to controls. One case was a 16-year-old male who had tau-immunoreactive neurites in the pons only. In addition, tau immunoreactivity has been demonstrated in oligodendrocytes in patients dying after stroke and TBI [11].

However, cytoskeletal pathology is observed in cases of repetitive relatively mild head injury including, for example, in boxing. Neurofibrillary tangles scattered throughout the cerebral cortex and the brainstem, being most prominent in the medial temporal cortex, were reported in elderly ex-boxers by Corsellis *et al.* [4]. In some cases this was associated with cognitive impairment, the condition being called *dementia pugilistica*. Geddes *et al.* [6] examined the brains of four young adults (age range: 23–28 years) with a history of repetitive head injury (two boxers, one footballer and one mentally subnormal patient with a history of self-inflicted head banging) and a frontal lobectomy specimen from an individual with intractable complex partial seizures with recurrent minor head injury. They identified widespread neocortical neurofibrillary tangles and neuropil threads not seen in age-matched controls which in areas showed a perivascular distribution. Schmidt *et al.* [23] have compared the molecular profiles of the neurofibrillary tangles in *dementia pugilistica* and AD. They found that *dementia pugilistica* and AD had a common tau isoform and phosphorylation profile. They concluded that the mechanisms underlying both these conditions might be similar.

In light of this background information the present study was designed to test the hypothesis that tau accumulation in humans in the acute phase after TBI is similar to that seen in animal models.

Materials and methods

Cases of TBI were selected from the archives of the Glasgow Neuropathology Department. For each case detailed clinico-neuropathological information was available, and a standardized protocol for tissue block sampling had been used. A total of 45 acute TBI cases with varying survival times ranging from < 24 h up to 1 month (Tables 1, 2 and 3) were included in this study. In general, the acute TBI cases had all suffered a severe head injury [Glasgow Coma Scale (GCS): ≤ 8] with only one case having a moderate injury (GCS: 9–12). Four cases had a mild head injury (GCS: 13–15) and died of pathology not directly related to the brain injury. In 12 cases GCS was not recorded as patients either died rapidly before hospital admission or were not formally assessed. In all of these cases the pathology suggests a significant head injury. The mechanisms of injury varied with road traffic accidents (RTAs) being more common in the < 20-year-old group, and falls more common in the > 50-year-old group. Fifteen cases with no significant neurological impairment or neuropathological abnormality were used as controls (Table 4). The Research Ethics Committee of the Southern General Hospital approved the study.

The brains had been fixed in 10% formal saline for a minimum of 3 weeks before dissection after which a stan-

Table 1. Details of traumatic brain injury cases aged < 20 years used in study

Survival	*Age*	*Documented survival*	*Severity of head injury (admission GCS)*	*Mechanism of head injury*	*Main pathology*
< 24 h	8 weeks	4 h	Severe (NA)	RTA	DVI
	1.5 years	22 h	Severe (NA)	Fall	TAI
	3 years	21 h	Severe (5)	RTA	TAI, swelling
	9 years	10 h	Severe (3)	RTA	TAI, swelling
	14 years	7 h	Severe (3)	RTA	DVI
24 h–1 week	3 years	48 h	Severe (NA)	RTA	Brain swelling
	5 years	3 days	Severe (NA)	RTA	TAI, swelling
	7 years	48 h	Severe (6)	RTA	Brain swelling
	12 years	7 days	Severe (5)	RTA	ICH, TAI
	14 years	48 h	Severe (3)	RTA	TAI, swelling
1 week–1 month	5 years	9 days	Severe (5)	RTA	Acute LSDH
	15 years	8 days	Severe (4)	RTA	TAI, swelling
	15 years	8 days	Severe (4)	RTA	TAI, swelling
	17 years	14 days	Severe (5)	RTA	TAI
	19 years	21 days	Severe (3)	RTA	TAI

GCS, Glasgow Coma Scale; NA, not available; RTA, road traffic accident; DVI, diffuse vascular injury; TAI, diffuse traumatic axonal injury; ICH, intracerebral haemorrhage; LSDH, left subdural haemorrhage.

Table 2. Details of traumatic brain injury cases aged 20–50 years used in study

Survival	*Age (years)*	*Documented survival*	*Severity of head injury (admission GCS)*	*Mechanism of head injury*	*Main pathology*
< 24 h	23	8 h	Severe (3)	RTA	TAI
	25	20 h	Severe (4)	RTA	Global ischaemia
	30	16 h	Severe (3)	RTA	Acute LSDH
	34	23 h	Severe (3)	Fall	Acute LSDH
	39	12 h	Severe (3)	RTA	DVI
24 h–1 week	23	3 days	Mild (15)	Assault	Drug overdose
	26	24 h	Severe (3)	Fall	Contusions, swelling
	26	4 days	Severe (6)	Fall	Contusions, swelling
	37	24 h	Severe (3)	Fall	Acute RSDH
	40	5 days	Severe (4)	Assault	Acute LSDH
1 week–1 month	21	9 days	Severe (NA)	RTA	TAI, acute LSDH
	23	28 days	Severe (3)	Assault	TAI
	27	11 days	Moderate (10)	Fall	TAI, R burst lobe
	30	18 days	Mild (15)	Fall	Extracranial pathology
	35	10 days	Mild (15)	Fall	Exsanguination

GCS, Glasgow Coma Scale; RTA, road traffic accident; DVI, diffuse vascular injury; TAI, diffuse traumatic axonal injury; ICH, intracerebral haemorrhage; R/LSDH, right/left subdural haemorrhage.

dardized brain cut and histological sampling were undertaken. The tissue was processed in a VIP tissue processor (Bayer Diagnostics, Newbury, UK) using a 60-h cycle and embedded in paraffin wax. Eight-micrometre-thick sections of temporal lobe including hippocampus were cut and stained with haematoxylin and eosin. In addition, immunohistochemistry was undertaken for tau (monoclonal antibody, Dako 1 : 15000). No pretreatment was required and the primary antibody was applied overnight at 4°C. This antibody reacts with both the phosphorylated and nonphosphorylated forms of tau protein, and labels the tau protein of neurofibrillary tangles. The antibody was detected using the ABC kit (Vecta Stain, Vector Laboratories, Peterborough, UK) and developed with diaminobenzidine. Immunostained sections were assessed 'blind' by C.S. and J.A.R.N.

Table 3. Details of traumatic brain injury cases aged > 50 years used in study

Survival	*Age (years)*	*Documented survival*	*Severity of head injury (admission GCS)*	*Mechanism of head injury*	*Main pathology*
< 24 h	51	12 h	Severe (3)	Fall	Acute RSDH
	59	20 h	Severe (3)	Fall	Bilateral acute SDH
	66	21 h	Severe (4)	RTA	Acute LSDH
	71	12 h	Severe (NA)	Fall	Acute LSDH
	88	7 h	Severe (NA)	Fall	Acute LSDH
24 h–1 week	53	2.5 days	Severe (NA)	Fall	Acute RSDH
	56	4 days	Severe (5)	Fall	Acute RSDH, TAI
	60	8 days	Severe (NA)	Fall	ICH
	68	3 days	Severe (4)	RTA	Acute LSDH, TAI
	73	7 days	Severe (NA)	RTA	Contusions, swelling
1 week–1 month	53	11 days	Severe (3)	Fall	Acute LSDH
	59	17 days	Severe (6)	Fall	LSDH
	60	10 days	Mild (14)	Fall	ICH, TAI
	79	8 days	Severe (NA)	RTA	Acute LSDH
	83	18 days	Severe (NA)	RTA	ICH, TAI

GCS, Glasgow Coma Scale; NA, not available; RTA, road traffic accident; TAI, diffuse traumatic axonal injury; ICH, intracerebral haemorrhage; R/LSDH, right/left subdural haemorrhage.

Table 4. Details of control cases (non-head injured controls with no neurological disease) used in study

<20 years		*20–50 years*		*>50 years*	
Age	*Cause of death*	*Age*	*Cause of death*	*Age*	*Cause of death*
4	Congenital heart disease	20	Drug overdose	50	Metastatic carcinoma
8	Viral infection	21	Septic shock	59	Disseminated Langerhan's histiocytosis
10	Burns	28	Drug overdose	68	Ruptured aortic aneurysm
18	Leukaemia	33	Ischaemic heart disease	69	Congestive cardiac failure
18	Systemic Hodgkin's disease	38	Congestive cardiac failure	71	Pneumonia

Results

Four patterns of tau immunoreactivity were seen:

1 neuronal perikaryal immunoreactivity (Figure 1a);
2 neuropil threads (Figure 1a);
3 glial cell immunoreactivity with associated punctate staining in white matter (Figure 1b); and
4 diffuse neuropil staining (Figure 1c).

Neuronal perikaryal immunoreactivity (Table 5) was seen in the form of fibrillary structures (neurofibrillary tangles). These were identified by anatomical region and analysed semiquantitatively as total number per × 10 fields. Neuronal perikaryal immunoreactivity was seen in both TBI and control cases and, in both groups, increased with age.

Neuropil threads (Table 5) were assessed as being either present or absent. Neuropil threads, like neuronal perikaryal immunoreactivity, was seen in both TBI and control cases and, in both groups, increased with age.

Glial cell immunoreactivity (Table 5) was assessed as either present or absent. Glial tau immunoreactivity was seen in some TBI cases but, in general, was a not a feature of control cases.

Diffuse neuropil staining (Table 5) was assessed as either present or absent. This pattern of immunoreactivity was seen at all ages in both TBI and control cases.

Discussion

We have assessed tau immunoreactivity in the hippocampus and adjacent temporal lobe in cases of fatal TBI with survival times ranging from < 24 h up to 1 month. We have shown that, unlike the previously reported animal studies, tau immunoreactivity is a feature of control brains, that is, brains with neither clinical nor gross neu-

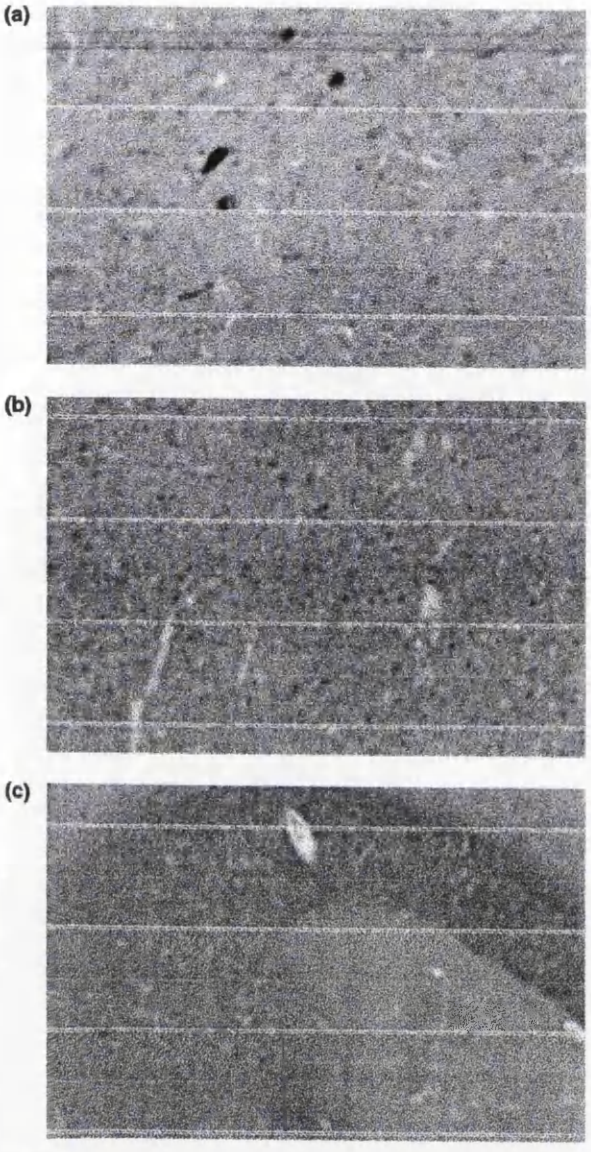


Figure 1. (**a**) Photomicrograph from the medial temporal cortex demonstrating both neuronal perikaryal immunoreactivity and neuropil threads. Tau-immunoreactive fibrillary structures (neurofibrillary tangles) are seen in four neuronal cell bodies, and neuropil threads are numerous. Tau immunostaining × 20. (**b**) Photomicrograph from cerebral white matter showing tau immunoreactivity within glial cell bodies. In addition, there is a punctate pattern of staining within the white matter. Tau immunostaining × 20. (**c**) Photomicrograph from the hippocampus showing diffuse tau immunoreactivity in the stratum pyramidalis of the cornu ammonis. Tau immunostaining × 20

ropathological abnormalities. This was seen with the tau perikaryal, neuropil thread and diffuse neuropil immunoreactivity. Tau perikaryal and neuropil thread immunoreactivity increased with age, while diffuse tau neuropil

Table 5. Comparison of tau immunoreactivity staining patterns at different ages and survival times

	<20 years				*20–50 years*				*>50 years*			
Tau immunoreactivity staining pattern	*Perikaryal*	*Threads*	*Glia*	*Neuropil*	*Perikaryal*	*Threads*	*Glia*	*Neuropil*	*Perikaryal*	*Threads*	*Glia*	*Neuropil*
Non-head injury controls with no neurological disease	0/5	0/5	1/5	1/5	1/5	1/5	0/5	1/5	4/5	1/5	0/5	3/5
Survival < 24 h	0/5	0/5	1/5	2/5	0/5	0/5	2/5	3/5	3/5	2/5	0/5	0/5
Survival 24 h–1 week	0/5	0/5	1/5	1/5	1/5	1/5	0/5	3/5	2/5	2/5	2/5	3/5
Survival 1 week–1 month	0/5	0/5	0/5	2/5	0/5	0/5	1/5	1/5	3/5	2/5	2/5	2/5

immunoreactivity was seen at all ages. Tau glial immunoreactivity, however, did appear to be associated with acute TBI and immunoreactivity could be detected in cases with a survival of < 24 h. We could not demonstrate perikaryal accumulation of phosphorylated tau after acute TBI in these human studies, a finding reported in the pig model [25]. This experimental study used controlled head rotational acceleration to induce injury, resulting in diffuse traumatic axonal pathology. A range of antibodies (Tau-2, PHF-1, PHF-6 and PHF-13.5) was used to assess tau pathology. The current study has examined human material derived from cases of fatal TBI with a variety of primary insults (RTA, fall and assault) producing mixed focal and diffuse pathologies, including diffuse traumatic axonal injury (TAI). It is probable that the loading conditions determine the brain response, and that the highly controlled laboratory situation accentuates a specific brain response. The current study used only a single antibody to detect tau (Dako monoclonal antibody), and a wider panel of antibodies, such as that used by Smith *et al.* [25], may be required to detect specific cytoskeletal abnormalities. In addition, there may be species differences, such that cytoskeletal disruption, if present in man, may develop over a different time course. Smith *et al.* [25] demonstrated axonal tau immunoreactivity at 3 days post-trauma, and consistently found expression up to 10 days post-trauma (latest time point examined). Our study looked up to 1 month post-trauma, but longer survival times may need to be examined to fully assess the possibility of tau expression after TBI.

The study by Newman *et al.* [19], although looking at TBI in man, utilized a different antibody against tau from this study. While the current study utilized an antibody that reacts with both phosphorylated and nonphosphorylated forms of tau, Newman *et al.* used Alz50, an antibody which selectively binds specific conformations of the tau protein [2]. The different antibodies may identify different stages in the temporal evolution of neurofibrillary pathology.

The cases of repetitive mild TBI reported by Geddes *et al.* [6] were all subjected to repetitive relatively mild blows to the head. These cases clearly differ from the mixed neuropathologies seen in most cases of human TBI, but demonstrate that in this rather specific type of insult TBI is associated with tau pathology. In cases of fatal TBI following a single blow to the head structural cytoskeletal disturbances involving tau may be initiated. The pathological response to human TAI is known to develop over a period of time [8], and the cytoskeletal response may be modified by coexistent primary and secondary pathologies. We may have to look at survival times beyond 1 month to fully assess the role of tau in the response to TBI, and the relationship between a single episode of TBI and the development of Alzheimer type pathology. A second pathological feature associated with AD is the deposition of Aβ plaques within the neuropil. Diffuse Aβ plaques have been identified in approximately 30% of patients who die shortly after a single episode of severe TBI [21,22]. This is a higher proportion than in non-head injury controls. Another group [1], however, has not confirmed this observation. Most of the deposit is in the form of Aβ42 [7,10], which is believed to be of pathological significance in AD.

In humans, genetic influences may play an important role in modifying the outcome after TBI. Possession of *APOE* ε4 is associated with a worse outcome after TBI [26], and with an increased severity of chronic neurological deficits in high-exposure boxers [12], with 'high-exposure' being defined as participation in at least 12 professional bouts. The role of *APOE* polymorphisms in modifying cytoskeletal responses after TBI was not addressed in the current study but may be an area of future research.

Acknowledgements

This work was supported by an MRC research grant. Dr C. Smith was supported by a Clinical Research Fellow grant from the Scottish Council for Postgraduate Medical and Dental Education.

References

1 Adle-Biassette H, Duyckaerts C, Wasowicz M, He Y, Fornes P, Foncin JF, Lecomte D, Hauw JJ. Beta AP deposition and head trauma. *Neurobiol Aging* 1996; 17: 415–9
2 Carmel G, Mager EM, Binder LI, Kuret J. The structural basis of monoclonal antibody Alz50s selectivity for Alzheimer's disease pathology. *J Biol Chem* 1996; 271: 32789–95
3 Corkin S, Rosen TJ, Sullivan EV, Clegg RA. Penetrating head injury in young adulthood exacerbates cognitive decline in later years. *J Neurosci* 1989; 9: 3876–83
4 Corsellis JAN, Bruton CJ, Freeman-Browne D. The aftermath of boxing. *Psychol Med* 1973; 3: 270–303
5 Fratiglioni L, Ahlbom A, Viitanen M, Winblad B. Risk factors for late-onset Alzheimer's disease: a population-based, case–control study. *Ann Neurol* 1993; 33: 258–66
6 Geddes JF, Vowles GH, Nicoll JAR, Revesz T. Neuronal cytoskeletal changes are an early consequence of repetitive head injury. *Acta Neuropathol* 1999; 98: 171–8

7 Gentleman SM, Greenberg BD, Savage MJ, Noori M, Newman SJ, Roberts GW, Griffin WS, Graham DI. A beta 42 is the predominant form of amyloid beta-protein in the brains of short-term survivors of head injury. *Neuroreport* 1997; 8: 1519–22
8 Gentleman SM, Roberts GW, Gennarelli TA, Maxwell WL, Adams JH, Kerr S, Graham DI. Axonal injury: a universal consequence of fatal closed head injury? *Acta Neuropathol* 1995; 89: 537–43
9 Guo Z, Cupples LA, Kurz A, Auerbach SH, Volicer L, Chui H, Green RC, Sadovnick AD, Duara R, DeCarli C, Johnson K, Go RC, Growdon JH, Haines JL, Kukull WA, Farrer LA. Head injury and the risk of Alzheimer's disease in the MIRAGE study. *Neurology* 2000; 54: 1316–23
10 Horsburgh K, Cole GM, Yang F, Savage MJ, Greenberg BD, Gentleman SM, Graham DI, Nicoll JA. beta-amyloid (Abeta)42(43), abeta42, abeta40 and apoE immunostaining of plaques in fatal head injury. *Neuropathol Appl Neurobiol* 2000; 26: 124–32
11 Irving EA, Nicoll J, Graham DI, Dewar D. Increased tau immunoreactivity in oligodendrocytes following human stroke and head injury. *Neurosci Lett* 1996; 213: 189–92
12 Jordan BD, Relkin NR, Ravdin LD, Jacobs AR, Bennett A, Gandy S. Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. *JAMA* 1997; 278: 136–40
13 Launer LJ, Anderson K, Dewey ME, Letenneur L, Ott A, Amaducci LA, Brayne C, Copeland JR, Dartigues JF, Kragh-Sorensen P, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A. Rates and risk factors for dementia and Alzheimer's disease: results from EURODEM incidence research group and work groups. European studies of dementia. *Neurology* 1999; 52: 78–84
14 Mayeux R, Ottman R, Tang MX, Noboa-Bauza L, Marder K, Gurland B, Stern Y. Genetic susceptability and head injury as risk factors for Alzheimer's disease among community-dwelling elderly persons and their first degree relatives. *Ann Neurol* 1993; 33: 494–501
15 Mehta KM, Ott A, Kalmijn S, Slooter AJ, van Duijn CM, Hofman A, Breteler MM. Head trauma and the risk of dementia and Alzheimer's disease: the Rotterdam study. *Neurology* 1999; 53: 1959–62
16 Mendez MF, Underwood KL, Zander BA, Mastri AR, Sung JH, Frey WH. Risk factors in Alzheimer's disease: a clinicopathological study. *Neurology* 1992; 42: 770–5
17 Mortimer JA, French LR, Hutton JT, Schuman LM. Head injury as a risk factor for Alzheimer's disease. *Neurology* 1985; 35: 264–7
18 Nemetz PN, Leibson C, Naessens JM, Beard M, Kokmen E, Annegers JF, Kurland LT. Traumatic brain injury and time to onset of Alzheimer's disease: a population-based study. *Am J Epidemiol* 1999; 149: 32–40
19 Newman SJ, Gentleman SM, Graham DI, Brown F, Roberts GW. Tissue distribution and cellular localisation of hyperphosphorylated tau in human head injury and age-matched controls. In *Research Advances in Alzheimer's Disease and Related Disorders*. Eds K Iqbal, JA Mortimer, B Winblad, HM Wisniewski. Chichester: Wiley, 1995; 397–403
20 Plassman BL, Havlik RJ, Steffens DC, Helms MJ, Newman TN, Drosdick D, Phillips C, Gau BA, Welsh-Bohmer KA, Burke JR, Guralnik JM, Breitner JC. Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology* 2000; 55: 1158–66
21 Roberts GW, Gentleman SM, Lynch A, Graham DI. β-A4 amyloid protein deposition in brain after head trauma. *Lancet* 1991; 338: 1422–3
22 Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI. β-amyloid protein deposition in the brain following severe head injury: implications for the pathogenesis of Alzheimer's disease. *J Neurol Neurosurg Psychiat* 1994; 57: 419–25
23 Schmidt ML, Zhukareva V, Newell KL, Lee VM-Y, Trojanowski JQ. Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. *Acta Neuropathol* 2001; 101: 518–24
24 Schofield PW, Tang M, Marder K, Bell K, Dooneief G, Chun M, Sano M, Stern Y, Mayeux R. Alzheimer's disease after remote head injury: an incidence study. *J Neurol Neurosurg Psychiat* 1997; 62: 119–24
25 Smith DH, Chen XH, Nonaka M, Trojanowski JQ, Lee VM, Saatman KE, Leoni MJ, Xu BN, Wolf JA, Meaney DF. Accumulation of amyloid beta and tau and the formation of neurofilament inclusions following diffuse brain injury in the pig. *J Neuropath Exp Neurol* 1999; 58: 982–92
26 Teasdale GM, Nicoll JA, Murray G, Fiddes M. Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* 1997; 350: 1069–71
27 Williams DB, Annegers JF, Kokmen E, O'Brien PC, Kurland LT. Brain injury and neurologic sequelae: a cohort study of dementia, parkinsonism, and amyotrophic lateral sclerosis. *Neurology* 1991; 41: 1554–7
28 Zemlan FP, Rosenberg WS, Luebbe PA, Campbell TA, Dean GE, Weiner NE, Cohen JA, Rudick RA, Woo D. Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins. *J Neurochem* 1999; 73: 437–8

Received 20 November 2002
Accepted after revision 14 May 2003

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

20

Head injury and dementia

Colin Smith[1], James A. R. Nicoll[2], and David I. Graham[2]

[1]University of Edinburgh, Western General Hospital, UK
[2]Institute of Neuropathological Studies, Southern General Hospital, Glasgow, UK

Introduction

Traumatic brain injury remains a significant cause of morbidity and mortality throughout the world. In the United Kingdom more than 150 000 patients are admitted to hospital each year with a head injury. Of this group more than 80% are classified as having a mild head injury, as defined by the Glasgow Coma Scale (GCS). The GCS (Teasdale & Jennett 1974, 1976) provides a means of quantifying the level of consciousness after traumatic brain injury based on the clinical features of verbal performance, eye opening and motor response. Using this scale three levels of severity of head injury are defined; mild (score 13–15), moderate (score 9–12), and severe (score 3–8).

Approximately 1–2% of patients admitted to hospital after traumatic brain injury die as a consequence of their injuries. The majority of fatalities are within the severe head injury group, with 40% of the cases resulting in death at 6 months (Murray *et al.*, 1999).

Among survivors of traumatic brain injury of all grades chronic disability may have a physical component although it is predominantly the cognitive and behavioural problems which provide the greatest challenge (Jennett *et al.*, 1981). Outcome may be assessed by the Glasgow Outcome Scale (GOS) (Jennett & Bond 1975) which defines four outcome states; death/vegetative state, severe disability, moderate disability, and good recovery. The GOS is based predominantly on assessment of social reintegration after traumatic brain injury involving a structured questionnaire-based interview. This has recently been modified as the extended GOS (Teasdale *et al.*, 1998). Predictors of neurobehavioural outcome in adults include age (greater than 50 years is a poor prognostic factor), the acute GCS, abnormal brain stem reflexes, subacute ventricular enlargement, neurological deficit, and the duration of post-traumatic amnesia (Capruso & Levin, 2000).

Somewhat surprisingly, recent studies have indicated that the incidence of moderate and severe disability in young people and adults one year after mild head injury is similar to that seen in survivors of moderate and severe head injury (Thornhill *et al.*, 2000). In addition there is evidence (discussed below) that traumatic brain injury may be associated with continuing cognitive decline in later years and with an increased incidence of Alzheimer's disease (AD). The mechanisms underlying the association between head injury and AD are unknown as yet, although, as we shall discuss in this chapter, the response to traumatic brain injury and the pathology of AD have some features in common not only in terms of a cellular and protein response but also striking parallels in the genetic influences.

In order to attempt to clarify the mechanisms underlying post-traumatic cognitive deficit a basic understanding of the pathology of traumatic brain injury is helpful.

Blunt force head injury results in both focal and diffuse pathologies involving the skull and the underlying brain and its coverings. Focal lesions can take the form of skull fractures, cerebral contusions (Fig. 20.1), focal ischaemic lesions secondary to raised intracranial pressure, and intracranial haematomas. Diffuse lesions may take the form of cerebral ischaemia or cerebral swelling, or may develop as a consequence of rotational forces (diffuse traumatic axonal injury) (Graham *et al.*, 1995b). The primary injury is related to mechanical damage, and can be focal or diffuse, or a combination of both. It is related to the effects of both the impact and inertial forces on the skull and brain. Delayed secondary events such as diffuse traumatic axonal

The Neuropathology of Dementia: Second Edition, ed. Margaret M. Esiri, John Q. Trojanowski and Virginia M.-Y. Lee.
Published by Cambridge University Press.

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

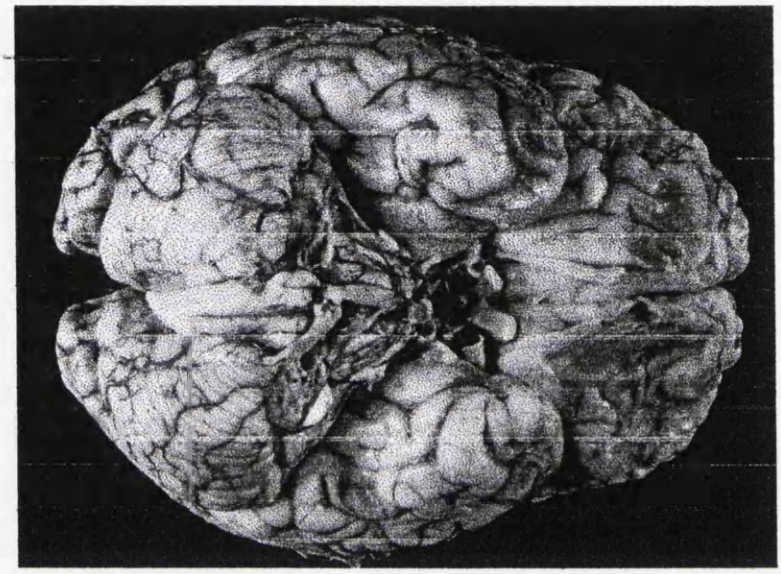


Fig. 20.1. Healed cerebral contusions. There are orange-brown scars on the under aspect of the frontal and temporal lobes.

injury and cerebral ischaemia develop over a period of hours, days, or weeks after the traumatic episode. The secondary events may be related to neurochemical alterations and the associated cellular and molecular alterations induced by trauma (Graham *et al.*, 2000).

Effects of head injury on cognitive function

Concussion refers to an immediate, usually reversible episode of brain dysfunction after traumatic brain injury. A clinical spectrum is recognized ranging from mild concussion, in which consciousness is often preserved, to severe diffuse axonal injury resulting in the vegetative state (Gennarelli, 1993). The anatomical basis of concussion syndromes is currently considered to be diffuse axonal pathology and, in particular, axonal disruption resulting in disconnection between areas involved in consciousness; cerebral cortex, brainstem reticular activating areas, thalamus and hypothalamus (Gennarelli, 1993). The vegetative state refers to a group of patients who have loss of meaningful cognitive function and awareness, but retain spontaneous breathing and periods of wakefulness. The neuropathological basis of the vegetative state has been defined in a study that examined 49 patients in the vegetative state, 35 of whom had experienced traumatic brain injury (Adams *et al.*, 2000) (Fig. 20.2). In the trauma-related cases diffuse traumatic axonal injury of grade 2 or 3 was found in 71% of cases, and thalamic pathology in 80% of cases. In cases with minimal brainstem and cerebral cortical pathology, thalamic pathology was always present. Therefore, damage to the thalamic nuclei and/or the afferent and efferent white matter pathways of the thalamus appear to play a major role in the genesis of the vegetative state after head injury. White matter (Wallerian) degeneration is a consequence of severe diffuse traumatic axonal injury (Fig. 20.3). The axonal loss results in gliosis and macrophage activation (Fig. 20.4), which may be under genetic control as discussed

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

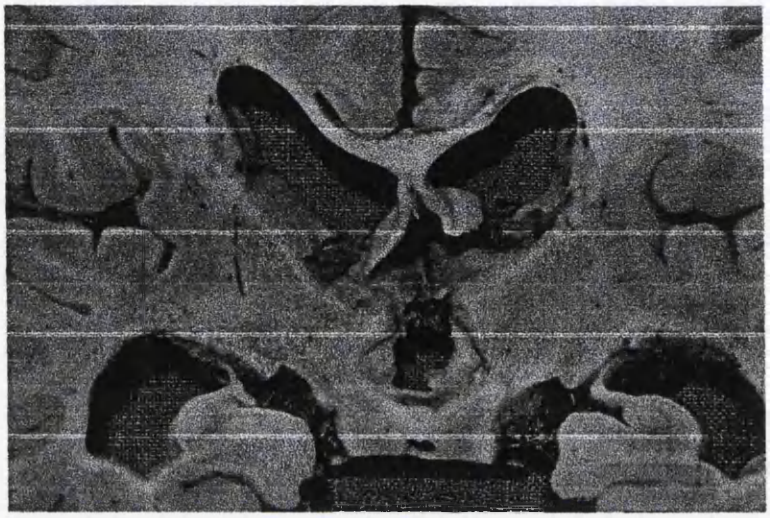


Fig. 20.1. Coronal slice-vegetative. 21-month survival post RTA. Note the thinning of the corpus callosum, ventricular enlargement and small rather granular thalami due to diffuse traumatic axonal injury.

later. In contrast, the structural basis of moderate disability after traumatic brain injury is more likely to be a focal lesion rather than diffuse brain pathology, usually an evacuated intracranial haematoma (Adams *et al.*, 2001). In a study of 30 severely disabled patients 50% had focal brain pathology only. Some severely disabled patients did show diffuse brain pathology similar to vegetative state patients, and it may be that there is a greater quantitative amount of damage in the vegetative cases (Jennett *et al.*, 2001).

Long-term outcome from head injury and chronic neurodegeneration

Clinical studies

Mild head injury (acute GCS 13–15) is associated with a higher than expected incidence of disability (GOS moderate or severe disability) at 1 year post-injury (Thornhill *et al.*, 2000). Of major interest in the context of this discussion are the longer-term effects on cognition many years after the injury, and the relationship between traumatic brain injury and AD.

There is a considerable epidemiological literature examining the relationship between traumatic brain injury and the development of AD in later life. Many of these studies take the form of retrospective case-control studies and are therefore subject to recall bias. A number of these studies have reported an association between traumatic brain injury and AD (French *et al.*, 1985; Graves *et al.*, 1990; Mortimer *et al.*, 1991; van Duijn *et al.*, 1992; Mayeux *et al.*, 1993; Rasmussen *et al.*, 1995; Salib & Hillier, 1997; O'Meara *et al.*, 1997; Nemetz *et al.*, 1999; Guo *et al.*, 2000), although some do not reach statistical significance (Chandra *et al.*, 1987, Amaducci *et al.*, 1986). In particular, the study by Mayeux *et al.* in 1993 reported an almost four-fold increased risk of developing AD after traumatic brain injury when compared to age-matched controls. Guo *et al.* (2000) studied 2233 individuals who met the criteria for probable or definite AD, and compared them with 14668 controls (first-degree relatives or spouses) as part of the MIRAGE (Multi-Institutional Research in Alzheimer Genetic

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

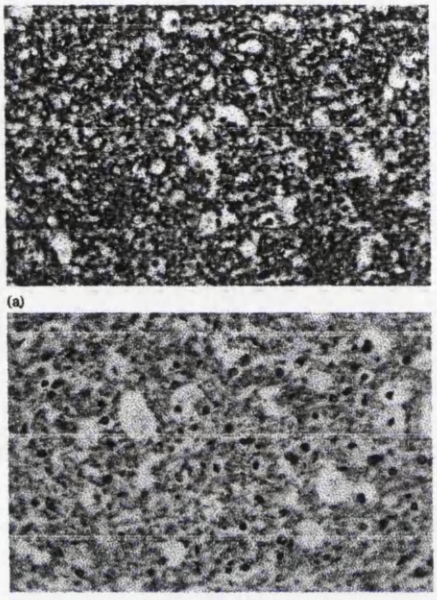


Fig. 20.3. Wallerian degeneration. Same case as Fig. 20.1. (*a*) Normal corticospinal tract. (*b*) Pallor of staining with increased cellularity. A&B Luxol fast blue/Cresyl violet (x 180).

Epidemiology) project. They reported that traumatic brain injury was a risk factor for AD and that the risk was proportional to the severity of the injury. For example, comparison of probands with unaffected spouses yielded odds ratios for AD of 9.9 for head injury with loss of consciousness and 3.1 for head injury without loss of consciousness. Comparison of probands with their parents and sibs were 4.0 for head injury with loss of consciousness and 2.0 for head injury without loss of consciousness. At age 93 years the lifetime risk of developing AD was 77.2% for those with and 40.1% for those without a history of head injury. Other retrospective case-control studies, however, have not confirmed that there is an association between traumatic brain injury and AD (Broe *et al.*, 1990; Ferini-Strambi *et al.*, 1990; Li *et al.*, 1992; Mendez *et al.*, 1992; Fratiglioni *et al.*, 1993).

To try and address the problems of recall bias inherent in case-control studies a number of prospective studies have been designed. Again, however, there is conflicting data. Corkin *et al.* (1989) performed neuropsychological assessment of 57 World War 2 veterans with a penetrating head injury at two time points 30 years apart, and compared their performance with 27 veterans who suffered a peripheral nerve injury only and who were assessed over the same 30-year period. They found that a penetrating head injury exacerbated the decline in cognitive performance over time when compared with the peripheral injury group. Schofield *et al.* (1997) reported a community-based longitudinal study of ageing in north Manhattan. 271 participants without significant cognitive impairment at the time of enrolment were interviewed in relation to previous head injury and associated loss of consciousness. Patients were then followed up for 5 years with annual evaluations. They reported that previous traumatic brain injury was a risk factor for AD with a three-fold increased risk. Plassman *et al.* (2000) examined 1776 World War 2 navy and marine veterans, with military medical records. 548 had a history of non-penetrating traumatic brain injury, 1228 did not. All individuals were assessed for AD. They found that in this group moderate head injury (Frankowski scale, Frankowski *et al.*, 1985) resulted in 2.3 × increased risk of AD, while severe head injury resulted in a four-fold increased risk. Against this data, however, there are a number of prospective studies which have failed to demonstrate an association between traumatic brain injury and AD (Katzman *et al.*, 1989; Aronson *et al.*, 1990; Williams *et al.*, 1991; Breteler *et al.*, 1995). Launer *et al.* (1999), as part of the European Studies of Dementia (EURODEM), analysed four European population-based prospective studies, with individuals aged 65 years or older at time of recruitment. This large study did not find an association between traumatic brain injury and AD. Mehta *et al.* (1999) reported the prospective population-based Rotterdam study, which looked at 6645 individuals aged 55 years or older and who did not have dementia when recruited. This study found that mild traumatic brain injury was not associated with an increased risk of AD, although the follow-up period was short being on average 2.1 years after initial assessment.

There are many difficulties in assessing the relationship between traumatic brain injury and AD as the conflicting results presented above clearly illustrate. Retrospective case-control studies have both recall and selection bias. The prospective studies retain a lesser degree of recall bias and do not rely on the recollections of cognitively impaired individuals. However, some of the prospective studies have only a short follow up period, 5 years in many cases, and

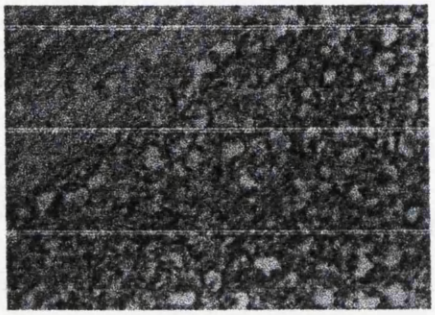
P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

this may bias the outcome. Comparisons between studies are difficult due to differences in definitions of severity of brain injury, post injury outcome status, and clinical definitions of AD. Also the age at the time of the injury and the age at the time of assessment are likely to be important variables.

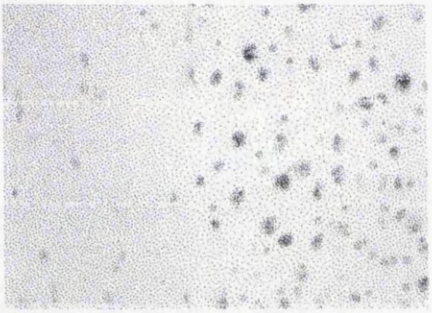
Boxers and *dementia pugilistica*

While the data relating to long term associations between traumatic brain injury and AD is currently conflicting, the association between relatively mild repetitive head injury and cognitive impairment has been established in the literature for many years. The 'punch drunk' state was first described by Martland in 1928 and was renamed *dementia pugilistica* by Millspaugh in 1937. *Dementia pugilistica* was fully reviewed in the previous edition of this book (Bruton 1997) and a summarized account will be presented here. This condition is described in boxers who have competed in many bouts over a long period of time. Clinically, they develop a degree of intellectual deterioration often with an associated movement disorder, usually parkinsonism but in some cases predominantly ataxia. The largest study of this disorder clinically (Roberts, 1969) examined 224 ex-boxers using neurological examination, electroencephalogram, and simple psychometric testing. He found that 17% had varying degrees of movement disorder involving the cerebellar, pyramidal and extrapyramidal systems. Minor degrees of intellectual function were seen in several of the ex-boxers, although only two required long term care as a result of their cognitive impairment. Roberts concluded that the occurrence of encephalopathy increased significantly with the number of bouts and the length of the boxer's career. He also concluded, however, that the rate of cognitive decline was not greater than that associated with ageing alone. More recent studies (Casson *et al.*, 1984; McLatchie *et al.*, 1987; Brooks *et al.*, 1987; Murelius & Haglund, 1991; Heilbronner *et al.*, 1991) suggest that full-blown *dementia pugilistica* is now rarely seen, although mild cognitive and movement disorders are still associated with boxing.

While *dementia pugilistica* was initially described in relation to boxing, cases have been described in National Hunt jockeys (Foster *et al.*, 1976). In addition, there is a considerable literature relating to the risks of repetitive mild traumatic head injury and other sports such as soccer (Matser *et al.*, 1999, Kirkendall *et al.*, 2001), rugby union and Australian rules football (McIntosh *et al.*, 2000), American football (Maroon *et al.*, 2000), and ice hockey (Biasca *et al.*, 1993). In the absence of large prospective studies the risk of cognitive impairment and movement disorders secondary to repetitive mild traumatic brain injury in relation to these contact sports, remains uncertain.



(a)



(b)

Fig. 20.4. Wallerian degeneration. Same case as Fig. 20.1. (*a*) Corticospinal tract in pons (bottom right) showing an astrocytosis compared with normal transverse fibres of pons (top left). GFAP (x180). (*b*) Corticospinal tract (right) in pons. There are many macrophages compared with normal transverse fibres of pons (left). CD 68 (x 180).

The largest pathological assessment of *dementia pugilistica* was the examination of the brains of 15 boxers, 11 of whom were diagnosed with *dementia pugilistica* in life (Corsellis *et al.*, 1973). This followed on from previous case reports (Brandenburg & Hallervorden 1954; Grahmann & Ule, 1957; Constantinidis & Tissot, 1967) and the

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

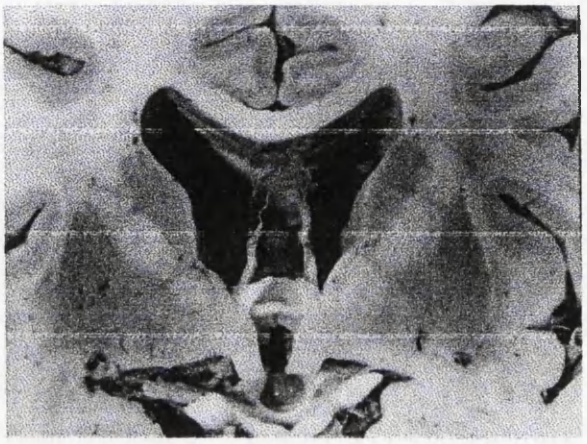


Fig. 20.5. High powered view of a boxer's septum. The septal leaves are widely separated forming a large cavum. Only a few strands of tissue remain (x 2.5).

descriptions by Mawdsley and Ferguson (Mawdsley & Ferguson, 1963; Ferguson & Mawdsley, 1965) of the brains of four ex-boxers. Corsellis *et al.* (1973) reported four principal features of the brain in *dementia pugilistica:*

(i) abnormalities of the septum pellucidum, (ii) cerebellar damage, (iii) degeneration of the substantia nigra, and (iv) cerebral cortex pathology.

(i) A fenestrated cavum septum pellucidum was seen in 77% of ex-boxers but in only 3% of non-boxers. One third of the non-boxers who had a fenestrated cavum septum pellucidum had evidence of a previous head injury (Fig. 20.5). The degree of separation of the two leaflets of the septum pellucidum may be related to repetitive injury being most pronounced in the ex-boxers.

(ii) Ataxia may be a feature of *dementia pugilistica*. Corsellis *et al.* (1973) described cortical scarring of the inferior aspects of the lateral cerebellar hemispheres adjacent to the tonsils in 10 of the 15 ex-boxers brains studied. Histologically there was gliosis and loss of both Purkinje cells and granule cells (Fig. 20.6).

(iii) Parkinsonism is a common feature of *dementia pugilistica* and substantia nigra pathology appears to be the underlying cause. Pigmented cell loss is often marked, both within the substantia nigra and the locus coeruleus, and neurofibrillary tangles can be seen in some of the remaining neurons (Fig. 20.7). Lewy bodies are not a feature (Corsellis *et al.*, 1973).

(iv) Gross cortical pathology, a common feature of acute traumatic brain injury in the form of contusions, does not appear to be a significant feature of *dementia pugilistica* (Corsellis *et al.*, 1973; Adams & Bruton, 1989). However, diffuse microscopic cortical pathology is a feature of *dementia pugilistica* (see below).

Pathological mechanisms which may underlie long term neurodegeneration after head injury

Over-representation of late cognitive decline in survivors of traumatic brain injury may simply reflect the additive effects of the acute damage and later age-related functional compromise. From this viewpoint the acute injury acts to decrease the 'functional reserve' of the brain and subsequent age-related neurodegeneration is more likely at an earlier age to result in traverse of the threshold of impairment required to manifest as dementia. However, there are remarkable parallels in the pathological processes involved both in the response to traumatic brain injury and AD (see below). It is possible that a component of the acute response to traumatic brain injury acts as a 'trigger' to initiate a positive feedback loop that smoulders away to become

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

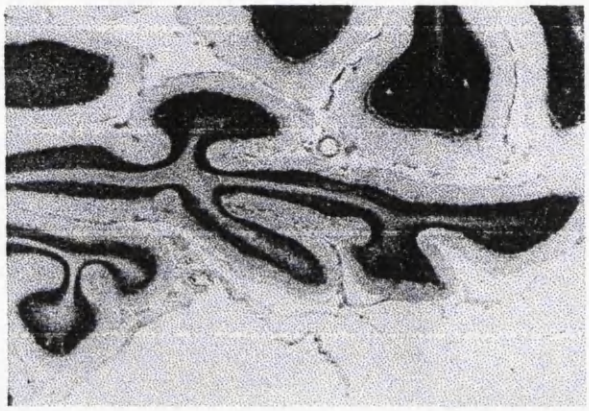


Fig. 20.6. Low power view of cerebellar folia in the tonsillar region of a boxer's cerebellum. The molecular layer of the scarred cortex is narrowed and intensely gliosed.

manifest in later years as frank neurodegenerative pathology and dementia (Nicoll *et al.*, 1995; Griffin *et al.*, 1998) (Fig. 20.8).

Detailed classical neuropathological descriptions of cohorts of long-term survivors of traumatic brain injury including immunohistochemical studies are lacking. However, some of the processes that are believed to be involved in chronic neurodegeneration, including AD, have been explored in the context of both acute injury and long-term survival after trauma.

(a) Cytoskeletal neurodegenerative pathology

Cytoskeletal pathology after diffuse traumatic brain injury has been examined experimentally using a pig model with injury induced via controlled head rotational acceleration (Smith *et al.*, 1999). Head-injured pigs were examined at days 1, 3, 7 and 10 post-injury, and compared to control animals without head injury. Within the experimental group tau and neurofilament accumulations were identified immunohistochemically within the white matter, co-localised with damaged axons (Aβ-PP immunoreactive), and within neuronal perikarya in the cerebral cortex. To date a similar observation has not been made in humans following a single episode of traumatic brain injury although cleaved forms of tau protein are elevated markedly in the CSF of brain-injured patients (Zemlan *et al.*, 1999). However, in

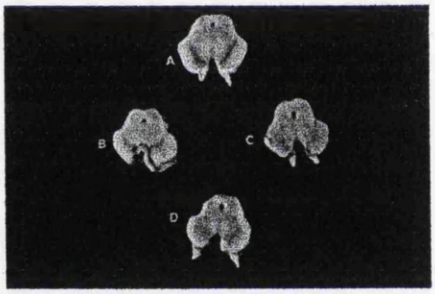


Fig. 20.7. (*a*) Transverse cut through the midbrain of an elderly male non-boxer to show the normal pigmentation of the substantia nigra (x 0.75). (*b*) (*c*) and (*d*). The substantia nigra of three punch drunk boxers. Some pigment is still visible in (*b*) but (*c*) and (*d*) are almost totally devoid of pigmentation (x 0.75).

cases of repetitive mild head injury cytoskeletal pathology is observed. Neurofibrillary tangles were reported in ex-boxers by Corsellis *et al.* (1973) scattered throughout the cerebral cortex and the brainstem, being most prominent in the medial temporal cortex (Fig. 20.9). Recently, Geddes *et al.* (1999) examined the brains of four young individuals

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

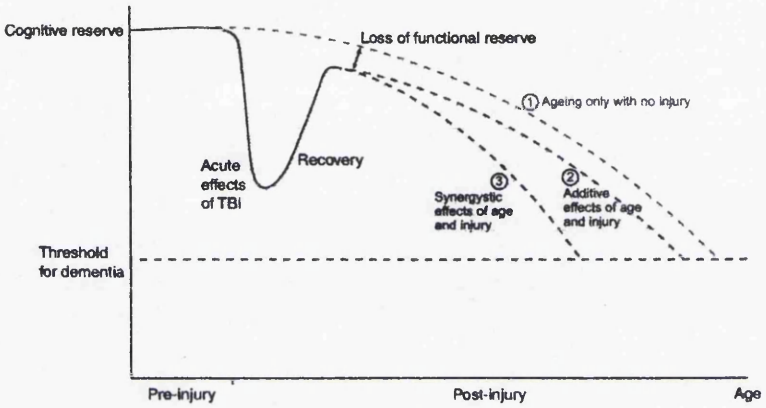


Fig. 20.8. Graphical illustration of potential mechanisms operating after head injury. See text for further explanation.

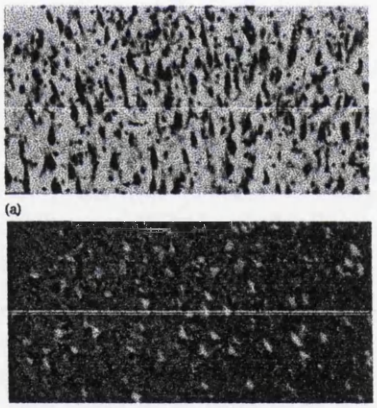


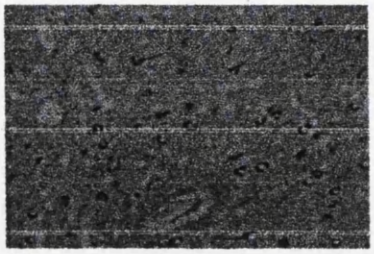
Fig. 20.9. Neurofibrillary tangles affecting most neurons in the parahippocampal gyrus in a case of *dementia pugilistica*. Note the absence of senile plaques. von Braunmuhl's silver stain (×100).

(age range from 23–28 years) with a history of repetitive head injury (two boxers, one footballer, and one mentally subnormal patient with a history of self inflicted head banging) and a frontal lobectomy specimen from an individual with intractable complex partial seizures with recurrent minor head injury. They identified widespread neocortical neurofibrillary tangles and neuropil threads not seen in age matched controls which in areas showed a perivascular distribution (Fig. 20.10). Schmidt *et al.* (2001) have compared the molecular profiles of the neurofibrillary tangles in *dementia pugilistica* and AD. They found that *dementia pugilistica* and AD had a common tau isoform and phosphorylation profile. They concluded that the mechanisms underlying both these conditions might be similar.

(b) Amyloid deposition

Diffuse Aβ plaques have been identified in approximately 30% of individuals who die shortly after a single episode of severe traumatic brain injury (Roberts *et al.*, 1991, 1994; Graham *et al.*, 1995a). This is a higher proportion than in non-head injury controls. Other groups (Adle-Biassette *et al.*, 1996), however, have not confirmed this observation. Most of this is in the form of Aβ42 (Gentleman *et al.*, 1997; Horsburgh *et al.*, 2000), which is believed to be of pathological significance in AD. The distribution of the plaques does

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0



(a)

(b)

Fig. 20.10. Neurofibrillary tangles in the neocortex from a case of repetitive head injury. (*a*) Neurofibrillary tangles showing a perivascular distribution. Tau (x 100). (*b*) Intraneuronal tau immunoreactivity in a neocortical neuron. Tau (x 180).

not correlate with focal traumatic lesions such as contusions but may be an expression of a diffuse acute phase response (e.g. hypoxia, acidosis, oedema, reduced cerebral blood flow) (Graham *et al.*, 1995). For example, the Aβ deposits may be the result of increased production or altered distribution of Aβ-PP, increased cleavage of Aβ-PP in a proteolytic environment to produce Aβ, an alteration in the balance of production of $A\beta_{40}$:$A\beta_{42}$, extracellular conditions which favour the precipitation of amyloid fibrils, or decreased removal or drainage of Aβ.

In the study by Corsellis *et al.* (1973) neurofibrillary tangles were found in the almost complete absence of senile plaques when examined using silver (Bielschowsky) and Congo red stains. However, when this was re-examined using immunohistochemistry with formic acid pre-treatment for the β-amyloid protein (Aβ), extensive immunoreactive plaque-like structures were seen in most cases of *dementia pugilistica* (Roberts *et al.*, 1990) although the neuritic plaques characteristic of AD were absent. In the study by Geddes *et al.* (1999) Aβ plaques were not seen despite using both a modified Bielschowsky silver stain and Aβ immunohistochemistry with formic acid pre-treatment. They concluded that neurofibrillary tangle formation in the absence of Aβ deposition is an early consequence of repetitive head injury and that, because of their striking perivascular distribution, neurofibrillary tangle formation may be related to damage to blood vessels.

The relationship between Aβ deposition and genetic polymorphisms is discussed in a later section.

(c) Neuronal loss

Neuronal loss after traumatic brain injury has been reported in the neocortex, the hippocampus, the cerebellum and the thalamus (Adams *et al.*, 1985; Kotapka *et al.*, 1992; Ross *et al.*, 1993). In the acute phase, neuronal loss is related to contusions or as a consequence of cerebral hypoxia/ischaemia, and bilateral hippocampal neuronal loss has been documented in 85% of cases in one study (Adams *et al.*, 1985). The mechanisms of cell death have been extensively studied and the processes of necrosis and programmed cell death have been considered to be separate mechanisms, although this view is being increasingly challenged and shared molecular pathways have been identified in both processes. The role of programmed cell death after traumatic brain injury has been reviewed by Raghupathi *et al.* (2000). Cell death has been identified *in situ* after traumatic brain injury in both animal models and in human material using the terminal deoxynucleotidyl transferase mediated dUTP nick end-labelling (TUNEL) technique (Rink *et al.*, 1995, Smith *et al.*, 1997). This technique identifies DNA fragmentation, a feature common to both necrosis and programmed cell death. Differentiation between necrosis and programmed cell death is possible by assessing other mechanisms seen in programmed cell death such as caspase activation, and identification of the morphological expression of programmed cell death, apoptosis. TUNEL positive neurons and oligodendroglial cells have been reported in human traumatic brain injury; Clark *et al.* (1999) demonstrated elevated levels of bcl-2 and caspase 3, increased cleavage of both caspases 1 and 3, and cells with the morphological appearances of apoptosis in 8 patients who had contusions removed surgically between 1 and 9 days after an episode of traumatic brain injury. Smith *et al.* (2000) and Shaw *et al.* (2001) studied a number of brain areas in human post-mortem tissue of 18 patients who survived between 6 hours and 10 days after traumatic brain

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

injury. TUNEL-positive cells were seen in both grey and white matter, peaking between 25 and 48 hours although still identifiable at 10 days post injury. There was a mixture of both apoptotic and necrotic morphology in neurons, although white matter TUNEL-positive cells more consistently showed an apoptotic morphology. They concluded that in human frontal lobe contusions both apoptosis and necrosis contributed to post-traumatic pathology, and that multiple cell types, including neurons, were involved.

Recent experimental studies suggest that the cellular pathology initiated by an episode of acute traumatic brain injury may indeed be progressive. Rats subjected to severe lateral fluid-percussion brain injury were studied for up to 12 months and showed long term cognitive and neurological motor dysfunction (Pierce *et al.*, 1998) accompanied by continuing cell loss (Smith *et al.*, 1997). Recent studies in human cases have demonstrated TUNEL-positive cells up to 12 months after traumatic brain injury (Williams *et al.*, 2001). Again, the majority of the cells were present in the white matter and were considered to be closely associated with Wallerian degeneration. Long-term DNA fragmentation therefore appears to be a feature of traumatic brain injury in humans.

(d)Cholinergic brain pathways

The nucleus basalis of Meynert within the basal forebrain provides cholinergic innervation of the cerebral cortex and the hippocampus, and damage to this pathway can result in attention, memory and emotional dysfunction (Everitt & Robbins, 1997). Abnormalities within the cholinergic projection system have been postulated to contribute both to the altered mental state in AD (Geula & Mesulam, 1994) and to the neurobehavioural sequelae which persist after a head injury (Cardenas *et al.*, 1994).

In rats there is a reduction in the number of choline acetyltransferase [ChAT] positive neurons after experimentally induced traumatic brain injury (Schmidt & Grady, 1995), and alterations of cholinergic innervation of the cerebral cortex and hippocampus have been detected (Dixon *et al.*, 1995, 1997).

Patients who die acutely as a consequence of traumatic brain injury have reduced levels of cortical ChAT when compared to age-matched controls (Dewar & Graham, 1996; Murdoch *et al.*, 1998). Recently, neuronal damage has been demonstrated within the nucleus basalis of Meynert in eight of twelve fatally head injured patients, with a median survival time of 27 hours (Murdoch *et al.*, 2001). Neuronal damage was a result of both mechanical distortion (tissue herniation) and focal ischaemia. The authors concluded that damage to cholinergic neurons may contribute to the dysfunction of memory and cognition in survivors of traumatic brain injury, although studies of the nucleus basalis of Meynert in long-term survivors has not been undertaken.

(e) Neuroinflammation

Recent studies have focussed attention on 'neuroinflammation' as a potential culprit both in AD and in the response to brain injury (Engel *et al.*, 2000; Nicoll *et al.*, 2000; Griffin *et al.*, 1998). The principal mediator of inflammatory processes in the central nervous system is the microglial cell. Microglia have a variety of functions including antigen presentation, synthesis and secretion of cytokines and phagocytosis. These cells are a source of several of the proteins upregulated both in AD and after traumatic brain injury, including apoE, and pro-inflammatory cytokines such as interleukin 1 (IL-1). This raises the question that patients who sustain a head injury may have a microglial response which plays a role both in influencing their outcome following injury and their increased susceptibility to AD later in life.

IL-1 is thought to orchestrate the inflammatory responses within the brain after an insult, resulting in a number of responses including: (a) microglial proliferation (Ganter *et al.*, 1992), (b) induction of neuronal production of Aβ-PP (Goldgaber *et al.*, 1989), and (c) astrocytic activation with upregulation of astrocytic-derived proteins (Das & Potter, 1995).

IL-1 is expressed in increased quantities in the cerebral cortex within hours of traumatic brain injury (Griffin *et al.*, 1994), and chronic overexpression of IL-1 is found in AD (Griffin *et al.*, 1989). Griffin *et al.* (1998) have proposed a 'Cytokine Cycle' in which traumatic brain injury, or other brain insults, can, in susceptible individuals, initiate an overexuberant sustained inflammatory response which can result in neurodegeneration. Aβ-PP and the astrocyte-produced molecule S-100β are upregulated in response to increased IL-1 levels, and are known to be upregulated in AD (Griffin *et al.*, 1989; Mrak *et al.*, 1996). Aβ-PP is not only upregulated in acute traumatic brain injury, but there is increased intraneuronal processing of the molecule (Buxbaum *et al.*, 1992) potentially resulting in Aβ production and deposition. The relation between IL-1 and Aβ-PP in the acute phase is uncertain, but increased levels of IL-1 may result in sustained Aβ-PP, and therefore Aβ, production. Positive feedback of this interaction may be provided by soluble fragments of Aβ-PP (sAPP) which are produced by the processing of Aβ-PP. sAPP promotes microglial activation by a mechanism that is modulated by apoE in an isoform specific fashion (Barger & Harmon, 1997).

IL-1 positive microglial cells lie in close relation to Aβ-PP positive neurons and dystrophic neurites in the

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

brains of head-injured patients (Griffin *et al.*, 1994) and are also found in close apposition to neurofibrillary tangle-containing neurons in AD (Sheng *et al.*, 1997). IL-1 is known to be trophic to neurons in low concentrations, but at higher concentrations IL-1 has a neurotoxic effect, inducing over-expression and phosphorylation of both neurofilaments and tau (Sheng *et al.*, 2000).

There is a considerable experimental literature relating to neuroinflammation and neuronal death. A recent study of mixed cultures of activated glia and neurons suggested that inflammatory neurodegeneration may be mediated by glial nitric oxide (NO) (Bal-Price & Brown, 2001). They proposed that NO produced by activated microglia or astrocytes inhibits the mitochondrial function of surrounding neurons, causing glutamate release from neurons (and possibly from astrocytes). Activation of NMDA receptors by glutamate triggers massive influx of Ca^{2+} into neurons, leading to cell death. Other potential mechanisms of NO mediated neuronal death include mitochondrial damage (Heales *et al.*, 1999) and poly(ADP-ribose) synthetase activation (Zhang *et al.*, 1994).

Evidence for genetic influences on outcome after head injury

The response to brain injury and AD have in common not only a cellular and protein response but there are striking parallels in the genetic influences. There is a polymorphism of the apolipoprotein E gene (*APOE*, gene; apoE, protein) of which there are 3 common alleles (*ε*2, *ε*3 and *ε*4). Possession of *APOE ε*4 allele is the major genetic susceptibility factor for sporadic AD (Saunders *et al.*, 1993). In addition, the *APOE* polymorphism influences neuropathological findings in patients who die from head injuries (Nicoll *et al.*, 1995). This study examined the brains of 90 individuals who died within 2 weeks of a head injury. Aβ deposits were found in 23 cases and the frequency of the *APOE ε*4 allele within this group was significantly greater than that seen in either control populations without neurological disease or in AD. In addition all individuals homozygous for the *APOE ε*4 allele had Aβ deposition. Furthermore, the density of these plaques is related to *APOE* genotype, with greater numbers of plaques being associated with the *APOE ε*4 allele in an allele dose dependant manner (i.e. homozygotes having greater numbers of plaques than heterozygotes) (Horsburgh *et al.*, 2000). The initial interpretation of these findings by Nicoll *et al.* was that in genetically susceptible individuals (i.e. those with an *APOE ε*4 allele) traumatic brain injury appears to act as a trigger for Aβ deposition. However, there are alternative explanations for these observations (Roses & Saunders, 1995); Aβ deposits may pre-date the injury, and patients with *ε*4, who are more likely to have age-related deposits, may have a higher mortality from traumatic brain injury and therefore be selected for an autopsy-based study. Until it is possible to image Aβ plaques during life it may not be possible to resolve this uncertainty. Subsequent clinical studies have indeed shown that head-injured patients (and patients with spontaneous intracerebral haemorrhage) who possess *APOE ε*4 have poorer outcome than non-carriers of *APOE ε*4 (Alberts *et al.*, 1995; Teasdale *et al.*, 1997; McCarron *et al.*, 1998). Jordan *et al.*, 1997 studied 30 professional boxers and assessed their cognitive status in relation to *APOE* genotype and number of professional bouts. They concluded that possession of an *APOE ε*4 allele is associated with increased severity of chronic neurological deficits in high-exposure boxers. A recent neuropathological study compared Aβ deposits in long term survivors of traumatic brain injury (survival time up to 20 years) with age-matched and *APOE* genotype-matched controls (MacFarlane *et al.*, 1999). They found Aβ deposits were more common in *ε*4 patients in both the long term survivors and the control groups, but were not more common among long term survivors than controls.

A further genetic polymorphism has recently been suggested to confer susceptibility to AD and this further implicates neuroinflammatory processes. Interleukin 1 (IL-1) exists in two distinct forms (IL-1α and IL-1β with *IL-1A* and *IL-1B* genes respectively). Polymorphisms have been identified in each of these genes (both have an allele 1 and an allele 2) and an association has been demonstrated between the *IL-1A* 2,2 genotype and AD (Nicoll *et al.*, 2000; Grimaldi *et al.*, 2000). Nicoll *et al.*, studied 232 pathologically confirmed cases of Alzheimer's disease and found the *IL-1A* 2,2 genotype in almost 13% of cases as compared to 6.6% of age-matched and *APOE*-matched controls. In addition they found that homozygosity for allele 2 of both *IL-1A* and *IL-1B* conferred an even greater risk, although homozygosity for allele 2 of *IL-1B* alone was not significant.

In AD there is evidence that patients with *APOE ε*4 have increased microglial activity compared to patients without *APOE ε*4 (Egensperger *et al.*, 1998). Although there is currently a lack of definitive information relating to *APOE* genotype, *IL-1* genotype and microglial activation in traumatic brain injury these observations raise the possibility that microglial activation ('neuroinflammation') may be under genetic influence. Specifically, they raise the question that individuals with the relevant alleles (*APOE ε*4 or *IL-1A* allele 2) who sustain a head injury may have a relatively overexuberant microglial response which is associated both with a poorer outcome from injury and greater

Ferguson, P. R. & Mawdsley, C. (1965). Chronic encephalopathy in boxers. *6th International Congress of Neurology, Vienna.* Wiener Medizinische Akademie, Vienna, Vol. 1: 81–4.

Fratiglioni, L., Ahlbom, A., Viitanen, M. & Winblad, B. (1993). Risk factors for late-onset Alzheimer's disease: a population-based, case-control study. *Ann Neurol*, 33: 258–66.

French, L. R., Schuman, L. M., Mortimer, J. A., Hutton, J. T., Boatman, R. A. & Christiana, B. (1985). A case-control study of dementia of the Alzheimer type. *Am J Epidemiol*, 121: 414–21.

Ganter, S., Northoff, H., Mannel, D. & Gebicke-Harter, P. J. (1992). Growth control of cultured microglia. *J Neurosci Res*, 33: 218–30.

Geddes, J. F., Vowles, G. H., Nicoll, J. A. R. & Revesz, T. (1999). Neuronal cytoskeletal changes are an early consequence of repetitive head injury. *Acta Neuropathol*, 98: 171–8.

Gennarelli, T. A. (1993). Cerebral concussion and diffuse brain injuries. In P. R. Cooper, (ed) *Head Injury*, 3rd edn. pp. 137–58. Baltimore, USA: Williams and Wilkins.

Getuk, C. & Mesulam, M. M. (1994). Cholinergic systems and related neuropathological predilection patterns in Alzheimer's disease. In R. D. Terry, R. Katzman, Bick, K. L. eds. Alzheimer's Disease, pp. 263–94. New York: Raven Press.

Goldgaber, D., Harris, H. W., Hla, T. *et al.* (1989). Interleukin-1 regulates synthesis of amyloid beta-protein precursor mRNA in human endothelial cells. *Proc Natl Acad Sci USA*, 86: 7606–10.

Gentleman, S. M., Greenberg, B. D., Savage, M. J. *et al.* (1997). A beta 42 is the predominant form of amyloid beta-protein in the brains of short-term survivors of head injury. *Neuroreport*, 8: 1519–22.

Graham, D. I., Gentleman, S. M., Lynch, A. & Roberts, G. W. (1995a). Distribution of beta-amyloid protein in the brain following severe head injury. *Neuropathol Appl Neurobiol*, 21: 27–34.

Graham, D. L, Adams, J. H., Nicoll, J. A. R., Maxwell, W. L. & Gennarelli, T. A., (1995b). The nature, distribution and cause of traumatic brain injury. *Brain Pathology*, 5: 397–406.

Graham, D. I., Mcintosh, T. K., Maxwell, W. L. & Nicoll, J. A. R. (2000). Recent advances in neurotrauma. *J Neuropath Exp Neurol*, 59: 641–51.

Grahmann, H. & Ule, G. (1957). Beitrag zur Kenntnis der chronischen cerebralen Krankheitsbilder bei Boxern. *Psychiat Neurol*, 134: 261–83.

Graves, A. B., White, E., Koepsell, T. D. *et al.* (1990). The association between head trauma and Alzheimer's disease. *Am J Epidemiol*, 131: 491–501.

Griffin, W. S. T., Stanley, L. C., Ling, C. *et al.* (1989). Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer's disease. *Proc Natl Acad Sci USA*, 86: 7611–15.

Griffin, W. S. T., Sheng, J. G., Gentleman, S. M., Graham, D. I., Mrak, R. E. & Roberts, G. W. (1994). Microglial interleukin-1α expression in human head injury: correlations with neuronal and neuritic β-amyloid precursor protein expression. *Neurosci Lett*, 176: 133–6.

Griffin, W. S. T., Sheng, J. G., Royston, M. C. *et al.* (1998). Glial–neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathol*, 8: 65–72.

Grimaldi, L. M., Casadei, V. M., Ferri, C. *et al.* (2000). Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism. *Ann Neurol*, 47: 361–5.

Guo, Z., Cupples, L. A., Kurz, A. *et al.* (2000). Head injury and the risk of Alzheimer's disease in the MIRAGE study. *Neurology*, 54: 1316–23.

Heales, S. J., Bolanos, J. P., Stewart, V. C., Brookes, P. S., Land, J. M. & Clark, J. B. (1999). Nitric oxide, mitochondria and neurological disease. *Biochim Biophys Acta*, 1410: 215–28.

Heilbronner, R. L., Henry, G. K. & Carson-Brewer, M. (1991). Neuropsychologic test performance in amateur boxers. *Am J Sports Med*, 19: 376–80.

Horsburgh, K., Cole, G. M., Yang, E *et al.* (2000). Beta-amyloid (Abeta)42(43), abeta42, abeta40 and apoE immunostaining of plaques in fatal head injury. *Neuropathol Appl Neurobiol*, 26: 124–32.

Jennett, B. & Bond, M. (1975). Assessment of outcome after severe brain damage. *Lancet*, 1: 480–7.

Jennett, B., Snoek, J. & Bond, M. R. (1981). Disability after severe head injury: Observations on the use of the Glasgow Outcome Scale. *J Neurol Neurosurg Psychiatry*, 44: 285–93.

Jennett, B., Adams, J. H., Murray, L. S. & Graham, D. I. (2001). Neuropathology in vegetative and severely disabled patients after head injury. *Neurology*, 56: 486–90.

Jordan, B. D., Relkin, N. R., Ravdin, L. D., Jacobs, A. R., Bennett, A. & Gandy, S. (1997). Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. *J Am Med Assoc*, 278: 136–40.

Katzman, R., Aronson, M., Fuld, P. *et al.* (1989). Development of dementing illnesses in an 80 year old volunteer cohort. *Ann Neurol*, 25: 317–24.

Kirkendall, D. T., Jordan, S. E. & Garrett, W. E. (2001). Heading and head injuries in soccer. *Sports Med*, 31: 369–86.

Kotapka, M. J., Graham, D. I., Adams, J. H. & Gennarelli, T. A. (1992). Hippocampal pathology in fatal non-missile human head injury. *Acta Neuropathol*, 83: 530–4.

Launer, L. J., Anderson, K., Dewey, M. E. *et al.* (1999). Rates and risk factors for dementia and Alzheimer's disease: results from EURODEM incidence research group and work groups. European studies of dementia. *Neurology*, 52: 78–84.

P1: MRM
052181915 6c20 October 18, 2003 9:30 Char Count= 0

Li, G., Shen, Y. C., Li, Y. T., Chen, C. H., Zhau, Y. W. & Silverman, J. M. (1982). A case-control study of Alzheimer's disease in China. *Neurology*, **42**: 1481–8.

McCarron, M. O., Muir, K. W., Weir, C. J. *et al.* (1998). The apolipoprotein E ε4 allele and outcome in cerebral vascular disease. *Stroke*, **29**: 1882–7.

MacFarlane, D. P., Nicoll, J. A. R., Smith, C. & Graham, D. I. (1999). APOE epsilon 4 allele and amyloid beta-protein deposition in long term survivors of head injury. *Neuroreport*, **10**: 3945–8.

McGeer, P. L., McGeer, E., Rogers, J. & Sibley, J. (1990). Anti-inflammatory drugs and Alzheimer disease. *Lancet*, **335**: 1037.

McGeer, P. L., Schulzer, M. & McGeer, E. G. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology*, **47**: 425–32.

McIntosh, A. S., McCrory, P. & Comerford, J. (2000). The dynamics of concussive head impacts in rugby and Australian rules football. *Med Sci Sports Exerc*, **32**: 1980–4.

McLatchie, G., Brooks, N. & Galbraith, S. *et al.* (1987). Clinical neurological examination, neuropsychology, electroencephalography and computed tomographic head scanning in active amateur boxers. *J Neurol Neurosurg Psychiatry*, **50**: 96–9.

McMillan, R., Strang, I. & Jennett, B. (1979). Head injuries in primary surgical wards in Scottish hospitals. Scottish head injury management study. *Health Bull*, **37**: 75–81.

Maroon, J. C., Lovell, M. R., Norwig, J., Podell, K., Powell, J. W. & Hartl, R. (2000). Cerebral concussion in athletes: evaluation and neuropsychological testing. *Neurosurgery*, **47**: 659–69.

Martland, H. S. (1928). Punch drunk. *J Am Med Ass*, **91**: 1103–7.

Matser, E. J., Kessels, A. G., Lezak, M. D., Jordan, B. D., Troost, J. (1999). Neuropsychological impairment in amateur soccer players. *JAMA*, **282**: 971–3.

Mawdsley, C. & Ferguson, F. R. (1963). Neurological disease in boxers. *Lancet*, **2**: 795–801.

Mayeux, R., Ottman, R., Tang, M. X. *et al.* (1993). Genetic susceptibility and head injury as risk factors for Alzheimer's disease among community-dwelling elderly persons and their first degree relatives. *Ann Neurol*, **33**: 494–501.

Mehta, K. M., Ott, A., Kalmijn, S. *et al.* (1999). Head trauma and the risk of dementia and Alzheimer's disease: The Rotterdam study. *Neurology*, **53**: 1959–62.

Mendez, M. E, Underwood, K. L., Zander, B. A., Mastri, A. R., Sung, J. H. & Frey, W. H. (1992). Risk factors in Alzheimer's disease: a clinicopathological study. *Neurology*, **42**: 770–5.

Millspaugh, J. A. (1937). Dementia pugilistica. *US Nav Med Bull*, **35**: 297–303.

Mortimer, J. A., French, L. R., Hutton, J. T. & Schuman, L. M. (1985). Head injury as a risk factor for Alzheimer's disease. *Neurology*, **35**: 264–7.

Mortimer, J. A., van Duijn, C. M., Chandra, V. *et al.* (1991). Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM risk factors research group. *Int J Epidemiol* **20** Suppl 2, S28–35. *Acta Neurol Scand*, **83**: 9–13.

Mrak, R. E., Sheng, J. G. & Griffin, W. S. T. (1996). Correlation of astrocytic S100β expression with dystrophic neurites in amyloid plaques of Alzheimer's disease. *J Neuropathol Exp Neurol*, **55**: 273–9.

Murdoch, I., Perry, E. K., Court, J. A., Graham, D. I. & Dewar, D. (1998). Cortical cholinergic dysfunction after human head injury. *J Neurotrauma*, **15**: 295–305.

Murdoch, I., Nicoll, J. A. R., Graham, D. I. & Dewar, D. (2001). Nucleus basalis of Meynert pathology in the human brain after fatal head injury. *J Neurotrauma*, **18**: 279–84.

Mureilius, O. & Haglund, Y. (1991). Does Swedish amateur boxing lead to chronic brain damage? A retrospective neuropsychological study. *Acta Neurol Scand*, **83**: 9–13.

Murray, G. D., Teasdale, G. M., Braakman, R. *et al.* (1999). The European Brain Injury Consortium Survey of Head Injuries. *Acta Neurochirg*, **141**: 223–36.

Nemetz, P. N., Leibson, C., Naessens, J. M. *et al.* (1999). Traumatic brain injury and time to onset of Alzheimer's disease: a population-based study. *Am J Epidemiol*, **149**: 32–40.

Nicoll, J. A., Roberts, G. W., Graham, D. I. (1995). Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury. *Nat Med*, **1**: 135–7.

Nicoll, J. A., Mrak, R. E., Graham, D. I. *et al.* (2000). Associations of Interleukin-1 gene polymorphisms with Alzheimer's disease. *Ann Neurol*, **47**: 365–8.

O'Meara, E. S., Kukull, W. A., Sheppard, L. *et al.* (1997). Head injury and the risk of Alzheimer's disease by apolipoprotein E genotype. *Am J Epidemiol*, **146**: 373–84.

Pierce, J. E., Smith, D. H., Trojanowski, J. Q. & McIntosh, T. K. (1998). Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience*, **87**: 359–69.

Plassman, B. L., Havlik, R. J., Steffens, D. C. *et al.* (2000). Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology*, **55**: 1158–66.

Raghupathi, R., Graham, D. I., McIntosh, T. K. (2000). Apoptosis after traumatic brain injury. *J Neurotrauma*, **17**: 927–38.

Rasmussen, D. X., Brandt, J., Martin, D. B., Folstein, M. E (1995). Head injury as a risk factor in Alzheimer's disease. *Brain Inj*, **9**: 213–19.

Rink, A. D., Fung, K. M., Trojanowski, J. Q., Lee, V. M.-Y., Neugebauer, E. & McIntosh, T. K. (1995). Evidence of apoptotic cell death after experimental traumatic brain injury in the rat. *Am J Path*, **147**: 1575–83.

Roberts, A. H. (1969). *Brain Damage in Boxers. A Study of Prevalence of Traumatic Encephalopathy Among Ex-professional Boxers*. London: Pitman.

Roberts, G. W., Gentleman, S. M., Lynch, A. & Graham, D. I. (1991). β-A4 amyloid protein deposition in brain after head trauma. *Lancet*, **338**: 1422–3.

Roberts, G. W., Gentleman, S. M., Lynch, A., Murray, L., Landon, M. & Graham, D. I. (1994). β-amyloid protein deposition in the brain following severe head injury: implications for the pathogenesis of Alzheimer's disease. *J Neurol Neurosurg Psychiat*, **57**: 419–25.

P1: MRM
0521819156c20 October 18, 2003 9:30 Char Count= 0

Roses, A. D. & Saunders, A. (1995). Head injury, amyloid beta and Alzheimer's disease. *Nat Med*, **1**: 603–4.

Ross, D. T., Graham, D. I. & Adams, J. H. (1993). Selective loss of neurons from the thalamic reticular nucleus following severe human head injury. *J Neurotrauma*, **10**: 151–65.

Salib, E. & Hillier, V. (1997). Head injury and the risk of Alzheimer's disease: a case-control study. *Int J Geriatr Psychiatry*, **12**: 363–8.

Saunders, A. M., Strittmatter, W. J., Schmechel, D. *et al.* (1993). Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology*, **43**: 1467–72.

Schmidt, M. L., Zhukareva, V., Newell, K. L., Lee, V. M.-Y., Trojanowski, J. Q. (2001). Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. *Acta Neuropathol*, **101**: 518–24.

Schmidt, R. H. & Grady, M. S. (1995). Loss of forebrain cholinergic neurons following fluid-percussion injury: implications for cognitive impairment in closed head injury. *J Neurosurg*, **83**: 496–502.

Schofield, P. W., Tang, M., Marder, K. *et al.* (1997). Alzheimer's disease after remote head injury: an incidence study. *J Neurol Neurosurg Psychiat*, **62**: 119–24.

Shaw, K., MacKinnon, M.-A., Raghupathi, R., Saatman, K. E., McIntosh, T. K. & Graham, D. I. (2001). TUNEL-positive staining in white and grey matter after fatal head injury in man. *Clin Neuropathol*, **20**: 106–12.

Sheng, J. G., Mrak, R. E. & Griffin, W. S. T. (1997). Glial–neuronal interactions in Alzheimer's disease: progressive association of IL-1α+ microglia and S100β+ astrocytes with neurofibrillary tangle stages. *J Neuropathol Exp Neurol*, **56**: 285–90.

Sheng, J. G., Zhu, S. G., Griffin, W. S. T. & Mrak, R. E. (2000). Interleukin-1 promotes expression and phosphorylation of tau protein in vivo. *Exp Neurol*, **163**: 388–91.

Smith, D. H., Chen, X. H., Nonaka, M. *et al.* (1999). Accumulation of amyloid beta and tau and the formation of neurofilament inclusions following diffuse brain injury in the pig. *J Neuropath Exp Neurol*, **58**: 982–92.

Smith, D. H., Chen, X. H., Pierce, J. E. *et al.* (1997). Progressive atrophy and neuron death for one year following brain trauma in the rat. *J Neurotrauma*, **14**: 715–27.

Smith, F. M., Raghupathi, R., MacKinnon, M.-A. *et al.* (2000). TUNEL-positive staining of surface contusions after fatal head injury in man. *Acta Neuropathol*, **100**: 537–45.

Teasdale, G. & Jennett, B. (1974). Assessment of coma and impaired consciousness: a practical scale. *Lancet*, **2**: 81–4.

(1976). Assessment and prognosis of coma after head injury. *Acta Neurochir*, **34**: 45–55.

Teasdale, G. M., Pettigrew, L. E., Wilson, J. T., Murray, G. & Jennett, B. (1998). Analyzing outcome of treatment of severe head injury: a review and update on advancing the use of the Glasgow Outcome Scale. *J Neurotrauma*, **15**: 587–97.

Teasdale, G. M., Nicoll, J. A., Murray, G. & Fiddes, M. (1997). Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet*, **350**: 1069–71.

Thornhill, S., Teasdale, G. M., Murray, G. D., McEwen, J., Roy, C. W. & Penny, K. I. (2000). Disability in young people and adults one year after head injury: prospective cohort study. *Br Med J*, **320**: 1631–5. *Am J Epidemiol*, **135**: 775–82.

van Duijn, C. M., Tanja, T. A., Haaxma, R. *et al.* (1992). Head trauma and the risk of Alzheimer's disease. *Am J Epidemiol*, **135**: 775–82.

Williams, D. B., Annegers, J. E., Kokmen, E., O'Brien, P. C. & Kurland, L. T. (1991). Brain injury and neurologic sequelae: a cohort study of dementia, parkinsonism, and amyotrophic lateral sclerosis. *Neurology*, **41**: 1554–7.

Williams, S., Raghupathi, R., MacKinnon, M.-A., McIntosh, T. K., Saatman, K. E. & Graham, D. I. (2001). In-situ DNA fragmentation occurs in white matter up to 12 months after head injury in man. *Acta Neuropathol*, **102**: 581–90.

Zemlan, F. P., Rosenberg, W. S., Luebbe, P. A. *et al.* (1999). Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins. *J Neurochem*, **73**: 437–8.

Zhang, J., Dawson, V. L., Dawson, T. M. & Snyder, S. H. (1994). Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science*, **263**: 687–9.

Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Forensic Science International 146 (2004) 97–104
Forensic Science International
www.elsevier.com/locate/forsciint

Long-term intracerebral inflammatory response after traumatic brain injury

S.M. Gentleman[a,*], P.D. Leclercq[a], L. Moyes[b], D.I. Graham[b], C. Smith[c], W.S.T. Griffin[d], J.A.R. Nicoll[e]

[a]*Department of Neuropathology, Division of Neuroscience and Psychological Medicine, Imperial College London, Charing Cross Campus, St Dunstan's Road, London W6 8RP, UK*
[b]*Academic Unit of Neuropathology, Division of Clinical Neuroscience, Institute of Neurological Sciences, Southern General Hospital NHS Trust, 1345 Govan Road, Glasgow G51 4TF, Scotland, UK*
[c]*Department of Pathology, University of Edinburgh, Alexander Donald Building, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*
[d]*Department of Geriatrics, University of Arkansas for Medical Sciences, 629 Jack Stephens Drive, 3103 Little Rock, AR 72205, USA*
[e]*Clinical Neurosciences, Department of Neuropathology, South Pathology Block, Southampton General Hospital, Southampton SO16 6YD, UK*

Available online 25 August 2004

Abstract

Epidemiological and pathological studies suggest that head injury is a significant risk factor for subsequent neurodegeneration and cognitive decline in later life. The precise mechanisms for the development of post-traumatic neurodegenerative change are unclear but we hypothesize that persistence of inflammatory processes in the brain may play a key role and that some individuals are more susceptible to such changes based on their genetic make-up. In support of this hypothesis we present evidence of persistent elevated microglial activity in long-term survivors of head injury and the suggestion of an association between the extent of this activity and interleukin-1 genotype.

Keywords: Traumatic brain injury; Inflammation; Neurodegeneration; Cytokine cycle

1. Introduction

Head injury is a significant risk factor for neurodegeneration and cognitive decline in later life. This observation is based largely on the results of long-term outcome studies of patients with head injury and epidemiological studies of the environmental risk factors for Alzheimer's disease (AD). One of the largest epidemiological studies, part of the MIRAGE (multi-institutional research in Alzheimer genetic epidemiology) project, involved nearly 17,000 individuals [1]. Within this cohort there were 2233 probands who met the criteria for probable or definite AD, while the rest were first-degree relatives or spouses with no signs of dementia. When the histories of the probands were compared with those of their relatives the authors confirmed that traumatic brain injury was a risk factor for AD and, furthermore, that the risk was proportional to the severity of the injury. To illustrate this, a comparison of probands with unaffected spouses yielded odds ratios for AD of 9.9 for head injury with loss of consciousness and 3.1 for head injury without loss of consciousness. In another study a large cohort of people who served in the armed forces in World War II were

* Corresponding author. Tel.: +44 20 8846 7680; fax: +44 20 8846 7680.
E-mail address: s.gentleman@imperial.ac.uk (S.M. Gentleman).

0379-0738/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2004.06.027

assessed for AD and their military medical records checked for evidence of a non-penetrating traumatic brain injury [2]. In this population a moderate head injury resulted in a 2.3-fold increased risk of AD, while for severe injuries this rose to a 4-fold increased risk. While these large cohort studies provide strong support for the link between head trauma and AD it must be realised that such retrospective studies can suffer from recall and selection bias and there are other studies that have not been able to find an association [3].

Further insight into a potential link between head injury and neurodegenerative change has come from the study of boxers with dementia pugilistica or 'punch-drunk' syndrome. In this case the association is between repetitive sub-clinical head injury experienced during the course of a boxing career and the emergence of a presenile dementing syndrome in later life. At the pathological level it has been known for some time that neurofibrillary tangles, similar to those seen in AD, can be found in the brains of boxers with this syndrome [4] and more recently the molecular composition of the tangles has been shown to be the same as that seen in AD [5]. In addition, Geddes et al [6] examined the brains of four young individuals (age range from 23 to 28 years) with a history of repetitive head injury (two boxers, one footballer, and one mentally subnormal patient with a history of self inflicted head banging) and a frontal lobectomy specimen from an individual with intractable complex partial seizures with recurrent minor head injury. They identified widespread neocortical neurofibrillary tangles and neuropil threads not seen in age-matched controls, which in areas showed a peri-vascular distribution. In the original study by Corsellis [4] and colleagues, using standard silver staining techniques, no amyloid plaques were seen. However, with the advent of specific antibodies raised against the β-amyloid peptide (Aβ) re-examination of the sections revealed widespread diffuse plaques with no neuritic components [7].

Surprisingly, the link between head injury and Alzheimer-type pathology is not restricted to long-term survivors. In a large cohort of patients who died within weeks of a severe head injury just under a third were found to have some degree of Aβ deposition in their brains at post mortem [8,9]. The patients in this cohort covered a large age range and undoubtedly some of these deposits will have been there before the injury and reflect the normal ageing process. However, in the younger patients it is unlikely that there would have been any age-related deposits, supporting the suggestion that they were produced as a result of the head trauma. Not all groups have been able to confirm this phenomenon [10] but further studies have added weight to this interpretation by showing that, after acute brain injury in man and in experimental models, there is an upregulation of many of the proteins thought to be involved in the pathogenesis of AD [11,12]. Upregulation of the β-amyloid precursor protein (APP), in particular, may favour an increase in processing and production of Aβ.

2. Neuroinflammation

The precise mechanisms involved in the development of neurodegenerative pathology in survivors of traumatic brain injury are unclear, but they are likely to include a combination of disrupted calcium homeostasis, free radical mediated damage and inflammatory changes. The last of these is the object of increasing interest in that many key inflammatory proteins have been found associated with the Aβ plaques in AD and are thought to play an important role in the evolution of the plaques and the progression of the disease [13]. There are some key differences between the inflammatory components of AD pathology and the changes seen in the brain in the immediate aftermath of a brain injury. While there is no evidence for a classical cell mediated immune response in AD, after brain injury one can observe infiltration and accumulation of T-lymphocytes and macrophages during the acute post-traumatic period when the blood brain barrier is compromised [14]. However, we have observed that the T-lymphocyte numbers decline relatively rapidly towards control levels after 48 h and do not persist in the tissue suggesting that they are unlikely to play a direct role in any chronic neurodegenerative process. By contrast CD68 positive macrophages were observed even at the longest survival times and were not restricted to areas of focal damage [15]. It therefore seems that resident microglial cells and/or invading macrophages are the key cells mediating the inflammatory processes in both AD and in long-term survivors of head injury. Taking this one stage further one might expect that the microglial response of an individual may affect both the immediate outcome following injury and the increased susceptibility to AD later in life.

When they are activated, microglial cells have a number of actions including phagocytosis of cell debris and the secretion of pro-inflammatory cytokines such as interleukin-1 (IL-1). IL-1 promotes microglial proliferation, activation of astrocytes resulting in the increased production of proteins such as apolipoprotein E (apoE) and it can also induce the neuronal production of APP [16,17]. IL-1 is chronically over-expressed in AD [18] and can be found in microglial cells close to APP-immunoreactive cells and neurites in head-injured patients [11]. These observations have given rise to the so called cytokine cycle in which AD, epilepsy, trauma or indeed any brain insult may initiate an inflammatory response in the brain, which is initially protective but in some susceptible individuals is self-perpetuating and goes on to cause neurodegenerative changes [19]. Precisely how this sustained glial response results in the death of neurons is not clear but, based on recent in vitro studies, one theory is that microglial derived nitric oxide may cause impairment of mitochondrial function in neighbouring neurons and release of glutamate, which acts on NMDA receptors to promote an influx of Ca2+ ions into the cell, eventually leading to cell death [20].

3. Genotype effects

One of the key aspects of the processes outlined in the cytokine cycle is that they may be influenced by the genetic make-up of an individual. Possession of the ε4 allele of the gene for apolipoprotein E (*APOE*) has been firmly established as a genetic risk factor for the development of AD (Saunders et al., 1993). Significantly, patients with this allele who die from a head injury are four times as likely to have evidence of cortical Aβ deposition than those without [21]. Furthermore the numerical density of the deposits appears to correlate with the ε4 allele dosage, with homozygotes having more than heterozygotes [22]. There are at least two different possible interpretations of this data. The first is that individuals with an ε4 allele are more likely to trigger Aβ deposition in their brains after a head injury. The second is that the Aβ deposits are age-related, pre-dating the injury, and that those with an ε4 allele have a higher mortality from head injury thereby biasing their selection in autopsy studies [23]. Deciding which of these interpretations is correct will require the development of in vivo imaging techniques for Aβ deposits.

An effect of genotype on outcome after head injury has been observed in a prospectively acquired patient series [24]. Around 57% of the patients with an ε4 allele had an unfavourable outcome 6 months after injury, compared with 27% in those patients without an ε4 allele. Subsequent studies have reinforced this with the observations that possession of an ε4 allele is an indicator of poor prognosis in patients with post-traumatic coma [25] and is a predictor of poor functional outcome in general after head injury [26].

Polymorphisms in the *APOE* gene are not the only genetic influences associated with AD. Single nucleotide polymorphisms in the promotor region (−889 C → T) of the *IL1A* gene and the coding region (+3953 C → T) of the *IL-1B* gene, which encode the two isoforms of IL-1, have previously been shown to modify the risk for certain inflammatory diseases [27,28]. The well-documented involvement of inflammation and IL-1 in the development of AD pathology prompted a number of studies to determine if the polymorphisms would modulate risk for AD [29–32]. Nicoll et al studied 232 neuropathologically confirmed cases of AD and found the *IL1A* −889 TT risk genotype in 12.9% of late onset AD (LOAD) cases as compared to 6.6% of 167 age-matched and *APOE*-matched controls from four centres in the UK and US. This yielded an odds ratio of 2.97 ($P = 0.011$) after controlling for age, for possession of the ε4 allele, and without regard to centre of origin. In addition they found that homozygosity for allele T of both *IL1A* and *IL-1B* conferred an even greater risk (odds ratio = 10.8, $P < 0.005$), although homozygosity for *IL-1B* +3953T allele alone was not significant. Others report that the *IL1A* −889 TT genotype can modulate the course of AD course by reducing the age of onset. These authors found more *IL1A* −889 CT and TT in early onset AD than in LOAD [33–36].

These findings strongly support the importance of IL-1 in the pathogenesis of AD. Demographic differences may contribute to some of the negative findings as most studies on Chinese/Korean populations failed to find any difference between the frequencies of *IL1A* −889 TT genotypes in AD and controls. In their population studies, TT genotypes were extremely rare to absent [37–39]. Other studies on white Americans [40] and European [35,41,42] populations failed to show *IL1A* −889 T allele as a risk factor for AD.

4. A hypothesis

Much of the evidence outlined above points to a central role for microglial cells in the pathology of AD and the development of neurodegenerative changes in the brains of long-term survivors of head injury. Taken with the findings that the genotype of an individual can affect outcome after a head injury we hypothesize that the genotype of an individual will affect the nature of their microglial response to head injury. Some of the possible ways in which one might envisage this genotype phenotype interaction are illustrated in a modified version of the cytokine cycle (Fig. 1). The simplest scenario might be that a rapid microglial response in the acute phase after an injury might be expected to be beneficial but that if this response is maintained over a long survival time it may lead to the later cognitive decline reported in the clinical studies. There is already some evidence to support this idea in AD, where it has been reported that patients with the ε4 allele of *APOE* have increased microglial activity in their brains at post mortem compared to patients without the risk allele [43].

5. Microglia and contusions

In a preliminary study designed to test the hypothesis that genotype might affect the microglial response we studied a series of 15 surgically removed contusions from patients with head injury. Controls comprised cerebral cortical tissue without specific neuropathological abnormality from 14 biopsies and 14 autopsies. *APOE* and *IL-1* genotypes were determined by PCR from paraffin sections as previously described [29]. Sections were immunostained with anti-CD68 to identify microglia (mouse monoclonal antibody to a macrophage-specific 110 kDa glycoprotein-Dako, 1:1,000) and CR3/43 which labels activated microglia (mouse monoclonal antibody to class II MHC: HLA-DR, -DQ and -DP β chains-Dako, 1:800). Immunoreactivity was assessed quantitatively by taking the mean number of cells stained in 5 × 20 objective fields from each case.

As might be expected, more immunoreactive microglia were seen in the contusion group than in either set of controls and this was true for both antibodies (Fig. 2). There was a trend towards an increase in microglial number with age but this didn't reach significance, possibly because of the rela-

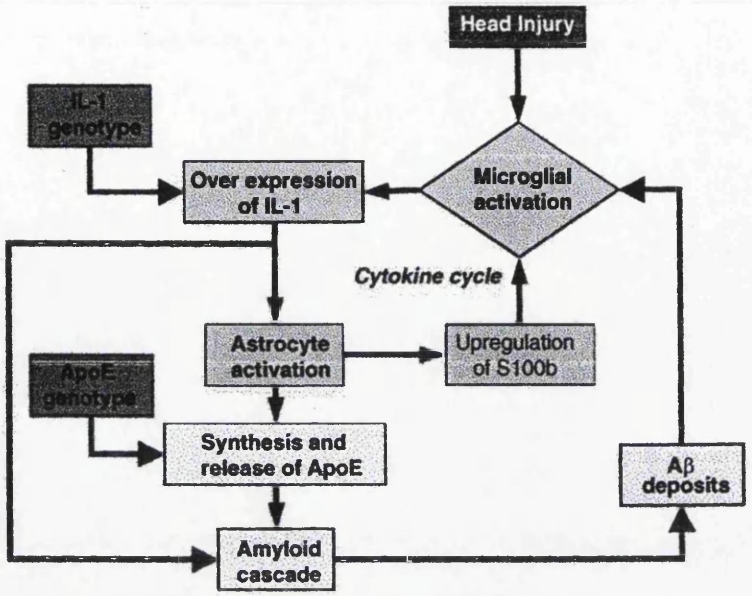


Fig. 1. A possible model for the influence of gene polymorphisms in the cytokine cycle and resultant amyloid cascade following head injury.

tively small numbers in the study. Likewise there was no association between survival time (in this case defined as the time between the trauma and the surgical excision of the contusion) and number of activated microglia. However, analysis of the cases in terms of their genotype revealed some interesting patterns. A simple analysis based on the presence or absence *APOE*ϵ4 revealed no difference in terms of microglial activation, but a similar subdivision in terms of the possession of none, 1 or 2 *IL1A* −889T alleles showed that those with the risk allele had greater microglial activation than those without (Fig. 3). Furthermore a plot of the time between head injury and surgery shows some stratification in terms of genotype (Fig. 4).

6. Microglia and head injury

Encouraged by the possibility that there might be genotype-based effects on pathological phenotype the studies were expanded to look at a large cohort of 81 consecutive

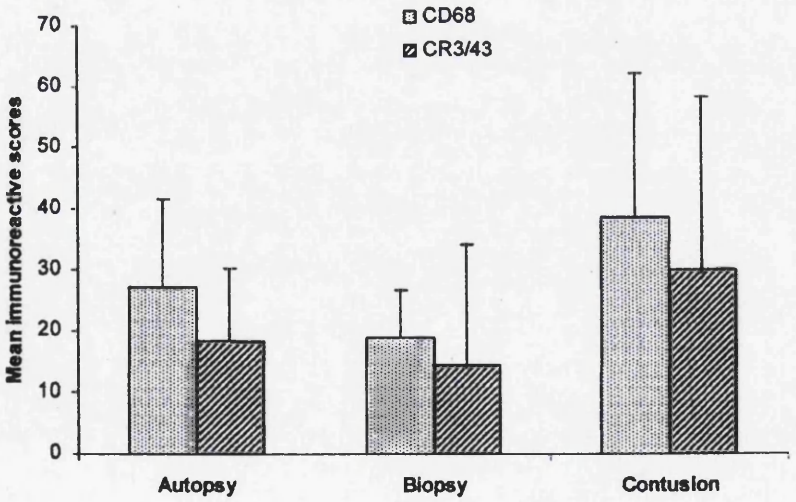


Fig. 2. A bar chart illustrating the extent of microglial immunoreactivity in three different groups: autopsy controls, biopsy controls and contusion biopsy samples. The contusion biopsy group shows an overall increase in the amount of immunoreactivity with both microglial markers CD68 (dotted) and CR3/43 (dashed) as compared to the two control groups.

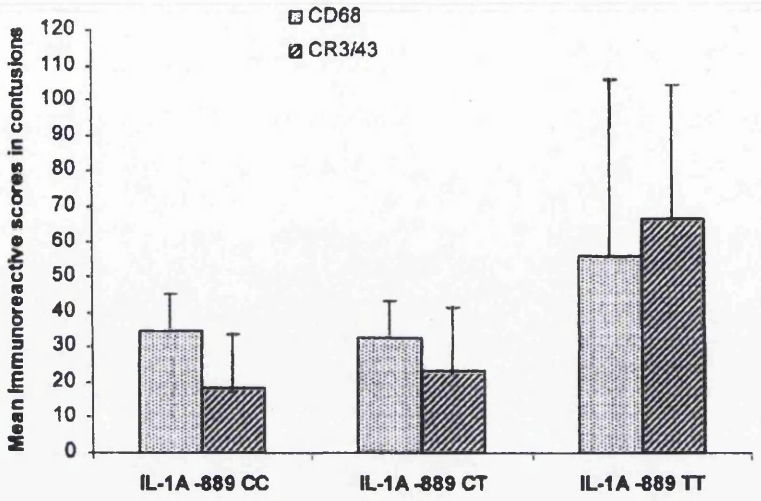


Fig. 3. A bar chart suggesting a possible IL-1A–889 T allele dose effect on CD68 and CR3/43 immunoreactivity scores in the contusion biopsy group.

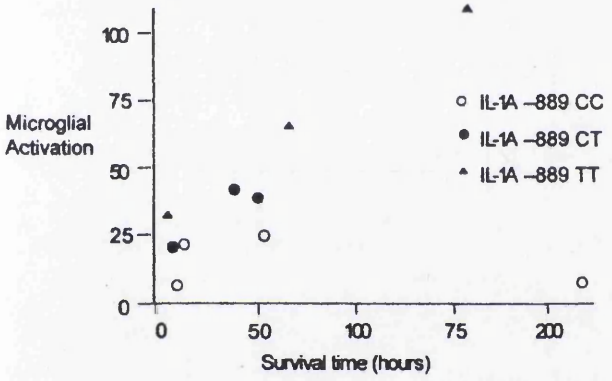


Fig. 4. A scatterplot illustrating the association of microglial activation and survival time in relation to IL-1A genotype.

cases of blunt head injury managed by the Department of Neuropathology, Institute of Neurological Sciences in Glasgow (60 males, 21 females; age range 0.15–79 years; survival times ranging from <11 to 334 h). CD68 immunoreactivity expressed by microglia and macrophages was quantified in contusion, grey and white matter using image analysis (Image-Pro Plus, Datacell) (Fig. 5).

As with the previous study, independent of patient genotype, CD68 loads were highest in the contusions and lowest in the grey matter regions remote from the site of contusion. When data were analyzed in terms of presence or absence of risk alleles (i.e. ϵ4 or allele T), there was no significant difference in the CD68 load between patients with or without the risk alleles in contusion and white matter. Subjects without ϵ4 allele had significantly higher CD68 loads in the grey matter than those with one or two ϵ4 allele (P = 0.049 using a Mann–Whitney test). There was a significant relationship between survival time and CD68 load in the contusion area (P = 0.002) and in the white matter (P = 0.038) of head-injured patients (one-way ANOVA on ranks). Spearman Rank Tests revealed no effect of age or post-mortem interval on CD68 loads.

The results of this study suggest that there is not a simple relationship between the extent of the microglial response after head injury, in terms of CD68 load, and patient *IL-1* genotype. Paradoxically, the absence of an ϵ4 risk allele was associated with an increased CD68 load in non-contused grey matter. However, for a number of reasons, these results should be interpreted with caution. First, the initial traumatic insult in these patients was very heterogeneous and this may mask any genotype variations. This is very difficult to control for, although in all cases, the injury was severe enough to cause death. Secondly, there were only relatively few cases homozygous for the risk alleles *APOE*ϵ4 (4), *IL1A* −889T allele (8) and *IL-1B* +3953 T allele (4). To reduce the risk of any bias in the statistical analysis it will be necessary to look at more cases with these genotypes. In terms of survival time there was a significant increase in the CD68 load in contusions and white matter 2–3 days after injury. However, again the results need careful interpretation because there was a skewed distribution in the survival times of the patients, with the majority dying within the first 48 h after head injury. Further studies are needed on cases with longer survival times.

7. Long-term microglial response

To address this, a new cohort of head-injured cases was identified from the archive in Glasgow. Cases were separated into two groups; the first with survival times of less than 12 months and the second with survival times from 12 months up to 22 years. In addition age-matched controls (n = 15),

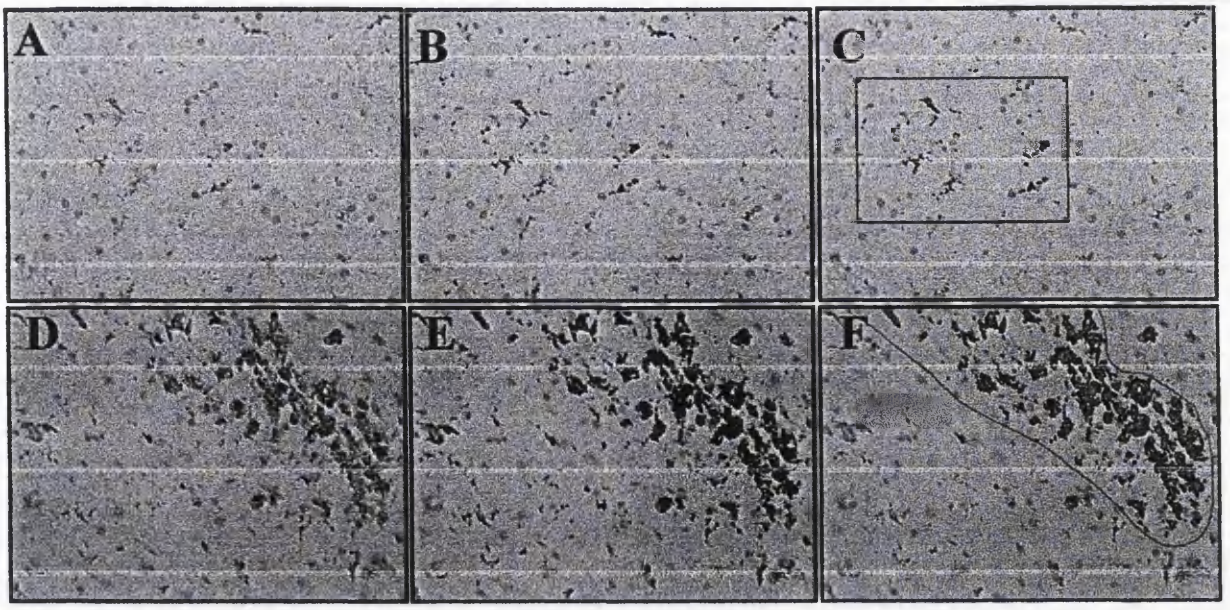


Fig. 5. Two examples illustrating the image analysis procedure in a non-contused (A–C) and a contused tissue area (D–F). Images of CD68 immunoreactive cells were captured using a X20 microscope objective (A,D). The images were thresholded on the basis of hue and intensity (B,E) and measurements expressed as area of immunostaining divided by area of interest sampled.

without significant neurological disease were identified. Sections of parasagittal cortex including underlying white matter, and hippocampus were immunostained for CD68 and CR3/43 to identify microglia and MHC class II activated cells, respectively.

Both CD68 and CR3/43 immunoreactivity increased with ageing in the controls, reflecting both hypertrophy and hyperplasia of microglia. In head-injured cases there was, however, a greater increase in CD68 immunoreactivity in both parasagittal and hippocampal white matter than was seen up to 4 years after the injury and this appeared not to be influenced by age. CR3/43 immunoreactivity had a greater load in both parasagittal and hippocampal white matter and was seen up to 16 years after the injury (Fig. 6).

In summary, we have described microglial hyperplasia and hypertrophy with both MHC class II upregulation and

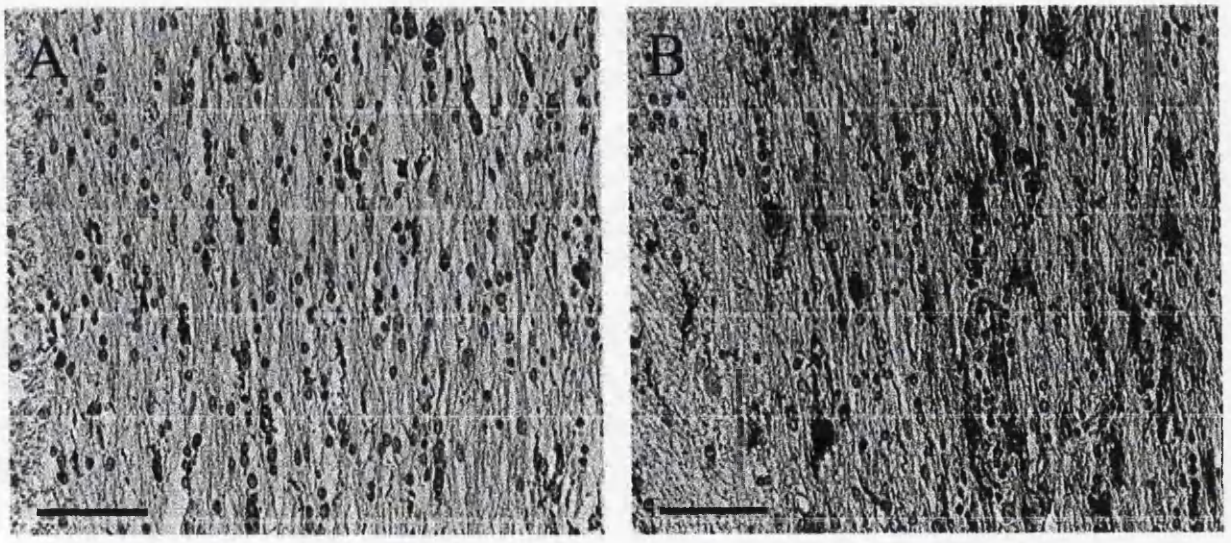


Fig. 6. White matter from the frontal lobe of a long term head injury survivor immunostained for the microglial markers CD68 (A) and CR3/43 (B). Note the disparity in the number and size of microglia stained with the lysosomal marker (A) as compared to the MHC class II marker (B). Bars = 50 μm.

increased phagocytosis in the white matter of cases of fatal head injury, and continued neuroinflammation for many years in survivors. This may be due to the consequences of both focal and diffuse pathologies after traumatic brain injury and may form the basis for the cognitive deficits seen in long-term survivors.

Acknowledgement

Much of this work was funded by NIH grant AG12411.

References

[1] Z. Guo, L.A. Cupples, A. Kurz, et al. Head injury and the risk of AD in the MIRAGE study, Neurology 54 (2000) 1316–1323.
[2] B.L. Plassman, R.J. Havlik, D.C. Steffens, et al. Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias, Neurology 55 (2000) 1158–1166.
[3] M.M. Breteler, R.R. de Groot, L.K. van Romunde, A. Hofman, Risk of dementia in patients with Parkinson's disease, epilepsy, and severe head trauma: a register-based follow-up study, Am. J. Epidemiol. 142 (1995) 1300–1305.
[4] J.A.N. Corsellis, C.J. Bruton, D. Freeman-Browne, The aftermath of boxing, Psych. Med. 3 (1973) 270–303.
[5] M.L. Schmidt, V. Zhukareva, K.L. Newell, V.M. Lee, J.Q. Trojanowski, Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease, Acta Neuropathol. (Berl) 101 (2001) 518–524.
[6] J.F. Geddes, G.H. Vowles, J.A. Nicoll, T. Revesz, Neuronal cytoskeletal changes are an early consequence of repetitive head injury, Acta Neuropathol. (Berl) 98 (1999) 171–178.
[7] G.W. Roberts, D. Allsop, C. Bruton, The occult aftermath of boxing, J. Neurol. Neurosurg. Psychiatry 53 (1990) 373–378.
[8] G.W. Roberts, S.M. Gentleman, A. Lynch, D.I. Graham, beta A4 amyloid protein deposition in brain after head trauma, Lancet. 338 (1991) 1422–1423.
[9] G.W. Roberts, S.M. Gentleman, A. Lynch, L. Murray, M. Landon, D.I. Graham, Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease, J. Neurol. Neurosurg. Psychiatry 57 (1994) 419–425.
[10] H. Adle-Biassette, C. Duyckaerts, M. Wasowicz, et al. Beta AP deposition and head trauma, Neurobiol. Aging 17 (1996) 415–419.
[11] W.S. Griffin, J.G. Sheng, S.M. Gentleman, D.I. Graham, R.E. Mrak, G.W. Roberts, Microglial interleukin-1 alpha expression in human head injury: correlations with neuronal and neuritic beta-amyloid precursor protein expression, Neurosci. Lett. 176 (1994) 133–136.
[12] A. Lewen, G.L. Li, P. Nilsson, Y. Olsson, L. Hillered, Traumatic brain injury in rat produces changes of beta-amyloid precursor protein immunoreactivity, Neuroreport 6 (1995) 357–360.
[13] W.S. Griffin, J.G. Sheng, G.W. Roberts, R.E. Mrak, Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution, J. Neuropathol. Exp. Neurol. 54 (1995) 276–281.
[14] S. Holmin, J. Soderlund, P. Biberfeld, T. Mathiesen, Intracerebral inflammation after human brain contusion, Neurosurgery 42 (1998) 291–298.
[15] S.M. Gentleman, J. Kanga, L. Christian, P.D. Leclercq, D.I. Graham, Is there a cellular immune response in the brain following head injury in man? J. Neurotrauma 16 (1999) 994.
[16] J.D. Buxbaum, M. Oishi, H.I. Chen, et al. Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor, Proc. Natl. Acad. Sci. U.S.A 89 (1992) 10075–10078.
[17] S.W. Barger, A.D. Harmon, Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E, Nature 388 (1997) 878–881.
[18] W.S. Griffin, L.C. Stanley, C. Ling, et al. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 7611–7615.
[19] W.S. Griffin, J.G. Sheng, M.C. Royston, et al. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression, Brain Pathol. 8 (1998) 65–72.
[20] A. Bal-Price, G.C. Brown, Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity, J. Neurosci. 21 (2001) 6480–6491.
[21] J.A. Nicoll, G.W. Roberts, D.I. Graham, Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury, Nat. Med. 1 (1995) 135–137.
[22] K. Horsburgh, G.M. Cole, F. Yang, et al. beta-amyloid (Abeta)42(43), abeta42, abeta40 and apoE immunostaining of plaques in fatal head injury, Neuropathol. Appl. Neurobiol. 26 (2000) 124–132.
[23] A.D. Roses, A. Saunders, Head injury, amyloid beta and Alzheimer's disease, Nat. Med. 1 (1995) 603–604.
[24] G.M. Teasdale, J.A. Nicoll, G. Murray, M. Fiddes, Association of apolipoprotein E polymorphism with outcome after head injury, Lancet 350 (1997) 1069–1071.
[25] S. Sorbi, B. Nacmias, S. Piacentini, et al. ApoE as a prognostic factor for post-traumatic coma, Nat. Med. 1 (1995) 852.
[26] G. Friedman, P. Froom, L. Sazbon, et al. Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury, Neurology 52 (1999) 244–248.
[27] T.L. McDowell, J.A. Symons, R. Ploski, O. Forre, G.W. Duff, A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism, Arthritis Rheum. 38 (1995) 221–228.
[28] K.S. Kornman, A. Crane, H.Y. Wang, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease, J. Clin. Periodontol. 24 (1997) 72–77.
[29] J.A. Nicoll, R.E. Mrak, D.I. Graham, et al. Association of interleukin-1 gene polymorphisms with Alzheimer's disease, Ann. Neurol. 47 (2000) 365–368.
[30] O. Combarros, M. Sanchez-Guerra, J. Infante, J. Llorca, J. Berciano, Gene dose-dependent association of interleukin-1A [−889] allele 2 polymorphism with Alzheimer's disease, J. Neurol. 249 (2002) 1242–1245.
[31] R. Hedley, J. Hallmayer, D.M. Groth, W.S. Brooks, S.E. Gandy, R.N. Martins, Association of interleukin-1 polymorphisms with Alzheimer's disease in Australia, Ann. Neurol. 51 (2002) 795–797.

[32] Y. Du, R.C. Dodel, B.J. Eastwood, et al. Association of an interleukin 1 alpha polymorphism with Alzheimer's disease, Neurology 55 (2000) 480–483.
[33] L.M. Grimaldi, V.M. Casadei, C. Ferri, et al. Association of early-onset Alzheimer's disease with an interleukin-1alpha gene polymorphism, Ann. Neurol. 47 (2000) 361–365.
[34] G.W. Rebeck, Confirmation of the genetic association of interleukin-1A with early onset sporadic Alzheimer's disease, Neurosci. Lett. 293 (2000) 75–77.
[35] H. Kolsch, U. Ptok, M. Bagli, et al. Gene polymorphisms of interleukin-1alpha influence the course of Alzheimer's disease, Ann. Neurol. 49 (2001) 818–819.
[36] F.L. Sciacca, C. Ferri, F. Licastro, et al. Interleukin-1B polymorphism is associated with age at onset of Alzheimer's disease, Neurobiol. Aging 24 (2003) 927–931.
[37] Y.M. Kuo, P.C. Liao, C. Lin, et al. Lack of association between interleukin-1alpha polymorphism and Alzheimer disease or vascular dementia, Alzheimer Dis. Assoc. Disord. 17 (2003) 94–97.
[38] S.J. Tsai, H.C. Liu, T.Y. Liu, K.Y. Wang, C.J. Hong, Lack of association between the interleukin-1alpha gene C(−889)T polymorphism and Alzheimer's disease in a Chinese population, Neurosci. Lett. 343 (2003) 93–96.
[39] C.S. Ki, D.L. Na, D.K. Kim, H.J. Kim, J.W. Kim, Lack of association of the interleukin-1alpha gene polymorphism with Alzheimer's disease in a Korean population, Ann. Neurol. 49 (2001) 817–818.
[40] L. Fidani, A. Goulas, V. Mirtsou, et al. Interleukin-1A polymorphism is not associated with late onset Alzheimer's disease, Neurosci. Lett. 323 (2002) 81–83.
[41] E.K. Green, J.M. Harris, H. Lemmon, et al. Are interleukin-1 gene polymorphisms risk factors or disease modifiers in AD? Neurology 58 (2002) 1566–1568.
[42] M. Pirskanen, M. Hiltunen, A. Mannermaa, et al. Interleukin 1 alpha gene polymorphism as a susceptibility factor in Alzheimer's disease and its influence on the extent of histopathological hallmark lesions of Alzheimer's disease, Dement. Geriatr. Cogn. Disord. 14 (2002) 123–127.
[43] R. Egensperger, S. Kosel, U. von Eitzen, M.B. Graeber, Microglial activation in Alzheimer disease: association with APOE genotype, Brain Pathol. 8 (1998) 439–447.

Association of APOE ε4 and cerebrovascular pathology in traumatic brain injury.

Colin Smith[1,2] MRCPath, David I Graham[1] PhD FRCPath , Lilian S Murray[3] PhD, and James AR Nicoll[1,4] MD FRCPath

1 Department of Neuropathology, University of Glasgow, Institute of Neurological Sciences, Southern General Hospital, Glasgow, G51 4TF, UK.

2 Neuropathology Laboratory, Department of Pathology, University of Edinburgh, Western General Hospital, Edinburgh, EH4 2XU.

3 Department of Medicine and Therapeutics, University of Glasgow, Western Infirmary, Glasgow, G12, UK.

4 Division of Clinical Neurosciences, University of Southampton, Southampton General Hospital, Southampton, SO16 6YD, UK.

Address for correspondence:

C Smith

Neuropathology Laboratory, Department of Pathology,

University of Edinburgh,

Western General Hospital,

Edinburgh, EH4 2XU.

Tel: 0131-537 1975

Fax: 0131-537 1013

e-mail:col.smith@ed.ac.uk

Dr C Smith was supported by a Clinical Research Fellow grant from the Scottish Council for Postgraduate Medical and Dental Education, UK.

Abstract

Previous studies have found the ε4 allele of the apolipoprotein E gene (*APOE* ε4) is associated with an unfavourable outcome after head injury. In order to clarify the mechanisms involved, in this study association was sought between carriage of *APOE* ε4 and specific pathological features of traumatic brain injury (TBI). Included in the study were 239 fatal cases of TBI (1987-1999) for which *APOE* genotypes were determined from archival tissue. For each case specific pathological features of trauma were recorded blind to the *APOE* ε4 status. Of the 238 cases examined, there were 83 *APOE* ε4 carriers (35 %) and 156 non-carriers (65 %).

Possession of *APOE* ε4 was associated with a greater incidence of moderate or severe contusions (ε4 carriers 42% versus ε4 non-carriers 30%; p= 0.05) and a greater incidence of severe ischaemic brain damage (ε4 carriers 54% versus ε4 non-carriers 42%; p= 0.08). Significant differences were not noted between the other pathological features examined.

Possession of *APOE* ε4 is associated with a greater incidence of moderate/severe contusional injury and severe ischaemic brain damage in fatal cases of TBI. This may be relevant to the relatively poor outcome from traumatic brain injury in patients with *APOE* ε4 identified in clinical studies.

Keywords: APOE, polymorphisms, head injury, contusions, ischaemic damage

Introduction

Clinical studies of traumatic brain injury (TBI) have shown that possession of the ε4 allele of the apolipoprotein E gene (*APOE* ε4) is associated with a relatively poor outcome (1, 2). In one such study 57% of *APOE* ε4 carriers had an unfavourable outcome (defined as dead, in the vegetative state or with severe disability) compared with 27% of non-carriers of *APOE* ε4 (1). There is evidence that *APOE* ε4 carriers also have worse outcome after spontaneous intracerebral haemorrhage (3, 4), cardiac bypass surgery (5, 6), cerebral ischaemia after cardiopulmonary resuscitation (7) and in boxing (8); in subarachnoid haemorrhage the evidence is conflicting (9-11) and the effect appears not to influence outcome from ischaemic stroke (12).

The specific mechanisms by which *APOE* genotype influences outcome after brain injury in humans are largely unclear. Much of the work relating to mechanisms involving apoE has been undertaken using *in-vitro* cell cultures and animal models, and the direct relevance of these studies to man is uncertain (13). Relevant mechanisms postulated range from basic cellular functions such as maintenance of cytoskeletal integrity (14), and protection from oxidative stress (15) and excitotoxcicity (16), to general systemic dysfunction such as increased risk of atherosclerosis (17), and altered blood coagulation (18).

The pathology of traumatic brain injury (TBI) can be classified as either focal or diffuse (19). Focal injuries include contusions, intracranial haemorrhages, and the vascular complications of raised intracranial pressure. Diffuse injuries include diffuse traumatic axonal injury (TAI), cerebral swelling, and ischaemic brain damage. Multiple factors influence the type and severity of the resulting brain injury and include the mechanism, location and magnitude of the primary injury, and host factors such as age and nutritional status.

We postulate that head-injured patients with *APOE* ε4, amounting to approximately a third of the population, are selectively predisposed to one or more of the different pathological features that constitute the response to TBI, and that this underlies the association of *APOE* ε4 with poor clinical outcome. We sought to test this hypothesis by identifying the prevalence of specific pathological features in *APOE* ε4 carriers, compared with non-carriers of *APOE* ε4, in a large tissue and data archive of fatal cases of head injury.

Materials and methods

Case selection

The study was approved by the Research Ethics Committee of the Southern General Hospital, Glasgow, Scotland. Cases were selected from the paraffin-embedded tissue archive of the Glasgow Neuropathology department. Initially all cases that died from TBI during the 13 year period, 1987-1999 within the archive and examined by the authors were selected. While many of the cases had been managed by the Department of Neurosurgery, Institute of Neurological Sciences, some patients had died at District General Hospitals or at the scene of the incident. A total of 259 cases were identified (are-range; 2 months-89 years). A prerequisite for inclusion in this study was successful *APOE* genotyping from the formalin-fixed paraffin embedded post mortem brain tissue.

APOE genotyping

APOE genotype was determined using a previously described PCR method (20). Of the initial 259 cases, 239 were successfully genotyped (92% success rate). Cases were discarded after four unsuccessful attempts at genotyping.

Pathological data

Archival data which had been gathered prospectively during the years 1987-1999 inclusive according to a uniform protocol was logged into a database and included the following information for each case: age, length of survival after episode of TBI, skull fractures, intracranial haemorrhages, diffuse traumatic axonal injury (TAI), ischaemic brain damage, raised intracranial pressure and associated infarcts, and contusions.

Skull fractures were documented as being either present or absent, and intracranial haemorrhages were recorded in relation to the anatomical compartment involved (extradural, subdural, intracerebral).

Traumatic axonal injury (TAI) was documented as being absent or present, and if present was graded as grade 1, 2 or 3 (21). Grade 1 lesions had widespread axonal damage in the corpus callosum, the cerebral hemispheres, and the brainstem. Grade 2 lesions, in addition, had focal haemorrhagic lesions in the corpus callosum, and in grade 3 there was in addition a haemorrhagic lesion in the rostral brain stem. The term diffuse axonal injury (DAI) was originally applied to traumatic damage exclusively. However, as immunohistochemical studies using β-APP as a marker of axonal damage have demonstrated, many brain insults can result in axonal damage. Therefore, it has been proposed that that the aetiology of any axonal damage should always be indicated, and that DAI (as originally defined) now be referred to as TAI (22).

Ischaemic brain damage was assessed using a grading system in which severe comprised those cases in which the lesions were diffuse, multifocal and large within arterial territories; moderate when the lesions were limited to the arterial boundary zones, singly or in combination with subtotal infarction in the distribution of the

cerebral arteries, or if there were 6-10 subcortical lesions; and mild if there were five or less subcortical lesions in the brain (23).

Raised intracranial pressure was considered to be present if there were tentorial hernias (either macroscopic or microscopic) (24), and associated vascular complications within the distributions of the anterior cerebral artery, the posterior cerebral artery, and in the cerebellum and brainstem.

Contusions were graded using the total contusion index (TCI) developed by Adams et al (25), and subsequently modified (26). This assesses the extent (0-3) and depth (0-4) of contusions in a variety of anatomical locators, producing a numerical score for each hemisphere which is then combined and interpreted as absent, mild, moderate, or severe. The anatomical locators are the frontal, temporal, parietal and occipital lobes, the cortex above and below the Sylvian fissure, and the cerebellum. The maximum score for an anatomical locator is 12 (4x3=12), and the TCI has a maximum value of 144 (each side 6x12=72, 2x72=144). For this study contusional injury was mild if the TCI was less than 20, moderate if the TCI was between 20 and 37, and severe if the TCI was greater than 37. These values were based on those used in previous studies (27).

Data analysis

The pathological features for each case documented on the database were then assessed in relation to *APOE* ε4 allele carriage presence or absence (see table 1). Comparison of the prevalence of the features was made using confidence intervals (CI) for the differences in proportions. Calculations were performed using Minitab (Version 12).

Results

Of the total number of 239 cases of fatal TBI examined there were 83 *APOE* ε4 carriers (35%) and 156 were non-carriers of *APOE* ε4 (65%). Differences were noted between *APOE* ε4 carriers and non-carriers of *APOE* ε4 in relation to contusions and ischaemic brain damage (table 1). 42% of ε4 carriers (35/83) had moderate or severe contusions (*i.e.* a total contusion index of >20) compared with 29% of non-carriers of ε4 (46/156, p=0.05). With regard to ischaemic brain damage a trend was noted between the possession of *APOE* ε4 and severe ischaemic brain damage which was present in 54% of *APOE* ε4 carriers and 42% of non-carriers of ε4 (p=0.08). No significant associations were demonstrated between possession of *APOE* ε4 and the presence of extradural haematoma, subdural haematoma, intracerebral haematoma, skull fracture, traumatic axonal injury or evidence of raised intracranial pressure.

Discussion

Among the pathological features which are present in fatal cases of TBI this study has identified an association between possession of *APOE* ε4 and contusion severity and a trend for an association with severe ischaemic brain damage. These findings suggest that cerebrovascular and haematological mechanisms may underlie, at least in part, the association of *APOE* ε4 with poor outcome after TBI. A limitation of this study relates to the fact that the pathology of only the most severe outcome from TBI group could be assessed (i.e. a fatal outcome). In addition this study is purely observational, based only on pathological assessment of injuries and does not take into account any possible changes in neurosurgical referral and treatment practice which may have occurred over the 13 year period. The cases studied had variable mechanisms of injury (RTA, fall, assault) which would result in variable forces being

applied to the head, and a wide range of ages; however, *APOE* ε4 was distributed across all the various types of injury and ages.

A fatal outcome occurs in only 5-10% of the total hospitalised TBI population, although approximately 50% of TBI related deaths occur before the patient can be transferred to hospital (28). An important question is whether the association identified in this study is of relevance to survivors of TBI. A recent study, performed in the same institute, of CT scans of survivors of TBI (29) showed that although patients with *APOE* ε4 were no more likely to have intracranial haemorrhages than non carriers of *APOE* ε4, if haemorrhages were present then they were of greater volume in those patients with *APOE* ε4. This study, therefore, provides a degree of clinical correlation with our autopsy based work, and suggests that our findings may well be relevant to survivors of TBI.

Although prospective clinical studies of outcome after TBI have identified *APOE* ε4 carriers as more likely to fall into poor outcome or poor recovery groups (1, 2), they have not yet specifically addressed the question of whether *APOE* ε4 carriers are more likely to have a fatal outcome. The previous clinical studies have looked at the prevalence ε4 carriers in poor outcome (severe, vegetative, or fatal) after TBI (1) or in vegetative state patients only (2). The present study, looking at only the fatal outcome group, did not find an over-representation of ε4 carriers, the *APOE* ε4 carriage rate (35%) being similar to that of all head-injured patients admitted to the same institution (33%, n=984, Teasdale et al, unpublished observations). Therefore, although *APOE ε4*-associated vascular pathology may influence the outcome in survivors it seems unlikely to significantly increase the probability of a fatal outcome after TBI. However, the situation after spontaneous intracerebral haemorrhage appears to be different; there is evidence that among patients with stroke due to

spontaneous intracerebral haemorrhage *APOE* ε4 carriers are substantially more likely to die in hospital (40% versus 25%) (30).

These findings point towards an important role for apoE in cerebrovascular and haematological mechanisms which are of relevance in the response to an episode of brain injury. More specifically, existing evidence indicates that apoE may play an important role in relation to both blood vessel wall integrity and coagulation of blood.

One role of apoE is as a lipid transport protein and apoE is therefore involved in the transport of the fat soluble vitamins together with lipids, from the small intestine to the liver. This mechanism is suggested to underlie the relatively low levels of plasma vitamin K in *APOE* ε4 carriers (31). Vitamin K is required by the liver for the synthesis of clotting factors and prothrombin times have been reported to vary with *APOE* genotype (32). Prolonged clotting times were also identified in *APOE* ε4 carriers after stroke (18) providing further evidence that *APOE* genotype is of relevance to the coagulation cascade. The possibility that contusions in head-injured patients with *APOE* ε4 are more severe as a result of relatively deficient clotting mechanisms provides the basis for a testable hypothesis.

A further mechanism of possible relevance to the findings of this study relates to the increased prevalence of atherosclerosis and cerebral amyloid angiopathy in carriers of *APOE* ε4 (13). Such vascular pathology might pre-date the head injury and promote contusional haemorrhage by increasing vascular fragility and decreasing the capacity for reactive vasoconstriction. Post mortem studies have confirmed the association of *APOE* ε4 with cerebral amyloid angiopathy in patients who died from TBI and have suggested that this is associated with increased severity of contusions (33) although the number of cases in this study was small.

Animal models, using *APOE* knockout and transgenic mice, have provided further information about apoE mechanisms and the response of the brain to injury (13). ApoE deficient mice were found to have larger infarcts than wild-type mice (34) and a greater extent of neuronal damage after controlled ischaemia (35), which can be ameliorated by continuous intracerebral infusion of apoE (36). Using transgenic mice differences have been demonstrated between the response to ischaemia and excitotoxicity in mice with human *APOE*ε3 and *APOE*ε4 genes, such that the *APOE*ε4 mice have larger lesions (37, 38) than *APOE*ε3 mice (39).

Further elucidation of potential vascular and haematological mechanisms which may underlie the role of apoE in response to brain injury could result in the development of new therapeutic interventions which may modify the outcome after TBI.

References

1. Teasdale GM, Nicoll JA, Murray G, Fiddes M Association of apolipoprotein E polymorphism with outcome after head injury. Lancet 1997;350:1069-71

2. Sorbi S, Nacmias N, Piacentini S, Repice A, Latorraca S, Forleo P, Amaducci L. ApoE as a prognostic factor for post-traumatic coma. Nat Med 1995;1:852

3. Alberts MJ, Graffagnino C, McClenny C, DeLong D, Strittmatter W, Saunders AM, Roses AD ApoE genotype and survival from intracerebral haemorrhage. Lancet 1995;346:575

4. McCarron MO, Hoffmann KL, DeLong DM, Gray L, Saunders AM, Alberts MJ. Intracerebral hemorrhage outcome: apolipoprotein E genotype, hematoma, and edema volumes. Neurology 1999;53:2176-9

5. Newman MF, Croughwell ND, Blumenthal JA, et al. Predictors of cognitive decline after cardiac operation. Ann Thorac Surg 1995;59:1326-30

6. Tardiff BE, Newman MF, Saunders AM, et al. Preliminary report of a genetic basis for cognitive decline after cardiac operations. The Neurologic Outcome Research Group of the Duke Heart Center. Ann Thorac Surg 1997;64:715-20

7. Schiefermeier M, Kollegger H, Madl C, Schwarz C, Holzer M, Kofler J, Sterz F. Apolipoprotein E polymorphism: survival and neurological outcome after cardiopulmonary resuscitation. Stroke 2000;31:2068-73

8. Jordan BD, Relkin NR, Ravdin LD, Jacobs AR, Bennett A, Gandy S. Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. JAMA 1997;278:136-40

9. Niskakangas T, Ohman J, Niemela M, Ilveskoski E, Kunnas TA, Karhunen PJ. Association of apolipoprotein E polymorphism with outcome after aneurysmal subarachnoid hemorrhage: a preliminary study. Stroke 2001;32:1181-4

10. Dunn LT, Stewart E, Murray GD, Nicoll JA, Teasdale GM. The influence of apolipoprotein E genotype on outcome after spontaneous subarachnoid hemorrhage: a preliminary study. Stroke 2002;33:548-52

11. Leung CH, Poon WS, Yu LM, Wong GK, Ng HK. Apolipoprotein e genotype and outcome in aneurysmal subarachnoid hemorrhage. Stroke 2002;33:548-52

12. McCarron MO, Muir KW, Nicoll JA, Stewart J, Currie Y, Brown K, Bone I. Prospective study of apolipoprotein E genotype and functional outcome following ischemic stroke. Arch Neurol 2000;57:1480-4

13. Horsburgh K, McCarron MO, White F, Nicoll JAR. The role of apolipoprotein E in Alzheimer's disease, acute brain injury and cerebrovascular disease: evidence of common mechanisms and utility of animal models. Neurobiol Aging 2000;21:245-55

14. Roses AD, Einstein G, Gilbert J, et al. Morphological, biochemical, and genetic support for an apolipoprotein E effect on microtubular metabolism. Ann N Y Acad Sci 1996;777:146-57

15. Lomnitski L, Kohen R, Chen Y, Shohami E, Trembovler V, Vogel T, Michaelson DM. Reduced levels of antioxidants in brains of apolipoprotein E-deficient mice following closed head injury. Pharmacol Biochem Behav 1997;56:669-73

16. Tolar M, Keller JN, Chan S, Mattson MP, Marques MA, Crutcher KA. Truncated apolipoprotein E (ApoE) causes increased intracellular calcium and may mediate ApoE neurotoxicity. J Neurosci 1999;19:7100-10

17. Hixson JE. Apolipoprotein E polymorphisms affect atherosclerosis in young males. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Arterioscler Thromb 1991;11:1237-44

18. Weir CJ, McCarron MO, Muir KW, Dyker AG, Bone I, Lees KR, Nicoll JA. Apolipoprotein E genotype, coagulation, and survival following acute stroke. Neurology 2001;57:1097-100

19. Graham DI, Gennarelli TA, McIntosh TK. Trauma. In: Graham DI, Lantos PL eds. Greenfield's Neuropathology, 7th edition. London:Arnold; 2002:823-98

20. Nicoll JA, Burnett C, Love S, et al. High frequency of apolipoprotein E epsilon 2 allele in hemorrhage due to cerebral amyloid angiopathy. Ann Neurol 1997;41:716-21

21.Adams JH, Doyle D, Ford I, Gennarelli TA, Graham DI, McLellan DR. Diffuse axonal injury in head injury: definition, diagnosis and grading. Histopathology 1989;15:49-59

22. Geddes JF, Whitwell HL, Graham DI. Traumatic axonal injury: practical issues for diagnosis in medicolegal cases. Neuropathol Appl Neurobiol 2000;26:105-16

23. Graham DI, Ford I, Adams JH, Doyle D, Teasdale GM, Lawrence AE, McLellan DR. Ischaemic brain damage is still common in fatal non-missile head injury. J Neurol Neurosurg Psychiatry 1989;52:346-50

24. Adams JH, Graham DI. The relationship between ventricular fluid pressure and the neuropathology of raised intracranial pressure. Neuropathol Appl Neurobiol 1976;2:323-32

25. Adams JH, Graham DI, Scott G, Parker LS, Doyle D. Brain damage in fatal non-missile head injury. J Clin Path 1980;33:1132-45

26. Adams JH, Doyle D, Graham DI, et al. The contusion index: a reappraisal in human and experimental non-missile head injury. Neuropathol Appl Neurobiol 1985;11:299-308

27. Graham DI, Lawrence AE, Adams JH, Doyle D, McLellan DR. Brain damage in fatal non-missile head injury without high intracranial pressure. J Clin Pathol 1988;41:34-7

28. Jennett B, MacMillan R Epidemiology of head injury. BMJ 1981;282:101-4

29. Liaquat I, Dunn LT, Nicoll JA, Teasdale GM, Norrie JD. Effect of apolipoprotein E genotype on hematoma volume after trauma. J Neurosurg 2002;96:90-6

30. Nicoll JAR, McCarron MO, Weir CJ, et al. Apolipoprotein E polymorphism and in-hospital mortality following intracerebral hemorrhage. Neurology 2000;54:Suppl 3:A386-7

31. Shearer MJ. Vitamin K. Lancet 1995;345:229-34

32. Giraud V, Naveau S, Betoulle D, et al. Influence of apolipoprotein E polymorphism in alcoholic cirrhosis Gastroenterol Clin Biol 1998;22:571-5

33. Leclercq PD, Murray LS, Graham DI, Smith C, Nicoll JAR, Gentleman SM. Cerebral amyloid angiopathy and traumatic brain injury: an association with

apolipoprotein E genotype. Neurobiol Aging 2002;23:S407

34. Laskowitz DT, Sheng H, Bart RD, Joyner KA, Roses AD, Warner DS. Apolipoprotein E-deficient mice have increased susceptibility to focal cerebral ischemia. J Cereb Blood Flow Metab 1997;17:753-8

35. Horsburgh K, Kelly S, McCulloch J, Higgins GA, Roses AD, Nicoll JA. Increased neuronal damage in apolipoprotein E-deficient mice following global ischaemia. Neuroreport 1999;10:837-41

36. Horsburgh K, McCulloch J, Nilsen M, McCracken E, Large C, Roses AD, Nicoll JA. Intraventricular infusion of apolipoprotein E ameliorates acute neuronal damage after global cerebral ischemia in mice. J Cereb Blood Flow Metab 2000;20:458-62

37. Horsburgh K, McCulloch J, Nilsen M, Roses AD, Nicoll JA. Increased neuronal damage and apoE immunoreactivity in human apolipoprotein E, E4 isoform-specific, transgenic mice after global cerebral ischaemia. Eur J Neurosci 2000;12:4309-17

38. Buttini M, Orth M, Bellosta S, Akeefe H, Pitas RE, Wyss-Coray T, Mucke L, Mahley RW. Expression of human apolipoprotein E3 or E4 in the brains of Apoe-/- mice: isoform-specific effects on neurodegeneration. J Neurosci 1999;19:4867-80

39. Buttini M, Akeefe H, Lin C, Mahley RW, Pitas RE, Wyss-Coray T, Mucke L. Dominant negative effects of apolipoprotein E4 revealed in transgenic models of neurodegenerative disease. Neuroscience 2000;97:207-10

Table 1

Pathological Feature	*APOE ε4* carriers n=83 (35%)	*APOE ε4* non-carriers n=156 (65%)	95% Confidence Interval for difference	p-value
Moderate/severe contusions	35 (42%)	46 (29%)	0 to25%	0.05
Severe ischaemic brain damage	45 (54%)	66 (42%)	- 1 to25%	0.08
Skull fracture	61 (73%)	105 (67%)	- 6 to18%	0.31
Traumatic axonal injury	31 (37%)	68 (44%)	- 19 to 7%	0.35
Extradural haemorrhage	13 (16%)	13 (8%)	-4 to 14%	0.31
Subdural haemorrhage	52 (60%)	98 (63%)	-13 to13%	0.98
Intracerebral haemorrhage	31 (37%)	47 (30%)	-5 to 20%	0.26
Raised intracranial pressure	58 (70%)	98 (63%)	-5 to 20%	0.27