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APOLIPOPROTEIN E AND RECOVERY FROM TRAUMATIC BRAIN INJURY

by

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ABSTRACT

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The outcome from traumatic brain injury (TBI) is variable and only partly explained by known prognostic factors. This is especially true for predicting long-term outcome. Genetic factors may influence the brain`s susceptibility to injury or capacity for repair and regeneration. To examine the association of apolipoproteinE (apoE) genotype with long-term outcome, hippocampal volumes and general brain atrophy, we determined the apoE genotype from 61 TBI patients who had been injured over on average 31 years earlier. The long-term outcome was evaluated with repeated neuropsychological testing and by applying various measures of everyday functioning and quality of life. Magnetic resonance imaging (MRI) based volumetric analyses of the hippocampus and lateral ventricles were performed.

In the prospective study, the purpose was to examine the association between apoE genotype and visibility of traumatic brain lesions during the first year after TBI and the ability of apoE genotype, the Glasgow Coma Score (GCS), MRI findings and duration of posttraumatic amnesia (PTA) to predict the one-year outcome. Thirty-three patients with TBI were studied and the outcome was evaluated with the Head Injury Symptom Checklist (HISC) and the Glasgow Outcome Scale extended version (GOS-E) scores one year after the injury. MRI and apoE genotyping were carried out.

After three decades, neither hippocampal nor lateral ventricle volumes differed significantly in those patients with the apoE ε4 allele vs those without this allele, but the TBI patients with the apoE ε4 allele showed significantly poorer general cognitive level than those without this allele. This decline was wholly accounted for by a subgroup of patients who had developed incident or clinical dementia.

In the prospective study the apoE genotype was not associated with visible MRI changes or outcome. The duration of PTA and acute MRI were the best predictors of one-year outcome in TBI.

A portion of the TBI patients with the apoE ε4 allele seem to be at risk of long-term cognitive decline. This association may involve mechanisms other than those responsible for the development of brain atrophy. The early MRI and PTA have an important role in assessing the injury severity and prognosis.

Key words: traumatic brain injury, apolipoprotein E, outcome, magnetic resonance imaging

TIIVISTELMÄ

Heli Hiekkanen

APOLIPOPROTEIINI E JA TOIPUMINEN AIVOVAMMASTA

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Annales Universitatis Turkuensis, Painosalama Oy – Turku, 2009

Toipuminen aivovammasta on vaihtelevaa ja selittyy vain osittain tunnetuilla ennustekijöillä. Tämä pätee varsinkin pitkäaikaistoipumista ennustettaessa. Perinnölliset tekijät saattavat vaikuttaa aivojen vaurioitumisherkkyyteen tai korjaantumiskykyyn. Tutkiaksemme apolipoproteiini E (apoE) genotyypin yhteyttä aivovammapotilaiden pitkäaikaistoipumisennusteeseen, hippokampusten tilavuuksiin ja yleiseen aivoatrofiaan määritimme apoE genotyypit 61 potilaalta, jotka olivat saaneet aivovamman keskimäärin 31 vuotta aikaisemmin. Pitkän aikavälin toipuminen määritettiin toistetulla neuropsykologisella tutkimuksella ja käyttäen erilaisia päivittäistoimintojen ja elämänlaadun mittareita. Aivojen magneettikuvauksella tutkittiin hippokampusten ja sivuaivokammioiden tilavuudet.

Prospektiivisessä tutkimuksessa tarkoituksena oli selvittää apoE genotyypin yhteyttä traumaattisten muutosten esiintyvyyteen aivojen magneettikuvauksessa vuoden kuluttua vammasta sekä apoE genotyypin, Glasgow'n kooma-asteikon (GCS), magneettikuvauslöydösten ja posttraumaattisen amnesian (PTA) kykyä ennustaa toipumista vuoden kuluttua vammasta. Työssä tutkittiin 33 aivovammapotilasta ja toipuminen arvioitiin vuoden kuluttua vammasta oirekyselyn (HISC, Head Injury Symptom Checklist) ja toipumismittarin (GOS-E, Glasgow Outcome Scale extended version) avulla. Lisäksi tehtiin aivojen magneettikuvaus ja määritettiin apoE genotyypit.

Hippokampusten tai sivuaivokammioiden tilavuudet eivät eronneet toisistaan merkitsevästi kolmen vuosikymmenen jälkeen sen mukaan oliko potilaalla apoE ε4 alleeli vai ei, mutta apoE ε4 alleelin omaavien aivovammapotilaiden kognitiivinen toipuminen oli merkitsevästi huonompaa kuin niiden, joilta se puuttui. Tämä heikentyminen selittyi kuitenkin kokonaan potilasryhmällä, jolle oli kehittynyt dementia.

Prospektiivisessä tutkimuksessa apoE genotyyppi ei ollut yhteydessä näkyviin magneettikuvausmuutoksiin tai toipumiseen vuoden seuranta-aikana. Posttraumaattisen amnesian kesto ja akuuttivaiheen magneettikuvaus löydökset kuvasivat parhaiten vuoden toipumisennustetta.

Osalla aivovammapotilaista, joilla on apoE ε4 alleeli, näyttää olevan pitkällä aikavälillä riski kognitiivisen toimintakyvyn heikkenemiseen. Yhteyteen saattavat vaikuttaa muut mekanismit kuin ne, jotka liittyvät aivoatrofian kehittymiseen. Aikaisessa vaiheessa tehdyllä magneettitutkimuksella ja posttraumaattisella amnesialla on tärkeä rooli arvioitaessa vamman vaikeusastetta ja ennustetta.

Avainsanat: aivovamma, apolipoproteiini E, toipuminen, magneettikuvaus

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ABBREVIATIONS

Aβ	β-amyloid peptide
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AD	Alzheimer`s disease
ADL	Activities of daily living
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
BBB	Blood-brain barrier
cAMP	Cyclic adenosine monophosphate
CERAD	Consortium to Establish a Registry of Alzheimer`s Disease
CBI	Chronic brain injury
CI	Confidence interval
CIQ	Community Integration Questionnaire
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DNA	Deoxiribonucleinic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
DAI	Diffuse axonal injury
EDTA	Ethylenediamine tetra-acetid acid
FIM	Functional Independence Measure
Flair	Fluid-attenuated inversion recovery
FOV	Field of view
FSE	Fast spin echo
FSEIR	Fast spin echo inversion recovery
FSPGR	Fast spoiled gradient echo
GCS	Glasgow Coma Scale
GHQ	Twenty-eight-item General Health Questionnaire
GOS	Glasgow Outcome Scale
GOS-E	Glasgow Outcome Scale extended version
HISC	Head Injury Symptom Checklist

4-HNE	4-Hydroxynonenal
HSV-1	Herpes simplex virus 1
ICD	International Classification of Diseases, Injuries and Causes of Death
ICH	Intracerebral hemorrhage
MDB	Mild Deterioration Battery
MMSE	Mini-Mental State Examination
MPR	Magnetization prepared rapid gradient echo
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
OR	Odds ratio
PCR	Polymerase chain reaction
PCRS	Patient Competency Rating Scale
PCS	Post-concussion syndrome
PTA	Posttraumatic amnesia
RHFUQ	Rivermead Head Injury Follow-up Questionnaire
RPQ	Rivermead Post-Concussion Symptoms Questionnaire
SAH	Subarachnoid hemorrhage
SCL-90	Symptom Checklist-90
SEM	Standard error of mean
SIP	Sickness Impact Profile
SD	Standard deviation
SR-related	Sport- and recreation-related
TAI	Traumatic axonal injury
TBI	Traumatic brain injury

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which are referred to in the text by the Roman numerals I-IV:

I Isoniemi H, Kurki T, Tenovuo O, Kairisto V, Portin R. Hippocampal volume, brain atrophy, and APOE genotype after traumatic brain injury. Neurology 2006; 67:756-760.

II Isoniemi H, Tenovuo O, Portin R, Himanen L, Kairisto V. Outcome of traumatic brain injury after three decades - relationship to APOE genotype. Journal of Neurotrauma 2006; 23:1600-1608

III Hiekkanen H*, Kurki T, Brandstack N, Kairisto V, Tenovuo O. Mri changes and APOE genotype, a prospective 1-year follow-up of traumatic brain injury: A pilot study. Brain Injury 2007;21:1307-1314.

IV Hiekkanen H*, Kurki T, Brandstack N, Kairisto V, Tenovuo O. Association of injury severity, MRI-results and APOE genotype with one-year outcome in mainly mild TBI - a preliminary study. (in press)

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1. INTRODUCTION

Traumatic brain injury (TBI) is the leading cause of death and disability in people under the age of 45 years in industrialized countries (Maas et al. 2000, Marshall 2000). Motor vehicle accidents account for the majority of fatal head injuries (Kraus 1993). Those individuals who survive TBI are often left with permanent neurological deficits, which adversely affect their quality of life, and contribute to the enormous social and economic costs of TBI that are borne by communities. Mild TBI is significantly underdiagnosed and the public health burden is therefore even greater (NIH Consensus Development Panel on Rehabilitation of Persons with Traumatic Brain Injury 1999).

Outcome following TBI is difficult to predict on the basis of clinical features or radiological findings at the time of injury. This is especially true for predicting long-term outcome (Zhou et al. 2008). Prognosis of outcome is important for clinical decision-making, rehabilitation planning, and communication with patients and their families. There is considerable variability in the outcome of acute head injury. An injury that initially appears to be severe can be followed by recovery, a mild injury may be followed by disability or even death, and injuries that are apparently similar in the acute stage can have markedly different outcomes (Thornhill et al. 2000). Although TBI may result in physical impairment, the more problematic consequences involve the individual`s cognition, emotional functioning, and behaviour (NIH Consensus Development Panel on Rehabilitation of Persons with Traumatic Brain Injury 1999).

Alzheimer`s disease (AD) is the most common neurodegenerative disorder of modern societies (Andersen et al. 2006). The possibility that TBI may predispose a person to developing AD in later life has significant social and medical implications, and reinforces the need for preventative efforts and health service planning to cope with the potential large increase in the number of AD patients (Lye and Shore 2000). It is therefore critical to establish whether any link between TBI and AD exists. Genetic factors probably contribute to the brain`s susceptibility to injury and capacity for repair and regeneration. ApolipoproteinE (apoE) genotype ε4 is a risk factor for AD (Cummings and Cole 2002). In the nervous system, apoE is engaged in the redistribution of cholesterol from cells during membrane synthesis, and neuritic extension growth and repair (Graham et al. 1999b, Mauch et al. 2001). Research in AD has provided evidence suggesting that there may be a genetic component to recovery or lack of recovery after TBI that is related to apoE genotype.

2. REVIEW OF THE LITERATURE

2.1 Traumatic brain injury (TBI)

2.1.1 Definition and classification

A patient with traumatic brain injury (TBI) is a person who has had a traumatically induced physiological disruption of brain function, which is manifested by at least one of the following: 1. any period of loss of consciousness, 2. any loss of memory for events immediately before or after the accident, 3. any alteration in mental state at the time of the accident (e.g. feeling dazed, disoriented, or confused), or 4. focal neurological deficit(s) that may or may not be transient (Kay et al. 1993). This definition of TBI includes the head being struck, the head striking an object, and the brain undergoing an acceleration/deceleration movement (i.e. whiplash) without direct external trauma to the head. In addition, traumatic changes in the brain detected with neuroimaging methods qualify for a diagnosis of TBI.

TBI can be classified as open or closed, depending on the presence of skull fracture and disruption of the dura mater. Neuroradiological techniques have generated an alternative classification of (1) focal damage, where a localized area of a brain tissue has been damaged, and (2) diffuse damage, where the damage of brain tissue is widespread and not accurately restricted. Focal brain injury is commonly the result of a direct force causing damage to underlying cerebral tissue and vessels. It includes contusions on the surface of the brain, intracranial haematomas and various types of secondary brain damage, including haemorrhage and infarction in the brainstem. The principal types of diffuse brain damage are brain swelling, hypoxic damage, and diffuse axonal injury (DAI). At present, the role of DAI is considered increasingly important in the outcome of TBI (Graham et al. 2002). Recently, the descriptor traumatic axonal injury (TAI) has been applied to experimental studies that have attempted to elucidate the mechanisms of axonal pathology after trauma (Graham et al. 2000). Focal damage is more likely to be sustained as the result of a fall, while diffuse damage is most commonly associated with acceleration/deceleration occurring after, for example, traffic accidents (Graham et al. 2002). The direct injuries are caused by direct contact on the skull, often as a result of a fall, and the indirect injuries by acceleration/deceleration occurring after, for example, motor vehicle accidents.

At the acute stage, the severity of TBI can be determined on the basis of the Glasgow Coma Scale (GCS) and the duration of posttraumatic amnesia (PTA). The GCS, measuring level of consciousness, includes three categories: eyes opening (score 1-4), best verbal response (score 1-5), and best motor response (score 1-6) (Teasdale and Jennett 1974). The total score of the GCS ranges from 3 to 15, low scores indicating

a low level of consciousness. PTA is a typical consequence of TBI, and it is defined as last up to the earliest time point after which continuous memory has returned. The Finnish Adult TBI Guidline recommends the use of both the GCS and length of PTA. The following categories are suggested by the Guideline: mild (GCS 13-15 and PTA < 24 hours), moderate (GCS 9-12 or PTA 1 to 7 days), severe (GCS $\leq$ 8 or PTA > 7 days), or very severe (PTA > 4 weeks) (Käypä hoito -suositus 2003).

2.1.2 Epidemiology

Data on hospital discharges and on deaths at national or local level do allow head injuries to be identified by the codes of the International Classification of Diseases (ICD) (Jennett 1996).

The incidence of TBI is difficult to estimate because only a part of individuals with TBI, possibly about 25%, are admitted to hospital (Sosin et al. 1996). Incidence reports in epidemiological studies also vary depending on the inclusion criteria: whether all grades of severity are included, deaths are counted or the study is limited to hospital admissions etc. Higher incidence rates are reported from surveys based on routine ICD coding than those based on hospital case records (Jennett 1996).

In Finland, the annual incidence of TBI during the years 1991-2000 was about 100 per 100 000 people on the basis of hospital discharge registers (Alaranta et al. 2000). Fifty-nine percent of these subjects were men. The largest age groups were men under 20 years and 40-49 years, and women under 10 years and over 70 years. The sex difference was greatest in favour of men from 10 to 49 years (men 69% vs. women 31%), and in favour of women over 70 years (men 37% vs. women 63%) (Alaranta et al. 2000). Winqvist et al. (2007) used the Northern Finland 1966 Birth Cohort to study the epidemiology of TBI. The annual incidence of TBI was 118 per 100 000 people. It has been estimated that, in Finland, 15 000-20 000 individuals sustain a TBI every year, and about 100 000 individuals have persistent symptoms of TBI (Käypä hoito -suositus 2003). In Sweden, the annual incidence in the Borås region was 546 per 100 000 people and fifty-nine percent of these subjects were men (Andersson et al. 2003). The incidence of TBI found in this study was high but well in accordance with earlier published Swedish studies (Silverbåge Carlsson 1986, Johansson et al. 1991). The reported incidences of TBI vary widely; between about 100 to over 3000 per 100 000 have been published (Jennett and MacMillan 1981, Frankowski 1986, Silverbåge Carlsson 1986, Kraus and Nourjah 1998).

The main causes of TBI are traffic accidents, falls, and assaults (Jennett 1996). There is, however, considerable variation from one study to another due to their different inclusion criteria and age and sex distributions. In Finland falls account for 65 % and traffic accidents for 20 % of all TBI cases treated in hospitals, followed by 5 % caused

by violence. At working age, however, traffic accidents are the leading cause of TBI. In Finland, about half of the TBI patients have been under the influence of alcohol at the time of injury (Käypä hoito -suositus 2003). In Sweden falls account for 58.1 % and traffic accidents for 16 % of all cases (Andersson et al. 2003). Road traffic accidents and falls are the most frequent causes of severe brain injuries (Hillier et al. 1997, Masson et al. 2001). Falls are a signinificant cause of TBI, particularly in young children and elderly people (Jennett 1996). Intentional injuries (homicide and suicide) occur particularly among young people, minorities, and males (Wagner et al. 2000). Those comprise about 1-15% of all external causes of TBI (Tagliaferri et al. 2005). Sport- and recreation-related (SR-related) brain injuries comprised 5.1% of all TBIs, and highest rates of SR-related TBI emergency department visits occurred among those aged 10-14 years, followed by those aged 15-19 years (Centers for Disease Control and Prevention (CDC) 2007).

Males are more than twice as likely as females to experience TBI. The highest incidence is among persons 15 to 24 years of age and 75 years or older. Alcohol is reported to be associated with half of all TBI, either in the person causing or in the person with the injury, or both (NIH Consensus Development Panel on Rehabilitation of Persons with Traumatic Brain Injury 1999). In addition lower socioeconomic status, previous TBI and psychiatric disorders are factors associated with TBI (Käypä hoito -suositus 2003). The risk of a second head injury has been found to be threefold compared to the uninjured population, and the risk rises to eight times the normal risk after two TBIs (Annegers et al. 1980).

2.1.3 Mechanisms and pathophysiology

The biomechanics of primary damage (i.e. injury at impact) are linked to the response of bone, blood vessels, and the brain to both impact and inertial forces. With direct impact to the skull, a local bending occurs with underlying tissue strain and gross movement of brain tissue. Conversely, inertial injury does not create local contact effects but produces a nonuniform distribution of pressure and tissue strain that cause primary tissue damage. Intracranial pressure changes and brain motion due to translational acceleration have been linked to specific focal lesions such as coup and contrecoup contusions, intracerebral and/or subdural hematomas, and brainstem lesions. DAI is related more to rotational acceleration forces (McIntosh et al. 1996).

Secondary injury (i.e. delayed injury) develops over a period of hours, days, or weeks after the initial trauma. This type of damage appears to be associated with trauma-induced neurochemical alterations, which can exert either direct pathogenic effects on regional cerebral blood flow, blood-brain barrier (BBB) function, cerebral metabolism, and ion homeostasis, or have direct neurotoxic effects on regional populations of neurons or glial cells (McIntosh et al. 1996). These post-traumatic changes may involve alterations

in the synthesis and release of both neuroprotective and autodestructive or neurotoxic cascades (McIntosh et al. 1998).

TBI represents a process that involves multiple interrelated physiological components that exert primary and secondary effects at the level of the individual neuron and at the level of neural networks. These include chemical changes to the basic molecules of metabolism (especially calcium), to mechamisms of the human cellular response to injury, and to the quantities of certain molecules that can be dangerous in excess (oxygen free radicals, nitric oxide) (NIH Consensus Development Panel on Rehabilitation of Persons with Traumatic Brain Injury 1999).

TBI triggers a complex neuroinflammatory cascade via the innate immune system resulting in the release of reactive oxygen and nitrogen species, glutamate, proteases, and cytokines to promote tissue survival and facilitate efficient clearance of cellular debris (Nguyen et al. 2002, Correlate and Villa 2004, Schmidt et al. 2005). This complex biochemical loop of mediators exerts both direct and indirect effects, with distinct time-dependent expression profiles and agonist/antagonistic interactions that are essential for the survival and function of neurons. However, these mediators possess neurotoxic effects when their presence is prolonged, unregulated, or excessive. Excessive glial activation results in metabolic and oxidative stress, and impairment of ionic homeostasis, thereby contributing to the neuropathological sequelae that are hallmarks of TBI, i.e. edema, blood-brain barrier (BBB) breakdown, delayed neuronal death, and the concomitant neurocognitive and motor impairments observed in TBI survivors (Allan and Rothwell 2001, 2003, Fujimoto et al. 2004).

A protein substance that is present in Alzheimer disease, amyloid β peptide (Aβ), may also be deposited in neurons. Neurotransmitters have either excitatory or inhibitory effects. The most prevalent of these excitatory molecules are the amino acids glutamate and aspartate, which can occur in massive amounts following TBI, leading to overexcitation and, ultimately, the death of neurons. At the cognitive level, alterations in neural networks and neurotransmitter systems (especially ones involving the transmitters acetylcholine, dopamine, and serotonin) can affect cognition and behaviour. The brain also has some capacity to recover, and adaptive structural changes occur in the adult brain, including the generation of new neurons and connections (neuroplasticity) (NIH Consensus Development Panel on Rehabilitation of Persons with Traumatic Brain Injury 1999).

2.1.4 Outcome

TBI is the leading cause of persistent neurological disability (World Health Organization 2006), and even in mild TBIs, a substantial number of patients complain about long term neuropsychological deficits or postconcussional symptoms (Andersson et al. 2007). The majority of patients who experience mild TBI recover without any residue symptoms

(Levin et al. 1987, Newcombe et al. 1994, Dikmen et al. 1995). However, 15 to 20% show long-lasting neuropsychological impairments (Rutherford et al. 1979, Bohnen et al. 1993, Alexander 1995). Of all mild TBI patients, about 50% at three months (Middleboe et al. 1992) and 15% at one year (Kushner et al. 1998) will have persistent post -concussion syndrome (PCS).

Most patients with moderate TBI tend to improve neurologically. In one series following 79 patients, 67% of patients improved to GCS 15 by time of discharge. At an average follow up of 27 months, Glasgow outcome scores were rated as good or moderate disability in the majority of survivors of moderate TBI, but only 74% of these patients who were employed prior to their TBI returned to work. The majority had significant cognitive and functional deficits, including memory problems, concentration difficulties and headaches (Vitaz et al. 2003). Other reports have confirmed good recovery in terms of general outcomes at three months but few patients were actually symptom-free. Patients tend to have difficulty with memory function and as many as 50 % have some emotional or behavioral difficulty at one-year post-injury (Stein 1996).

Becker et al. (1977) demonstrated a mortality rate in the 30% range in severe TBI patients managed with neurocritical care principals, including ICP monitoring, when compared with the same era management at other institutions resulting in a mortality rate in the 50% range. Other series report similar mortalities from severe TBI in patients undergoing aggressive neurocritical care management (Marshall et al. 1979, Miller et al. 1981). The European Brain Injury Consortium survey revealed a GOS score in patients with severe TBI at six months post-injury of 4-5 (moderate disability-good recovery) in 45% a score of 2-3 (persistent vegetative state - severe disability) in 19%, and a score of 1 (death) in 36% (Murray et al. 1999). Kersel et al. (2001) followed 65 patients with severe TBI and assessed their GOS and neuropsychological outcomes at six months and at one year. Despite 51% of patients having a good recovery and 20% having moderate disability on the GOS, at best 31% and up to 63 % had difficulty in neurocognitive tests administered one year post-injury. Nevertheless, few patients sustaining severe TBI return to their premorbid functioning, and the majority have significant cognitive and emotional sequelae (Marion 1996). Depression, decreased social contact and loneliness remain persistent long-term problems for the majority of individuals with severe TBI (Morton and Wehman 1995). Marsh et al. (1998) found that anger, dependency and apathy were the most difficult emotional problems in severe TBI patients for caregivers to deal with.

Predicting outcome after TBI is difficult and it is not always consistent with the estimate of severity at the acute stage. The limited self-awareness frequently associated with TBI (Hart et al. 2004), can further complicate the identification of TBI symptoms and their consequences. Despite the vast burden of TBI, no optimal long-term outcome

predictors have been developed. Especially in mild to moderate TBI, there is only a weak association between the clinical neurological signs, intracranial abnormalities in CT and subtle long-term neurobehavioural disorders (Culotta et al. 1996). The force and direction of impact and more generally the injury biomechanics are known to influence the outcome (Margulies et al. 1990, McIntosh et al. 1996, Nishimoto and Murakami 1998, Zhang et al. 2001, Ommaya et al. 2002). Other factors include the size, location, and nature of the brain lesion, PTA, GCS, earlier brain insults (Gronwall and Wrightson 1975), the psychological and emotional state of the victim (King 1996), sex (Farace and Alves 2000), level of education, and age at injury (Rothweiler et al. 1998).

2.2 Apolipoprotein E (apoE) and TBI

2.2.1 ApoE

The gene for apoE is located on chromosome 19 and is highly polymorphic. The three most common alleles are ε2, ε3, and ε4, which encode the main three isoforms of apoE: E2, E3, and E4 (Mahley et al. 1984, Mahley 1988, Rall and Mahley 1992). The isoforms differ by single amino acid interchanges at residues 112 and 158: E3 (Cys112 -Arg158), E4 (Arg112 -Arg158), and E2 (Cys112 -Cys158) (Weisgraber 1994). This polymorphism leads to the occurrence of six different phenotypes: E3/3, E2/2, E4/4, E3/2, E3/4, E2/4. Apolipoproteins are lipid carrier proteins that transport and redistribute lipids among cells (Graham et al. 1999b, Mauch et al. 2001). The allele frequencies vary between different populations. Northern Europeans have higher frequencies (0.17-0.23) of the ε4 allele than subjects in most populations studied (Ehnholm et al. 1986, Lehtimäki et al. 1990, Gerdes et al. 1992) and, in addition, a gradient for the ε4 allele frequency decreasing from Finland to the south of Europe has been reported (Tiret et al. 1994). ApoE is mainly synthesised in the liver but also in many other organs including brain, spleen and kidney (Siest et al. 1995).

2.2.2 ApoE in the CNS

ApoE is produced within CNS primarily by astrocytes, although neurons and microglia may also contribute to apoE synthesis (Linton et al. 1991). In the nervous system, apoE is engaged in the redistribution of cholesterol from cells during membrane synthesis, and neuritic extension growth and repair (Graham et al. 1999b, Mauch et al. 2001). It seems that the apoE ε4 allele has a detrimental effect when compared with the apoE ε3 allele. In various cell lines, apoE3 has been shown to increase the growth and branching of neurites, whereas apoE4 was found to have the opposite effect (Handelmann et al. 1992, Nathan et al. 1994, Bellosta et al. 1995). It is not known whether apoE ε2 has a protective effect except in the case of Alzheimer`s disease (Corder et al. 1993).

At present, all the mechanisms underlying associations between apoE genotype and CNS disease and injury are not known. Increasing evidence suggests that apoE influences outcome after brain injury by apoE isoform differences in synaptic repair, remodelling, and protection. Proposed mechanisms by which the apoE E4 isoform magnifies neurological injury severity or impedes recovery include: less efficient transport of lipids (Poirier et al. 1993, Kay et al. 2003); more accumulation of beta amyloid and more widespread pre-existing amyloid deposits (Nicoll et al. 1995, Graham et al. 1999a); more brain inflammation (Laskowitz et al. 1997a, Laskowitz et al. 1998, Grocott et al. 2001); poorer protection against oxidative injury (Lee et al. 2004); poor brain perfusion after injury (Laskowitz et al. 1997b, Kerr et al. 1999); a cytoskeleton more vulnerable to damage (Strittmatter et al. 1994); diminished growth and branching of neurites resulting in poorer repair (Strittmatter et al. 1994, Bellosta et al. 1995, Fullerton et al. 2001); poorer cerebral edema (Fullerton et al. 2001, Lynch et al. 2002); poorer pre-existing atherosclerotic vascular disease (Ti et al. 2003); slower recovery to anaerobic metabolism (Kerr et al. 2003); and poorer N-methyl-D-aspartate excitotoxicity (Aono et al. 2002).

Several protective mechanisms induced by apoE ε4 have also been described. The apoE ε4 protein was shown to activate an extracellular signal-regulated kinase cascade that results in activation of cAMP-response element binding protein and induction of many genes, including the cell-protective gene Bcl-2 (Ohkubo et al. 2001). Cholesterol is another potential protective mechanism. ApoE ε4 carriers are known to have elevated low-density lipoprotein and total cholesterol levels (Sing and Davignon 1985), which lead to an increase in γ -glutamyl-transferase that is protective against neurotoxic effects of excitotoxic amino acids (Dyker et al. 1997). ApoE ε4 allele may have a positive effect on neurogenesis. Neurogenesis not only occurs in developing nervous systems but also in adults (Altman and Das 1965, Eriksson et al. 1998, Gould et al. 1999). A study in transgenic mice found that apoE ε4 positive mice had increased neurogenesis compared to apoE ε3 positive mice (Levi and Michaelson 2007). Additional studies are needed to identify the factors that induce neurons to express apoE.

2.2.2.1 ApoE and Aβ

The association of apoE with Aβ deposits in the brains of AD patients led to the hypothesis that apoE influences the rate of cerebral amyloid deposition (Wisniewski and Frangione 1992). ApoE is found in amyloid plaques and neurofibrillary tangles, two neuropathological hallmarks of AD, but its role in their pathogenesis is unclear (Namba et al. 1991, Selkoe 1991, Wisniewski and Frangione 1992, Crowther 1993, Strittmatter et al. 1993ab, Tanzi and Bertram 2001). ApoE modulates the deposition and clearance of amyloid β peptides and plaque formation (Strittmatter et al. 1993ab, LaDu et al. 1994, Ma et al. 1994, Sanan et al. 1994, Wisniewski et at. 1994, Bales et al. 1999, Irizarry et al. 2000).The ε4 allele of apoE is associated with increased Aβ deposition, and increased

risk and earlier onset of AD (Corder et al. 1993, Saunders et al. 1993). Horsburgh et al. (2000a) showed that apoE4 binds more avidly than apoE3 to Aβ, and enhances aggregation of Aβ - a cleavage product of amyloid precursor protein (APP). It has also been shown that there are apoE-isoform differences in the ability of apoE to transport Aβ across the BBB (Martel et al.1997); apoE E2 and E3 prevent the transport of Aβ, whereas apoE E4 binds Aβ and crosses the blood brain barrier. However, the mechanisms of these effects are still largely unknown, and it is not known which are the primary effects and which are subsequent or downstream effects.

Data from experimental and human post-mortem studies suggest that Aβ deposition is promoted by apoE, and this effect is apoE isoform-dependent. Nicoll et al. (1995) showed that individuals carrying the apoE ε4 allele have a greater extent of Aβ deposition after TBI compared with those without an apoE ε4 allele. Doubly transgenic mice expressing mutant APP and apoE ε4 have increased amyloid β -protein deposits compared with apoE ε3 mice (Hartman et al. 2002). Transgenic mice that overexpress a mutant APP gene, that causes familial AD, deposit Aβ in the brain in an age- and region-dependent manner similar to that seen in AD brain (Games et al. 1995). When these mice were bred onto an apoE deficient background there was a significant reduction in the extent of Aβ deposited (Bales et al. 1997). In contrast, when these APP transgenic mice were bred onto apoE ε3 and apoE ε4 transgenic mice expressing human apoE by astrocytes there was a marked reduction in Aβ deposition in the mice at 39 weeks of age (Holzman et al. 1999). It is unclear whether human apoE inhibits early deposition of Aβ, and that with longer survival the mice may exhibit Aβ deposits, or whether there is a critical concentration of Aβ at which apoE would promote the deposition of Aβ. The question of whether apoE represents a gain of negative or a loss of protective function is unresolved. Trommer et al. (2005) suggested that apoE4 confers a gain of negative function synergistic with Aβ1-42, apoE2 is protective, and the apoE- Aβ interaction is specific to oligomeric Aβ1-42. These studies serve to highlight the complexity of these interactions between apoE isoforms and Aβ. A better understanding of the regulation of neuronal production of apoE is important for unravelling the mechanisms underlying apoE4-related neurodegenerative disorders.

2.2.2.2 ApoE and inflammation

There is increasing evidence that apoE plays a biologically relevant role in modulating immune responses, but the mechanisms by which this occurs remain unclear. Astrocytic and microglial activation, with the resultant secretion of imflammatory mediators, is believed to promote breakdown of the blood brain barrier and subsequent development of cerebral edema. In the injured central nervous system, apoE may downregulate glial activation and the endogenous inflammatory response (Barger et al. 1997, Laskowitz et al. 1997a, Hu et al. 1998, Laskowitz et al. 1998, Laskowitz et al. 2001, Lynch et al.

2001). The apoE4 isoform is less effective than apoE3 at downregulating inflammatory cytokines in the peripheral circulation, as well as in the brain (Laskowitz et al. 2001, Lynch et al. 2003). Lynch et al. (2003) found an apoE isoform specific brain inflammatory response in transgenic mice expressing human apoE3 versus human apoE4, with the apoE4 transgenic mice having a greater inflammatory response than the apoE3 transgenic mice. Laskowitz et al. (2001) found similar microglial down-regulation and decreased inflammatory cytokine release in mouse and human microglial cultures administered with human apoE protein (2, 3, or 4). The apoE is also capable of binding high affinity receptors and initiating a calcium-dependent signalling response in immunocompetent cells (Misra et al. 2001). In humans, isoform-specific differences are suggested by autopsy reports demonstrating increased numbers of scattered microglia and microglial activation in AD patients carrying the apoE ε4 allele (Egensperger et al. 1998).

2.2.2.3 ApoE and oxidative injury

ApoE can protect against oxidative injury and subsequent lipid peroxidation that contribute to the pathogenesis of neuronal damage associated with acute brain injury and AD (Markesbery and Carney 1999). This is consistent with the observation that plasma lipoproteins from apoE deficient mice are more susceptible to in vitro oxidation than those of wild-type mice (Hayek et al. 1994). In addition, apoE deficient mice have been shown to recover poorly from closed head injury compared to controls (Chen et al. 1997), and their diminished recovery has been shown to be related to a reduction in their ability to counteract oxidative damage (Lomnitski et al. 1997). 4-Hydroxynonenal (4-HNE) is a toxic byproduct of lipid peroxidation. 4-HNE accumulates in the neuronal cytoplasm and in neurofibrillary tangles in the brain of AD patients (Montine et al. 1997), the cellular distribution of which is influenced by apoE genotype (Montine et al. 1998). A reduction was observed in the extent of lipid peroxidation using 4-HNE immunoreactivity in apoE deficient mice infused with lipid-conjugated apoE after global ischemia (Horsburgh et al. 2000b). In addition, vitamin E, an antioxidant, reduces the extent of atherosclerosis in apoE deficient mice (Pratico et al. 1998). This anti-oxidant activity of apoE has also been shown to be isoform-specific in vitro: apoE can protect neurons from hydrogen peroxide toxicity in an isoform-specific manner, with apoE E4 being less effective compared to apoE E3 and E2 (Miyata and Smith 1996).

2.2.2.4 ApoE and synaptic repair, remodelling and regeneration

The apoE may influence AD and the longer term outcome after brain injury by apoE-isoform differences in synaptic repair, remodelling and regeneration. After injury to the entorhinal cortex, an area vulnerable in AD and in some cases after head trauma, it has been shown that the increased expression of apoE in astrocytes and apoE receptors parallels dendritic proliferation and synaptic regeneration (Poirier et al. 1991, Poirier

et al. 1993, Poirier 1994, White et al. 2001a). The co-ordinated increased expression of apoE and receptors after injury, is thought to represent the transport of cholesterol and lipids necessary for dendritic remodelling and synaptogenesis (Poirier 1994). In support of this, the ability of synapses to regenerate after entorhinal cortex lesion is impaired in apoE deficient mice (Masliah et al. 1996). Intracerebroventricular administration of recombinant human apoE E3 or E4 reverses the impaired synaptic response and alleviates the cognitive deficit in apoE deficient mice with ageing (Masliah et al. 1997). It is also suggested that apoE plays a role in the clearance of degeneration products after injury (Fagan et al. 1998, White et al. 2001a). Alterations in apoE expression parallel the removal of degeneration products after injury, and this system is impaired in apoE deficient mice that exhibit a persistence of degeneration products (Fagan et al. 1998). It is suggested that individuals with an apoE ε4 allele (Arendt et al. 1997) are unable to compensate for age-related neuronal cell loss. In keeping with this, the neuronal reorganization is more severely affected in patients with AD carrying an apoE ε4 allele (Arendt et al. 1997). Low brain concentrations of apoE have been shown in AD patients carrying an apoE ε4 allele compared to normal control patients and AD individuals with apoE ε3 genotype (Bertrand et al. 1995). The lower levels of apoE may compromise lipid homeostasis and underly the poor compensatory synaptogenesis (Poirier and Sevigny 1998). More specifically, the cholinergic system is dependent on phospholipid homeostasis, and apoE isoform differences have been suggested to underly dysfunction of the cholinergic system in AD, apoE ε4 individuals being more severely affected (Poirier et al. 1995, Poirier and Sevigny 1998).

In vitro evidence is consistent with an isoform-dependent neurotrophic role of apoE. Rabbit dorsal root ganglion neurons and neuroblastoma cells incubated with lipoproteins alone have enhanced neurite outgrowth that is further enhanced in the presence of apoE E3 lipoproteins, and inhibited in the presence of apoE E4 lipoproteins (Handelmann et al. 1992, Nathan et al. 1994). Similar results are obtained when cells are stably transfected to secrete apoE E3 or apoE E4; in the presence of lipoproteins, cells expressing apoE ε3 have extensive neurite outgrowth, whereas neurite extension is suppressed in cells expressing apoE ε4 (Bellosta et al. 1995). One of the cellular events associated with neurite outgrowth is an apoE-isoform- specific effect on the cytoskeleton. ApoE E3 stabilizes the formation of microtubules in vitro in contrast to apoE E4 that is associated with destabilization of the microtubule assembly (Fleming et al. 1996).

White et al. (2001b) found decreased synaptic plasticity associated with the apoE4 isoform presence in transgenic mice producing human apoE4 isoform compared to those producing human apoE3 isoform. Human apoE3 protein, expressed in mice deficient in endogenous mouse apoE, is protective against kainic acid-induced neurodegeneration, while human apoE4 is not protective (Buttini et al. 1999). A similar difference was observed between human apoE3- and apoE4-expressing mice and chronic age-related

neurodegeneration (Buttini et al. 1999). Of potential relevance to longer term outcome, apoE is involved in the delivery of cholesterol to neurones required for neurite outgrowth and synaptogenesis (Mauch et al. 2001), clearance of degeneration products, microglial activation, and the maintenance of the cholinergic system (Graham et al. 1999b, Horsburgh et al. 2000a).

2.2.2.5 ApoE and normal aging

Furthermore, most (Haan et al. 1999, Bretsky et al. 2003, Mayeux et al. 2001), but not all (Dik et al. 2000, Collie et al. 2001) longitudinal studies of nondemented persons have reported that apoE ε4 carriers show greater cognitive decline with aging. Among humans with age-associated memory impairment, women performed less well on memory testing (Bartres-Faz et al. 2002). Healthy older adults with apoE4 of both sexes without dementia or neurological disease, in several studies, have demonstrated deficits in specific areas of neuropsychological function, especially visual attention, memory, and psychomotor rapidity (Bondi et al. 1995, Berr et al. 1996). ApoE ε4 carriers performed significantly more poorly than non-carriers when assessed for learning and memory abilities (Schmidt et al. 1996). Normal male twins with the apoE ε4 allele performed more poorly on cognitive testing when compared to their co-twins without the apoE ε4 allele (Reed et al.1994). Memory declines more rapidly with age in apoE ε4 carriers than in apoE ε4 non-carriers (Caselli et al. 2004). These results provide support for the hypothesis that the apoE genotype can affect cognition even in the absence of disease in aging adults. It is assumed that the expression of apoE is not significantly altered during normal aging, although this has not been determined experimentally. Recent studies have shown an age-related increase in apoE expression in the liver, implying that alterations in apoE expression may be an important factor in maintaining normal homeostasis during aging in some tissues (Gee et al. 2005).

2.2.3 The role of apoE in neurological diseases

ApoE E4 isoform is genetically associated with late-onset familial and sporadic Alzheimer`s disease (Corder et al. 1993, Saunders et al. 1993). AD is characterized by loss of synapses and neurons, the accumulation of amyloid plaques and the occurrence of neurofibrillary tangles. The risk of AD was further increased and the age of onset decreased in subjects with two ε4 alleles, compared with those with only one ε4 allele (Corder et al. 1993).

Seven hundred stroke patients were retrospectively studied, and the apoE ε4 allele did not influence the dichotomized three-month outcome (good if alive at home, bad if dead or in care) after ischemic stroke. However, in those patients who had suffered a hemorrhagic stroke there was a nonsignificant trend towards poorer outcome in those patients with the ε4 allele (McCarron et al. 1998). In a prospective study, McCarron et al.

(2000) followed ischemic stroke patients, and the apoE ε4 allele was not associated with three-month outcome. MacLeod et al. (2001) examined apoE genotype in 266 ischaemic stroke patients and found no association between apoE ε4 allele and ischaemic stroke. The apoE ε4 allele did not predict outcome after ischemic stroke at the time of discharge from an inpatient rehabilitation programme (Treger et al. 2003). Although the outcome measures vary between different studies, it appears that apoE ε4 carriers have a poorer outcome after spontaneous intracerebral haemorrhage (Alberts et al. 1995, McCarron et al. 1999, McCarron et al. 2003, Martinez-Gonzales and Sudlow 2006). The apo ε4 allele was not associated with larger hematomas (McCarron et al.1999), but may be associated with relatively prolonged coagulation times (Weir et al. 2001).

The apoE ε4 allele seems to predispose a patient to a poor outcome after subarachnoid hemorrhage (SAH). Leung et al. (2002) demonstrated a significant negative influence on six-month outcome in a study of 72 patients. Niskakangas et al. (2001) showed that 40 % of patients with the apoE ε4 allele had an unfavourable outcome, compared with 19% of those without. Tang et al. (2003) found that 28% of those with the apoE ε4 allele had an unfavourable outcome compared with 8% without. Two linked studies did not demonstrate an association between the apoE ε4 allele and outcome after SAH (Dunn et al. 2001, Morris et al. 2004). However, the patients who sustained the most severe hemorrhage showed a trend towards poorer outcome in apoE ε4 -carriers. A meta-analysis of apoE genotype and SAH demonstrated that in patients with SAH, the expression of the apoE ε4 allele was associated with a higher risk of a negative outcome and delayed ischemia (Lanterna et al. 2007). SAH- affected mice expressing the apoE ε4 allele also had a greater functional deficit, mortality and vasospasm as compared with their ε3 counterparts (Gao et al. 2006). In summary, the results suggest that apoE may affect outcome after ICH and SAH, but not after ischemic stroke (Martinez-Gonzales and Sudlow 2006).

Evidence derived from several clinical studies suggests an unfavourable course of multiple sclerosis (MS) in carriers of the apoE ε4 allele (Sylantiev et al. 1998, Chapman et al. 1999, Evangelou et al. 1999, Fazekas et al. 2000, Masterman et al. 2002, Chapman et al. 2001, Fazekas et al. 2001, Enzinger et al. 2003), although such an association was not seen in all studies (Ferri et al. 1999, Weatherby et al. 2000, Schmidt et al. 2002, Schreiber et al. 2002).

The apoE ε4 allele is associated with poorer neurological outcome after cardiopulmonary arrest (Schiefermeier et al. 2000), and carriers of the apoE ε4 allele have been shown to recover neuropsychological and cognitive functions less well following cardiac bypass surgery (Tardiff et al. 1997). The apoE ε4 allele seems to be associated with an increased risk of developing diabetic neuropathy and human immunodeficiency viral neuropathy (Blacman et al. 2005). Reports on apoE genotype and Parkinson`s disease

are inconsistent. Some studies have reported an association between the apoE ε4 allele and Parkinson`s disease or Parkinson`s disease with dementia, whereas others have not (Whitehead et al. 1996, Inzelberg et al. 1998). To date, there is little evidence to support the role of gene polymorphisms in outcome from CNS infections. ApoE interacts with HSV-1 and may facilitate its transport within the CNS but it does not appear to affect outcome from herpes simplex encephalitis (Nicoll et al. 2001).

2.2.4 ApoE and outcome of TBI

ApoE has been implicated in modifying neurological outcome after TBI, although the mechanisms by which this occurs remain poorly defined. Several studies have shown that patients with apoE ε4 have a poorer outcome after TBI in the acute and subacute stage. Sorbi et al. (1995) showed that the apoE ε4 allele was a prognostic factor for posttraumatic coma. Teasdale et al. (1997) demonstrated a significant association between apoE ε4 and poor outcome after head injury. Patients with the ε4 allele were more than twice as likely to have an unfavourable outcome six months after head injury than those without. Subsequent studies have shown a similar association, although in all cases the subject numbers have been small. Jordan et al. (1997) suggested that possession of an apoE ε4 allele may be associated with increased severity of chronic neurological deficits in high-exposure boxers. Friedman et al. (1999) reported that only 3.7% of patients with the ε4 allele made a good recovery, compared with 31 % of patients without the allele. The outcome of patients who were able to complete a course of neurorehabilitation after TBI was also found to be associated with apoE genotype (Lichtman et al. 2000). Overall and motor recovery, as assessed by the Functional Independence measure (FIM), were both significantly lower in patients with the ε4 allele than in those without. Crawford et al. (2002), while demonstrating poorer outcome in ε4 patients, found that memory post TBI was poorer in these subjects. In their study, 110 active and veteran American military personnel were assessed using a number of memory and cognitive measures. Although all patients displayed impaired performance, those who had the ε4 allele were significantly worse. This relationship did not extend to all aspects of cognitive functioning, as there was no relationship between measures of executive functioning and presence of the ε4 allele. The memory performance of subjects without ε4 was poorer after a more severe head injury than after a milder one, while the performance of subjects with ε4 was as poor as the severely injured subjects without ε4, regardless of TBI severity.

An important point in studies of genetic variation is the ethnicity of the population. Chiang et al. (2003) extended the findings of the above studies to a Chinese population, in whom the ε4 allele is relatively uncommon in comparison to European and North American populations. One hundred consecutive patients admitted with TBI were prospectively followed to six months post-injury. More than twice as many patients with ε4 had an unfavourable outcome at that stage than those without (52.6% vs. 24.1%). Nathoo et

al. (2003) reported that apoE ε4 allele had no significant effect on TBI outcome in an African cohort.

The ε4 allele adversely affects outcome even after mild head injury. Sundström et al. (2004) performed a series of neuropsychological tests on a small cohort of individuals both pre- and post-head injury. Those with the ε4 allele performed significantly more poorly on three tests post-head injury, while those without the ε4 allele performed no differently. Liberman et al. (2002) showed that ε4-carriers had lower neuropsychological outcome scores on 12 out of 13 neuropsychological outcomes three weeks after injury compared with non -ε4 carriers. Two of the differences were significant. Six weeks after injury, apoE ε4 -positive patients had lower adjusted mean scores on 11 of the 13 neuropsychological outcomes. None of the differences was significant. The conclusions were that apoE genoptype may influence the severity of the acute injury. However, with no consistent pattern to the recovery curves, the influences on the rate of recovery are unclear. In a large prospective study, 1094 subjects were included and the outcome was assessed using the GOS (Teasdale et al. 2005). The apoE genotype did not affect the overall outcome, but the prognostic influence of apoE was restricted only to younger TBI victims. In a cohort of moderate and severe TBI patients, subjects with the ε4 allele showed poorer learning and long-term memory, and had significantly more neurobehavioural disturbances (Ariza et al. 2006). TBI patients with the ε4 allele had a slower recovery rate than those without this allele over a two-year period, and the presence of the apoE ε4 allele influenced the recovery rate from severe TBI irrespective of other covariates (Alexander et al. 2007). A recent meta-analysis indicated that the presence of the apoE ε4 allele is not associated with the initial severity of brain injury following TBI but is associated with increased risk of poor outcome at six months after injury (Zhou et al. 2008).

The very long-term outcome of TBI patients is poorly known, with few studies dealing with this problem. In a cross-sectional study, middle-aged and elderly patients had persisting cognitive impairments 30 years after a mild-to-moderate TBI compared to age-, gender- and education-matched controls (Klein et al. 1996). Other studies dealing with truly long-term outcome have mostly included veterans of war (Plassman et al. 2000), who differ markedly both in trauma mechanism and population background from modern civilian injuries. In a study of mainly severe TBI patients, cognitive decline 15 to 25 years after injury was not related to apoE genotype (Millar et al. 2003). The subjects assessed were relatively young (mean age 42.1 years) in the context of age-related congitive decline and the onset of sporadic Alzheimer`s disease.

Animal studies, using apoE knockout and transgenic mice, have also provided further information about apoE mechanisms and the response of the brain to injury. Some studies have shown that the apoE molecule may have a direct neurotoxic role (Neve and

Robakis 1998), whereas others have speculated that apoE ε4 may directly interact with Aβ and impact on the metabolism of APP (Growdon 1998). Transgenic mice expressing human apoE ε4 had increased mortality and worsened neurological scores compared with apoE ε3 transgenic mice after experimental closed-head injury (Sabo et al. 2000). Ezra et al. (2003) found increased mortality and poorer outcome in apoE ε4 transgenic mice, and greater recovery and less cortical damage in apoE ε3 transgenic mice.

However, the effect of apoE is still controversial, since some studies have failed to show any association with the outcome of TBI (Plassman et al. 2000, Jellinger et al. 2001, Diaz-Arrastia et al. 2003, Millar et al. 2003, Nathoo et al. 2003, Chamelian et al. 2004, Smith et al. 2006, Ponsford et al. 2007, Willemse-van Son et al. 2008). Polymorphism within the promoter region may increase the apoE expression and thus exacerbate the response to TBI. To date, several polymorphisms within the promoter region of the apoE gene have been identified at -491 (A/T transversion), -427 (T/C transversion), and -219 (G/T transversion), and these are proposed to affect the transcriptional activity of the apoE gene in both the periphery and the CNS (Artiga et al. 1998).

Table 1. Studies of relationships between apoE genotype and outcomes from TBI in adults

Study	Sample size	Outcome measure	Effect	Time of assessment
Sorbi et al. 1995	16	Coma duration	e4 +	1 year
Teasdale et al. 1997	89	GOS	e4 +	6 months
Jordan et al.1997	30	CBI scale	e4 +	unclear
Friedman et al. 1999	69	Functional and cognitive assessment	e4 +	6-8 months
Lichtman et al. 2000	31	FIM	e4 +	6 months
Crawford et al. 2002	110	Learning/fluency test	e4 +	6 months
Liberman et al. 2002	80	Neuropsychological tests	e4 No consistent pattern	6 weeks
Liaquat et al. 2002	129	Haematoma volume/GOS	e4 +	6 months
Chiang et al. 2003	100	GOS	e4 +	6 months
Diaz-Arrastia et al. 2003	106	Seizure frequency/GOS	e4 associated with increased risk for seizures but not outcome	6 months
Millar et al. 2003	396	GOS/Neuropsychological tests	e4 -	18 years
Nathoo et al. 2003	110	GOS	e4 -	6 months
Chamelian et al. 2004	90	GOS/Neuropsychological tests/RHFUQ/ RPQ/GHQ	e4 -	6 months
Sundström et al. 2004	34	Neuropsychological tests	e4 +	unclear
Teasdale et al. 2005	984	GOS	e4 + only younger patients	6 months
Ariza et al. 2006	77	Neuropsychological tests	e4 +	6 months
Alexander et al. 2007	123	GOS	e4 +	2 years
Ponsford et al. 2007	120	Neuropsychological tests	e4 -	1 year
Willemse-van Son et al. 2008	79	GOS, SIP-68, CIQ	e4-	3 years

e4+ =e4 associated with poorer outcome, e4- = no association with poorer outcome; CBI, chronic brain injury; FIM, Functional Independence Measure; RHFUQ, Rivermead Head Injury Follow-up Questionnaire; RPQ, Rivermead Post-Concussion Symptoms Questionnaire; GHQ, Twenty-eight-item General Health Questionnaire; GOS, Glasgow Outcome Scale score; SIP-68, Sickness Impact profile-68; CIQ, Community Integration Questionnaire

2.2.5 Relation of TBI, apoE and dementia

The association between TBI and dementia is still controversial. TBI can lead to overexpression of the APP resultting in deposition of the β-amyloid in the brain, similar to that seen in brains of AD subjects (Graham et al. 1996). This finding has led to the hypothesis that TBI may increase the risk of AD. The mechanisms by which TBI is associated with the formation of Aβ plaque depositions are still unknown. However, significant discrepancies exist in neuropathological studies. The epidemiological studies that have reported on the relationship between TBI and dementia are also contradictory. The apoE ε4 allele is a risk factor for AD (Corder et al. 1993). Of special interest is the possible influence of TBI on the risk of developing later Alzheimer`s disease, and the eventual interaction with the apoE genotype. Although epidemiological studies and retrospective autopsy data provide evidence that a later cognitive decline may occur after severe TBI, the relationship between dementia after TBI and apoE status is still ambiguous (Jellinger 2004). The role of apoE genotype for the prognosis of TBI is also contradictory. Most studies supports the view that the ε4 allele of apoE adeversely affects outcome after TBI in the first six months post injury (Zhou et al. 2008). Its effects over a much longer period are less clear. In contrast to the studies demonstrating an association between apoE ε4 and an unfavourable outcome following TBI, a number of recent studies examining both severe and mild TBI have failed to support such findings (Guo et al. 2000, Plassman et al. 2000, Jellinger et al. 2001, Chamelian et al. 2004, Willemse-van Son et al. 2008). A cohort study (Guo et al. 2000) and a neuropathological study (Jellinger et al. 2001) have suggested that TBI might be a risk factor for AD in those lacking the ε4 allele of apoE.

Preliminary evidence indicating a possible role for TBI in the development of AD came from an early case report documenting classic AD pathology in a 38-year-old man who had suffered a severe head trauma 16 years earlier (Rudelli et al. 1982). Neuropathological studies of the brains of boxers suffering from dementia pugilistica have also demonstrated AD-like pathology with diffuse Aβ plaque depositions (Roberts et al. 1990). Histopathological studies of individuals who have died after suffering a severe TBI demonstrate widespread cerebral Aβ deposition in short (Roberts et al. 1991, 1994, Gentleman et al. 1997, Ikonomovic et al. 2004) and long-term (Clinton et al. 1991) survivors, irrespective of age. However, significant discrepancies exist in neuropathological studies. Adle-Biassette et al. (1996) were unable to detect any Aβ deposits in the head injuries of individuals below the age of 63 years, in a similar age range and survival time to those studied by Roberts et al. (1991). Also, the non-selective autopsy investigation by Braak and Braak (1997) rarely detected Aβ plaques in the younger subjects (below the age of 40 years).

Studies using different forms of experimental TBI have given some insight into how brain injury may lead to AD, although the results remain somewhat inconclusive. In

support of the argument that increased APP levels following TBI may potentiate AD pathology, experimental TBI in rats induced overexpression and accumulation of APP in the cerebral cortex and hippocampus, which subsequently led to neuronal degeneration in the CA3 region of the hippocampus as early as three days post-injury (Murakami et al. 1998). Also, experimental trauma studies in pigs have demonstrated rapid Aβ accumulation, manifested in axonal bulbs and diffuse plaques three days after trauma (Smith et al. 1999). However, post-traumatic Aβ deposition has not been observed in the majority of non-transgenic animal studies, unlike human studies (Pierce et al. 1996, 1998, Masumura et al. 2000, Laurer et al. 2001, Ciallella et al. 2002, Hamberger et al. 2003). In the transgenic models, rapid Aβ deposition has not been demonstrated (Roberts et al. 1991,1994, Gentleman et al. 1997, Ikonomovic et al. 2004), and increased severity of injury did not result in increased Aβ deposition; if anything, it actually seemed to correlate with reduced Aβ deposition or even resolution of already established plaques (Szczygielski et al. 2005). In humans, both the risk (Plassman et al. 2000) and the post-traumatic Aβ deposition increased with TBI severity (Roberts et al. 1994).

Some case-control studies suggest that head injury may increase the risk of dementia (Heyman et al. 1984, French et al. 1985, Mortimer et al. 1985, Graves et al. 1990, van Duijn et al. 1992, Mayeux et al. 1993, O`Meara et al. 1997, Salib and Hillier 1997, Guo et al. 2000) but not all case-control studies (Amaducci et al. 1986, Chandra et al. 1987, 1989, Shalat et al. 1987, Broe et al. 1990, Ferini-Strambi et al. 1990, Fratiglinoni et al. 1993). The MIRAGE study analysed 2233 definite and probable AD patients, and 14,668 first-degree relatives, and showed that head injury with loss of consciousness significantly increased the AD risk (Guo et al. 2000). Specifically, the meta-analysis of seven case-control studies by Mortimer et al. (1991) provided the first convincing evidence in support of a strong association between TBI and AD. A relative risk of 1.82 was reported (95% confidence interval (CI) 1.26 to 2.67) for head injury with loss of consciousness. The relative risk, when adjusted for a family history of dementing illness, education, and alcohol consumption, remained significant but was only true for males (2.67, 95% CI 1.64 to 4.41). Later, a review of case-control studies sought to replicate those findings, and supported an association between head injury and AD in males (Fleminger et al. 2003). The possible explanation for the gender differences in the risk of AD following TBI may be attributed to the neuroprotective and neuroregenerative effects of the female hormones oestrogen and progesterone (Stein 2001), or it could be merely the fact that men typically suffer more severe injuries than women.

There have also been conflicting reports in cohort studies. In two early studies, no significant association was found between head injury and the risk of developing AD (Katzman et al. 1989, Williams et al. 1991). A history of head trauma with unconsciousness was excluded as a risk factor for AD in both the Rotterdam Study (Mehta et al. 1999) and the large EURODEM study (Launer et al. 1999). Plassman et

al. (2000) examined the association between early adult head injury and dementia in late life. In the study of 548 World War II (US Navy) brain-injured veterans and 1228 age-matched non-injured controls, follow up after 50 years revealed that moderate to severe head injuries in young men may be associated with increased risk of AD and other dementias in late life.

Several studies have shown that there is a greater risk of developing AD when the head injury has occurred in later life (within 10 years of onset of AD) as opposed to a head injury occurring earlier in life (beyond 10 years of onset of AD) (Graves et al. 1990, Mortimer et al. 1991, van Duijn et al. 1992). In contrast, other studies have shown that a head trauma occurring mainly in earlier childhood is associated with an increased risk of AD in later life (Schofield et al. 1997, Plassman et al. 2000). After examining the incidence of AD pathology in 58 consecutive patients with residual closed TBI lesions and the frequency of TBI residuals in 57 age-matched autopsy controls, Jellinger et al. (2001) concluded that severe TBI may have some influence on the development of AD, irrespective of the age at which the TBI occured. According to cohort studies TBI may interact with other risk factors to hasten the onset of AD in persons susceptible to the disease (Sullivan et al. 1987, Gedye et al. 1989, Schofield et al. 1997, Nemetz et al. 1999). Rasmusson et al. (1995) did not find any effect of head injury on age of onset of AD in a cohort of 68 AD cases, and nor did the MIRAGE study (Guo et al. 2000).

Mayeux et al. (1995) reported a synergistic interaction between head injury and apoE ε4 on the risk of AD. In their study, head injury alone did not increase the risk, but head injury in persons with ε4 increased the risk 10-fold compared with those who lacked both factors. O`Meara et al. (1997) showed an increased risk of AD in individuals with a history of TBI but no modification by the apoE ε4 allele. Cohort studies from selected (Plassman et al. 2000), retrospective population -based (Guo et al.2000), or prospective population -based (Mehta et al. 1999) materials have also yielded very different results. A prospective study of 1776 World War II navy veterans showed that moderate and severe TBI, rated by duration of loss of consciousness or PTA in early adult life, was associated with increased risk of AD and dementia in late life. This risk increased with the severity of TBI, and showed a non-significant trend towards a stronger association between AD and TBI in men with apoE ε4 alleles (Plassman et al. 2000). In the MIRAGE study, head injury as a risk factor for AD appeared greater amongst subjects lacking apolipoprotein apoE ε4 (Guo et al. 2000). Mehta et al. (1999) did not find either a connection between TBI and AD or an interaction with the apoE genotype. Fleminger et al. (2003) found an association between head injury and AD in males, but this review was unable to determine the relationship with apolipoprotein E gene status. A long-term follow-up of cognitive outcomes after head trauma showed that a late decline may occur after head injury, but no association with the apoE genotype. However, the majority of patients were still too young (mean 42.1 years) to assess the risk of AD (Millar et al. 2003). A 30-year

follow-up study of mental disorders after TBI indicated that apoE ε4 predicted dementia but not other psychiatric disorders (Koponen et al. 2004). Luukinen et al. (2005) showed that fall-related TBI predicted earlier onset of dementia, with the effect being especially high amongst subjects who carried the apoE ε4 allele.

There is no conclusive evidence linking apoE genotype with the development of AD following TBI (Jellinger 2004). However, it is still unclear whether TBI might really increase the risk of AD by, for example, triggering a process whereby Aβ accumulates in the brain, or whether the connection between TBI and AD evolves only from the lower cerebral reserves after TBI, thus causing the earlier appearance of eventual AD (Nemetz et al. 1999). ApoE4 -related long-term cognitive decline may also appear through increasing cholinergic hypofunction (Kleifeld et al. 1998, Champagne et al. 2005).

3. AIMS

The specific aims of the study were:

I. To investigate the outcome of TBI after three decades and its relationship to apoE genotype

II. To evaluate the association between hippocampal volume, brain atrophy and apoE genotype

III. To evaluate the relationship of mri changes during the first year after TBI to apoE genotype

IV. To investigate the association of injury severity, MRI results and apoE genotype with one-year outcome in mainly mild TBI

4. MATERIAL AND METHODS

4.1 Subjects

4.1.1 Studies I and II

This study was a part of a more extensive TBI research project coordinated by the Department of Neurology of Turku University. The study group was selected from 210 patients who had suffered TBI between 1950 and 1971, and who had been referred for neuropsychological evaluation to one of the authors (RP) at Turku University Central Hospital between the years 1966 - 72. The reason for the referral to the neurologist and neuropsychologist was either a recent injury or significant disability after an earlier injury, and all referred patients were included in the original material. Inclusion criteria in our current study were: (1) a blunt injury severe enough to cause TBI and causing neurological symptoms and signs lasting at least one week after the injury (including headache and nausea); and (2) at least one of the following: loss of consciousness for at least one minute (eye-witnessed); posttraumatic amnesia (PTA) for at least 30 minutes; neurological symptoms and signs of recent brain injury during the first three days after the trauma (excluding headache and nausea); or neuroimaging findings suggesting TBI (e.g. skull fracture, intracerebral hemorrhage). Exclusion criteria were: (1) significant (i.e causing possibly permanent damage) brain disease prior to TBI; (2) clinical symptoms of a nontraumatic brain disease developed after TBI (excluding degenerative dementia); (3) insufficient cooperation; (4) refusal to participate in the study; and (5) medical records not available.

From the original sample of 210 patients, 76 patients had died, 13 patients did not meet the inclusion criteria, one patient was excluded because of brain disease prior to TBI, and the medical records were missing for two patients. After this screening, the remaining 118 patients were contacted by mail, and 88 of them responded. Of these, 83 patients met the inclusion criteria, but seven of them were excluded because of a later non-traumatic brain disease, and 14 patients refused to participate in the study. The remaining 62 patients were examined between January 1998 and April 1999. MRI was not available for four patients and one patient refused the neuropsychological examination. The formation of the study groups is presented in Figure 1. To test the representativeness of the study group (n = 61), the deceased subjects plus the combined group of subjects who either refused or could not be reached were compared with the study group in terms of age, gender, education and severity of TBI. The representation analyses were restricted to the same age range as in the study group (age at TBI < 53 years). The only significant differences between the groups, according to analysis of variance (ANOVA) were that the deceased subjects were significantly older ($p < 0.0001$) and had less education ($p < 0.01$) compared to the other groups.

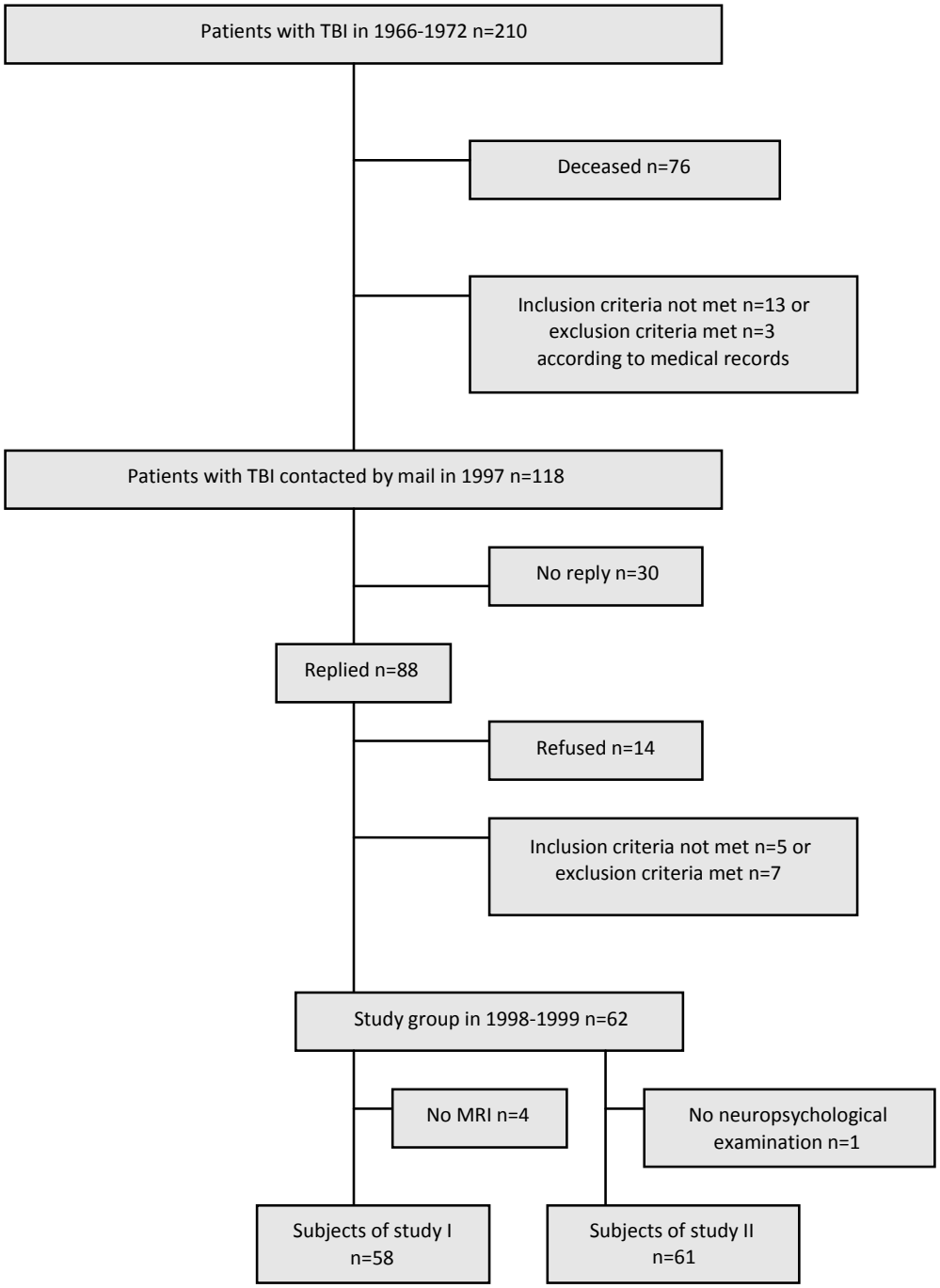

Figure 1. Formation of the stydy groups.

4.1.2 Studies III-IV

Thirty-three consecutive patients attending the Emergency Department of the Turku University Central Hospital were recruited for the study from January 1998 to February

2000. The inclusion criteria for the patients were: 1) acute (< 3 days) brain trauma that included one or more of the following: a) loss of consciousness for at least 1 minute, b) at least half an hour posttraumatic amnesia, c) neurological symptoms or signs of brain injury during the first three days (excluding headache and nausea), and d) neuroradiological findings indicating acute TBI: 2) age 16-70 years and 3) informed consent. Patients with other central nervous system diseases or contraindications to MRI were excluded. The severity of TBI was assessed both by the GCS on arrival and by the duration of PTA (Kay et al. 1993, McFarland et al. 2001) using the Rivermead protocol (King et al. 1997). Table 2 shows the characteristics of the subjects.

Table 2. Demographic features of the patient material

Variable	Study I	Study II	Studies III-IV
	n=58	n=61	n=33
Age at injury,y	28.9±10.5	28.9±10.7	44.2±16.6
Age at examination,y	60.2±10.2	60.3±10.6	44.2±16.6*
Time from injury,y	31.3±3.9	31.4±3.9	0
Education**,y	9.3±2.2	9.3±2.2	10.6±2.4
Gender, m/f (%)	67/33	69/31	70/30
Mechanism of injury (%)			
Traffic accident	60	62	27
Fall	24	23	73
Assault	2	2	0
Other	14	13	0

* Age at first examination

** Education was recorded as the total number of school years

4.2 Methods

4.2.1 Rating scales and questionnaires

In studies I and II, information regarding the TBI was obtained retrospectively from medical records and patient interview. Background data were collected with a questionnaire containing information on the demographic characteristics and TBI. All patients suffered from closed head injuries. Subjects reported education as the total number of school years. The mechanism of injury was grouped into four categories: motor vehicle accident, fall, assault, and other. The severity of TBI was classified according to the duration of PTA as follows: < 1 hour = mild, 1-24 hours = moderate, 1-7 days = severe, and > 7 days = very severe (Rusell and Smith 1961). In study II, physical outcome was estimated with the physical category (ambulation, mobility, body care and movement) of the Sickness Impact Profile (SIP) (Bergner et al. 1981). Subjective symptoms and ADL functioning were assessed by the Symptom Checklist-90 (SCL-90) (Degoratis et al. 1973) and the Patient Competency Rating Scale (Prigatano 1986). Vocational outcome was assessed by a detailed interview covering the whole working history before and after the TBI, in terms of duration, level of demand,

and continuity. For the analysis, the original seven employment categories were grouped into two categories: not employed after the injury and employed after the injury.

In study IV, to assess outcome at one year after injury, the Head Injury Symptom Checklist (HISC) (McLean et al. 1984) and the Glasgow Outcome Scale, extended version (GOS-E) (Wilson et al. 1998) were used.

4.2.2 Neuropsychological assessment

Cognitive outcome was assessed in study II by comparing the score of the Mild Deterioration Battery (MDB) (Kujala et al. 1994) in the original examination to the follow-up study about 30 years later. The MDB measures general cognitive impairment in episodic memory and in verbal and visuomotor performance. It consists of eight tests: Similarities, Digit span, Digit symbol, and Block design from the Wechsler Adult Intelligence Scale (Wechsler 1955), the Benton Visual Retention test (Benton 1963), immediate recall of 30 paired word associates, and naming time and immediate recall of 20 common objects. A patient received one deterioration point, if his or her performance on any of the eight subtests was 1.5 standard deviations below the norm, two points if the score was 2.0 standard deviations below the norm, and three points if the score was 3.0 standard deviations below the norm. Thus the maximum total score on the MDB was 24 points. The results were rescored as described earlier, and only the total score was used for the analysis. The MDB was the only outcome measure that was also registered at baseline, but in the follow-up examination, parts of CERAD (Verbal fluency test, modified Boston naming test, Mini-Mental State Examination, Word-list memory, Word-list recall and Word-list recognition) (Welsh et al. 1994), and a memory questionnaire (Sunderland et al. 1983) were also applied.

In studies I and II, in addition to the diagnosis of dementia (definite) according to DSM-IV criteria, a diagnosis of subclinical dementia was made in those cases where the DSM-IV criterion ”significant impairment in social or occupational functioning” was not met unequivocally.

4.2.3 Magnetic resonance imaging (MRI)

4.2.3.1 Study I

The MRI scans were acquired with a 1.5 T Siemens Magnetom system using a standard head coil. In addition to routine T2-weighted axial sequence, a sagittal three-dimensional MPR (magnetization prepared rapid gradient echo; TR 10, TE 4, flip angle 10˚, matrix 192 x 256, contiguous 1.5 mm slices, 1 acquisition) sequence was obtained in all cases. All measurements were evaluated blindly by an experienced neuroradiologist.

Hippocampal volumes were measured according to a previously described method (MacKenzie et al. 2002). The hippocampus included the Ammon’s horn, the gyrus

dentatus, the subiculum and the uncus ventral to the caudal amygdala: the slice in which the fornices were visible in their full length determined the posterior end of the hippocampus. Intracranial coronal area was measured at the level of the anterior commissure; this area was used to normalize the volumes with respect to head size.

Primary intracranial lesions were classified by their location into cortical contusions and traumatic axonal injury (TAI). The volume of contusions was assessed by a previously published method to measure intracranial lesions (Pasqualin et al. 1991). Lesions were considered as TAI based on their location and characteristics. In general hemorrhagic lesions or lesions at the grey-white matter junction, in the corpus callosum and the dorsolateral upper brain stem were considered as TAI.

4.2.3.2 Studies III and IV

Head MRI was performed one week (7± 2 days) and again one year after the trauma. Nine of the patients were studied on a Siemens Magnetom 1.5 T system. The following sequences were used for the analysis: (a) T2-weighted axial turbo spin echo (TSE) 3500/93 ms (repetition time/ echo time) with one acquistion, slice thickness 5.0 mm, data acquisition matrix 192 x 256, and field of view (FOV) 23.0 cm; (b) T1-weighted coronal SE 600/15 with two acquisitions, slice thickness 7.0 mm, data acquisition matrix 192x 256, and FOV 23.0 cm; (c) T2/PD-weighted coronal TSE 3500/93/19 with one acquisition, slice thickness 4.0 mm, data acquisition matrix 192x 256, and FOV 23.0 cm.

Twenty-four of the MRI studies were obtained with a General Electric Signa 1.5 T-system. The analysis was performed using the following sequences: (a) T2-weighted axial fast spin echo (FSE) 4520/81.6 with two acquisitions, slice thickness 5.0 mm, data acquisition matrix 512 x 224, and FOV 24 x 18 cm; (b) fluid-attenuated T2-weighted fast spin echo inversion recovery (FSEIR) 10002/172.5 with inversion time 2200 ms, one acquisition, slice thickness 7.0 mm; data acquisition matrix 256 x 192, and FOV 24.0 cm; (c) 3D fast spoiled gradient echo (FSPGR) 11.3/4.2, flip angle 20˚, one acquisition, data acquisition matrix 256 x 192, slice thickness 1.2 mm, FOV 22 x 17.6 cm, and 124 contiguous axial slices with no inter-slice gap.

The MRI scans were analysed by an experienced neuroradiologist, with early and late scans reviewed on different days. Rating of contusions was done according to their number in different anatomical locations and their size. The extent of contusions was rated on a scale of 0-3, in which 0 indicated absence of contusions; 1 indicated contusions that include cortex and white matter (WM), together less than 0.5 cm; 2 indicated contusions that included 0.5 – 2 cm of the WM; and 3 indicated contusions that included over 2 cm of the WM (Brandstack et al. 2006). To determine ventricular size in successive MRI scans the maximum distance between the frontal horns (A) and the maximum inner

diameter of the skull (B), measured from the same slice as (A) were evaluated. These measurements were used to obtain the Evans's index (Evans 1942) (EI) (EI=A/B).

Other intraparenchymal lesions consisted of TAIs and traumatic deep grey matter injuries. Hemorrhagic white matter changes without adjacent cortical lesion or lesions at the grey-white matter junction, in the corpus callosum, corona radiata and the dorsolateral upper brain stem were considered to be TAI. Visual rating of these traumatic lesions on T2-weighted images was done using the modified Scheltens semiquantative rating scale (Scheltens et al. 1993), so that both T2-hyperintense and T2-hypointense changes were included.

4.2.4 ApoE genotyping

To perform the apoE genotyping assays 3 mL of EDTA anticoagulated blood was collected from each patient. Genomic DNA was extracted from fresh or frozen whole blood either by the Nucleon DNA-extraction kit (Amersham) or by a modified salting-out procedure (Donohoe et al. 2000). The quality and quantity of DNA was then measured by spectrophotometry, and samples were diluted to a final concentration of 20 mg/L. The determination of the six major genotypes of apoE was done using the multiplex amplification refractory mutation system PCR as previously described (Donohoe et al. 1999). Each PCR run included negative controls and three to five samples of known apoE genotypes as positive controls.

4.2.5 Statistical analyses

For analytical purposes, the patients with various apoE genotypes were grouped into apoE ε4 carriers (ApoE4+, including patients with one or two ε4 alleles) and apoE ε4 non-carriers (ApoE4-, including patients without ε4 allele). The significance of differences between the apoE groups for continuous variables was evaluated by one-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA) in post hoc pairwise comparisons, with Bonferroni corrected p values. Age, sex, education, and the severity of brain injury were used as covariates. Association of categorical variables and apoE status was tested using the Chi-square test and the Fisher exact test when needed. In study I, volumes normalized for the intracranial area were used in all statistical analyses. In study IV, multiple linear regression analyses were used to examine the association between response and explanatory variables. Correlations were tested according to Pearson. The level of significance was $p < 0.05$ in all analyses. For data management and statistical calculations, SPSS software was used.

4.2.6 Ethical considerations

After the subjects had been given a complete description of the study, written informed consent was obtained. The protocol was approved by the joint Ethics Committee of Turku University and Turku University Hospital.

5. RESULTS

5.1 Association between hippocampal volumes, brain atrophy, and apoE genotype after TBI (I)

The apoE genotypes were distributed among the 58 patients as follows: ε2/3, eight patients (13.8%); ε2/4, one patient (1.7%); ε3/3, 31 patients (53.4%); ε3/4, 13 patients (22.4%); and ε4/4, five patients (8.6%). Accordingly, 32.8% of the patients were apoE4+ and 67.2% were apoE4-. The left and right hippocampal volumes did not differ significantly between the apoE4+ and apoE4- patients. Neither did the volumes of the lateral ventricles differ significantly between these groups. The presence of the apoE4 was not associated with the presence or number of focal visually detected MRI abnormalities, whether defined as contusions (p=0.51) or signs of TAI (p=0.47) (I: Table 2).

In multivariate analysis, the MRI findings in the apoE4+ and apoE4- groups were compared taking into account the effect of age, education, gender, and severity of injury. None of the assessed MRI volumes showed differences between the apoE4+ and apoE4- patients (p= 0.13 to 0.40) (I: Table 3).

Six of the TBI patients developed subclinical or clinical dementia according to DSM-IV criteria, all of whom were apoE4 positive. One of them had mild, one moderate, two severe, and two very severe TBI according to the classification used. Their volumetric measurements were compared both to the other apoE4+ and the apoE4- patients. These patients showed more global atrophy as measured by lateral ventricular volume (p=0.038), but other radiological features analyzed did not differentiate them from the other groups (I: Table 4). However, the degree of ventricular enlargement was largely explained by the larger contusion volume in these demented patients because, after taking contusion volume as a covariate, these patients did not differ from the other groups (p=0.641). Those apoE4+ patients who had not developed subclinical/clinical dementia did not show any atrophy either in the hippocampuses or globally compared to apoE4- patients.

5.2 ApoE genotype and outcome of TBI after three decades (II)

At baseline, the apoE4+ group performed more poorly than others, but the difference was not significant. In the follow-up assessment, the cognitive outcome according to the MDB score was significantly poorest in the apoE4+ group (p=0.034) after adjusting for confounding variables (II: Table 2). Six patients in this material had developed clinical or subclinical dementia according to the DSM-IV criteria during the 30-year follow-up, all of them being apoE4 positive. Without these six patients, the cognitive level (measured with the MDB score) of the apoE4+ patients did not show any decline

with age, and their performance was slightly, although not significantly, better at the follow-up examination.

There was no association between the apoE genotype and physical outcome, vocational outcome, or subjective symptoms/ADL functioning using the outcome measures applied in this study.

5.3 ApoE genotype and MRI changes during the first year after TBI (III)

Nineteen patients had traumatic lesions on MRI, 30.3% of them being apoE4+ and 69.7 % apoE4-. The number and extension of contusions after injury, and at one year did not differ significantly between the apoE4+ and apoE4- patients. Neither did the results from the analysis of other intraparenchymal lesions differ significantly between these groups, and nor did the evolution of ventricular size index (III: Table 2).

In ANCOVA, the MRI findings in the apoE4+ and apoE4- groups were compared taking into account the effect of age, gender, and severity of injury but the results remained insignificant (III: Table 3).

5.4 Association of injury severity, MRI results and apoE genotype with one-year outcome in mainly mild TBI - a preliminary study (IV)

The HISC and GOS-E values did not differ significantly between the the apoE4+ and apoE4- patients, although the HISC scores tended to be poorer in the apoE4+ group. Thus, there were no significant association between the apoE genotype and outcome. The GCS and PTA did not correlate with the HISC values, but PTA correlated significantly with the GOS-E values (r= -0.458, p=0.007).

Nineteen patients had traumatic lesions on MRI. The number of contusions correlated significantly with the HISC (r=0.669, p=0.003), and GOS-E scores (r=-0.665, p=0.004). The number of other intraparenchymal lesions was significantly correlated with the HISC scores (r=0.723, p=0.008), but not with the GOS-E. In a multiple linear regression model, the number of intraparenchymal lesions was the only significant predictor of HISC (p=0.001), indicating an association between the presence of TAI and poor outcome. No significant association was found between apoE genotype, GCS, PTA, number of contusions and the HISC score. The results remained significant after adjustment for age at injury (R square (intraparenchymal lesions) = 0.764, B=1.078; p=0.019). The only significant explanatory variables for GOS-E were PTA (R square 0.253, B=0.478; p=0.018) and number of contusions (R square 0.557, B = 0.157: p=0.014). All the other above-mentioned explanatory variables remained insignificant for GOS-E. In this study, the duration of PTA and acute MRI were the best predictors of one-year outcome in TBI.

6. DISCUSSION

6.1 Material and methods

6.1.1 Subjects (studies I,II)

Our material was clearly selected, as the patients were drawn from a group of patients referred on a clinical basis for neurological and neuropsychological evaluation. The reason for the referral was either a recent injury or significant disability after an earlier injury. Thus, the subjects have probably had more symptoms and disability than individuals with TBI in general. It is unlikely that fully unselected TBI materials can be collected. Moreover, such a material might not even be clinically relevant because most patients have mild injuries without long-term sequences. We believe that our population represents well a spectrum of patients with TBI and long-lasting symptoms, but due to the selection bias, our results cannot be generalized to all TBIs.

The representativeness of the study groups was tested in terms of age, gender, education, and severity of TBI. The representation analyses were restricted to the same age range as in the study group (age at TBI younger than 53 years). The only significant differences between the groups were that the deceased subjects were older and less well educated compared to the other groups. The characteristics of the present subjects were not essentially different from TBI populations in general, as the majority of the subjects were men, and in early adulthood at the time of injury.

Other weaknesses in our study include the relatively small material and the uncertainty in assessing TBI severity. Although the size was moderate, it proved to be small for subgroup analyses. At the time of the collection of this material, neither the Glasgow Coma Scale nor assessment of PTA was in clinical use. Thus, we were forced to use a retrospective assessment of trauma severity by using all obtainable data. The reliability of the retrospective assessment of TBI severity may have been affected by patients` memory disturbances, inadequately maintained hospital records in the 1960s, and the lack of systematic brain imaging technology at that time. Because of this and the still persisting variability in classifying TBI severity using PTA, the association of our results with TBI severity must be regarded with caution.

A systematic bias is a well-known problem in studies on the outcome of TBI. At one-year follow-up, about 40% of the subjects were lost, and at two-year follow-up, about 60% were lost (Corrigan et al. 2003). In follow-up studies of TBI patients among individuals lost to follow-up, three categories have been described: persons from socioeconomically disadvantaged groups, persons with a preinjury history of substance abuse, and persons injured because of self-or other-directed violence (Corrigan et al. 2003).

The main weakness of our study I is the lack of controls. We were unable to collect retrospective controls because the MRI equipment was changed soon after our patients were selected, thus making impossible a reliable comparison. Consequently, we could not analyze whether our patients with TBI showed more atrophic changes than healthy controls. However, the main point was to evaluate the effect of apoE4, and the lack of controls does not prohibit evaluating its effect among patients with TBI. In fact, studies have been done in a normal population where the effect of apoE4 on developing hippocampal atrophy with age has been studied. As these studies have shown apoE4 to predispose to hippocampal atrophy in a normal population, it gives even more weight to the lack of such a connection in our patients with TBI, especially as the majority of our apoE4+ patients did not show any sign of atrophy.

In study II, the lack of an age-matched control group is the most important shortcoming. Such a comparison would have given a significantly more reliable answer to whether the time profile in cognitive performance is similar in patients with TBI and in controls in relation to the apoE genotype.

6.1.2 Subjects (studies III,IV)

We wanted to study the outcome of TBI patients in an unselected population (excluding the most severely injured patients who were unable to participate in the examination one week after the trauma, and trivial injuries) because predicting their outcome is the most challenging. In unselected material, where most injuries are mild, many patients fail to show visible lesions with conventional MRI sequences. Generalization of the present results is restricted by the small sample size.

6.2 Hippocampal volume, brain atrophy, and apoE genotype after TBI (I)

To our knowledge, there are no earlier studies specifically evaluating the association between apoE polymorphism with the degree of hippocampal and global brain atrophy in TBI partients. Hippocampal or lateral ventricle volumes did not differ significantly in patients with apoE ε4 compared to those without this allele. Even after multivariate analysis the results remained insignificant. Our study suggests that the presence of apoE ε4 in patients with TBI does not have a major influence on the development of atrophic changes, either in the hippocampus or in general. If the apoE ε4 allele is associated with an unfavourable outcome after TBI, this association may involve mechanisms other than those responsible for the development of brain atrophy. The results do not support a connection between apoE genotype and AD-type atrophic changes in TBI patients. We aimed to clarify in particular the long-term effects of TBI on brain structure, and to relate these to the apoE genotype. If a significant TBI were a risk factor for the development of AD in later life involving the predisposing effect of apoE ε4, one would

expect to find at least some of the following long-term consequences: a) development of more hippocampal atrophy in patients with apoE ε4, at least when adjusted for trauma severity; b) more signs of general brain atrophy in patients with apoE ε4, after adjusting for trauma severity; or c) a positive interaction between trauma severity, apoE ε4, and other demographic variables to predict long-term structural changes.

It may be that our material was too small or selected to show these connections, or that such connections after TBI do not exist. The former explanations remain possible but the significance in our material was fairly low, and our patients represented rather a wide range of TBI. Moreover, the patients seemed to be unselected according to the distribution of the apoE genotypes compared to the general Finnish population, where 38.7% have the ε4 allele (Ehnholm et al. 1986).

We cannot rule out that the interaction between TBI and apoE4 could lead to hippocampal or global atrophy, especially in certain types and severities of TBI. Indeed, our earlier studies show that only a portion of the apoE4+ patients developed long-term cognitive decline, and in these patients the global atrophy was significant (Koponen et al. 2004, Himanen et al. 2006). As most of these patients had grade 2 to 3 contusions, and most of them also severe injuries, it is uncertain whether the presence of apoE4 had any effect on the development of atrophy, or whether it was mainly determined by trauma-related factors, such as the contusion volume and severity of TAI, as largely suggested by the multivariate analysis. However, as all patients developing incident or manifest dementia were apoE4+, our results are in accordance with an earlier report suggesting synergistic effects between TBI and apoE4 for the development of dementia (Mayeux et al. 1995). It should be noted that without neuropathological examination it is difficult to determine whether the patient has posttraumatic atrophy and generalized cognitive decline superimposed by an aging effect, or an ongoing AD process.

The association between TBI and AD has been criticized, and some analyses have suggested that TBI does not predispose to AD but may hasten its development to a clinically evident stage (Nemetz et al. 1999, Fleminger et al. 2003). Our study may be seen to support this view. The undisputedly strong predisposing effect of apoE4 on the development of AD (Farrer et al. 1997) did not receive any additive features from coexisting TBI in our material, if hippocampal or general atrophy is thought to be a sign of, or to predispose to the development of AD. According the cognitive reserve theory (Satz et al. 1993), all processes diminishing the reserves of the brain may hasten the appearance of dementia in later life, and significant TBI may have a strong influence on these reserves. A possible explanation for these and our earlier results is that the apoE4-related long-term cognitive decline appears through increasing cholinergic hypofunction (Kleifeld et al. 1998, Champagne et al. 2005), and not by premature neuronal loss. The relationship between dementia after TBI and apoE status is still ambiguous. In contrast to

the studies demonstrating an association between apoE ε4 and an unfavourable outcome following TBI, a number of recent studies have failed to support such findings (Guo et al 2000, Plassman et al 2000, Jellinger et al 2001, Chamelian et al. 2004, Ponsford et al. 2007, Willemse-van Son et al. 2008). Therefore, there is no conclusive evidence linking apoE genotype with the development of AD following TBI (Jellinger 2004). Further prospective human clinicopathological and experimental studies are warranted to clarify the relationship between TBI, apoE genotype and AD. Moreover, the exact mechanism by which apoE genotype influences outcome is unclear.

6.3 Outcome of TBI after three decades -relationship to apoE genotype (II)

The main finding of the present study was that those with an apoE ε4 allele showed a significantly lower cognitive level three decades after TBI than patients with the other apoE genotypes. The Mild Deterioration Battery (MDB) was used to describe the overall cognitive functioning of the subjects (Portin et al. 2001). This method has earlier been applied in several studies (e.g. Kujala et al. 1997, Polo-Kantola et al. 1998). The apoE ε4 allele increased the risk of poorer overall cognitive presentation, but not of poorer subjective memory, well-being or ADL functioning. The apoE genotype was not associated with physical or vocational outcomes, either. However, this cognitive decline was wholly caused by a subgroup of the apoE4+ patients, who developed subclinical or clinical dementia during the follow-up.

Although some degree of recovery is a general rule after TBI, other studies also suggest that some patients may actually show a steady decline even at a younger age (Teasdale et al. 1997, Kesler et al. 2003, Teasdale et al. 2005). Our results suggest that this decline is largely restricted to the apoE4+ patients, or to a subgroup of them, at least using the outcome measures of this study. Interestingly, in our results, the apoE3 patients showed only a mild cognitive decline during the follow-up, while the apoE2 patients showed no decline at all. This is in accordance with the view that apoE4 increases the risk of poor recovery, while apoE2 is protective and apoE3 intermediate (Horsburgh et al. 2000a).

The poorer outcome of patients with apoE4 after TBI may be related to the deposition Aβ-protein (Graham et al. 1999b). However, it is still unclear whether TBI really increases the risk of AD by, e.g. triggering a process in which Aβ accumulates in the brain, or whether the connection between TBI and AD evolves only from the lower cerebral reserves after TBI, thus causing the earlier appearance of eventual AD (Nemetz et al. 1999). Our study does not give an answer to this, but suggests that those TBI patients possessing the apoE ε4 allele may have poorer long-term cognitive outcome. Because our study lacks a 30-year follow-up of normal controls, it is impossible to say whether this age-related decline in the apoE4+ patients merely reflects the earlier appearance of cognitive aging phenomena, or whether it represents a more specific TBI-related process. The prevalence

of dementia in those patients who had reached 65 years of age was 30 % in this material, which is many times higher than that found in random population samples of the same age group in Finland (Juva et al. 1993, Hänninen et al. 2002). This supports the view that TBI has contributed to the appearance of dementia.

The fact that the apoE4+ patients did not differ from the others in CERAD scores thirty years after the injury does not support the view that the apoE ε4 positive TBI patients are generally at special risk of developing AD. Indeed, as the latest results both in AD and in patients with multiple sclerosis suggest, the role of the apoE4 seems to predispose to a disease-related decline rather than to the disease itself (Enzinger et al. 2004, Khachaturian et al. 2004). On the other hand, all our patients fulfilling either of the criteria for subclinical or clinical dementia were apoE4 positive. Without neuropathological confirmation, it is impossible to say whether these patients had an Alzheimer's disease or whether the DSM-IV criteria were fulfilled due to the combined effect of TBI and ageing.

Very interestingly, the cognitive decline in the apoE4+ patients was wholly restricted to those patients developing subclinical or clinical dementia. Indeed, the majority (68 % of apoE4+ patients) did not show any cognitive decline during the 30-year follow-up, and all their CERAD subtests were actually non-significantly better than, e.g. in the apoE3 patients. The fact that 32 % of the apoE4+ patients had developed significant cognitive decline by the mean age of 72.7 years is not especially different from what is expected in the general population (Raber et al. 2004), at least when considering that most of these patients definitely had a low cognitive level already before their seventies because of the former TBI, and possibly also because of their low level of education. It is actually more interesting that the combined effect of apoE4 and TBI does not cause any long-term cognitive decline in the majority of these patients. However, it should be noted that these patients who maintained their cognitive level were on average 56.2 years at follow-up, so it may still be possible that they will develop significant cognitive decline during the next fifteen years. In this material, the combination of apoE4+ and poor cognitive level at baseline were strong predictors of old-age dementia.

It is necessary to compare our results to the study of Millar et al. (2003) which dealt with the very same issue altogether. 396 TBI patients were reassessed at a mean of 18 years after the TBI. Their analysis could not find a statistically significant association between outcome and apoE genotype. There are several possible explanations for the seemingly discordant result compared with the present study. First, they used slightly different methods to measure the outcome. As seen in the present study, only a certain combination of cognitive measures – the MDB – showed a significant association. This suggests that if the apoE genotype affects long-term brain functioning, not all processes are similarly affected; there may be certain functions that are selectively vulnerable to the detrimental effect of apoE4, at least in TBI patients. Indeed, this view has got support

from both human (Crawford et al. 2002) and animal studies (Kleifeld et al. 1998). Secondly, their patient material included mainly severe TBIs, where a lower remaining reserve might not allow the effects of apoE to become evident. However, although in our material the apoE4-positive patients had, on average, the mildest injuries, those six patients who developed a clear decline had mainly severe injuries. Thirdly, their patient material was also markedly younger at the time of the follow-up study, which may mean that the eventual combined effect of ageing and apoE4 on the cognitive performance after TBI could not yet be detected.

Our study has couple of clear advantages. First, this is a truly long follow-up study after TBI, which included the effect of apoE genotype and other parameters in multiple logistic models. The possible association between TBI and AD is most reliably assessed with long-lasting follow-up protocols. The second advantage of our study is that the evaluation of cognitive functioning using a sensitive and wide neuropsychological test battery was done both at baseline and at follow-up.

6.4 MRI changes and apoE genotype - a prospective one-year follow-up of TBI (III)

ApoE has been implicated in modifying neurological outcome after TBI, although the mechanisms by which this occurs remain poorly defined. Most of the published literature supports the view that the ε4 allele of apoE increases the risk of poor outcome during the first six months after TBI (Zhou et al. 2008). Early MRI is important for the detection of traumatic lesions. Both the number and extent of lesions diminish significantly with time (Brandstack et al. 2006). Our study suggests that the presence of apoE4 in TBI patients does not have a major influence on the development of MRI changes during the first year. The findings of this prospective study on acute injuries are in agreement with our earlier study, suggesting that the processes responsible for the development of atrophy or for the repair of visible lesions are not dependent on apoE. Our results must be viewed with caution, bearing in mind the relatively small sample size. Also, in unselected material, where most injuries are mild it can be expected that many patients fail to show visible lesions with conventional MRI sequences. Our findings merit further confirmation with a larger sample size, in only patients with severe injuries, and with new imaging techniques.

6.5 Association of injury severity, MRI results and apoE genotype with one-year outcome in mainly mild TBI (IV)

The assessment of TBI severity is complicated, because of its complex pathophysiology, and because multiple variables affect the outcome. Hitherto, the GCS score and duration

of PTA have been the main indices of TBI severity. However, we still do not sufficiently know which phenomena of brain physiology these variables reflect, or whether they may be caused by variable pathologies. Therefore, there is a major need for reliable markers of TBI severity. This is especially true for "mild" injuries, which constitute the vast majority of all TBIs. A reliable marker would also be helpful to solve the medico-legal problems that often arise when neuropsychological impairment occurs after a seemingly mild traumatic event.

GOS is the most widely used method for assessment of outcome in TBI patients (Teasdale et al. 1998). It is used to rate a patient`s overall outcome, taking into consideration cognitive and physical impairments, as well as disability in everyday activities, whereas HISC has been used to describe the overall subjective symptoms. We wanted to evaluate the outcome taking account of both these aspects, so instead of GOS, we used the extended version of the scale (GOS-E) because patients with a mild or moderate head injury in general end up with good recovery or moderate disability, and this scale describes this upper range of outcome in more detail.

Early MRI is important for the detection of traumatic lesions. Both the number and extent of lesions diminish significantly with time (Brandstack et al. 2006). The use of acute MRI in investigating the TBI pathology offers clear advantages over the use of CT alone, particularly in regard to the visualization of basal lesions and TAI. In accordance with many earlier studies (Paterakis et al. 2000, Hughes et al. 2004), we found a significant correlation between the acute MRI findings and outcome, especially concerning the TAI lesions.

The presence of the apoE ε4 allele may have a negative effect on response to injury and recovery in patients with TBI. In this study, we did not find such a connection. Perhaps our sample size was too small to show the connection, or else the methods used to measure the outcome were unable to reveal it. However, our results are consistent with other recent findings suggesting the need for caution in accepting the hypothesis that apoE status is a factor in recovery from TBI (Millar et al. 2003, Chamelian et al. 2004, Teasdale et al. 2005, Ponsford et al. 2007, Willemse-van Son et al. 2008).

Due to the complex interaction of various factors in determining the final TBI outcome, a multivariate analysis is necessary to give more reliable estimates of the role of various explanatory variables. Therefore, we compared the predictive values of apoE genotype, GCS, PTA, and MRI findings using multiple regression analysis. After adjusting for age, only the presence of TAI lesions and duration of PTA were predictive of the one-year outcome. The presence of TAI lesions explained about 76 % of the variance in the outcome scores using the HISC, while duration of PTA explained 52 % of the variance using the GOS-E. These results suggest that the duration of PTA is the best predictor of global functional outcome after TBI, whereas the presence of TAI lesions in acute

imaging may better predict the overall subjective symptoms. In this material, apoE genotype and GCS were not found to predict the outcome, which at least in the case of GCS, probably stems from the small number of subjects.

The implications of these results are important, since they, like the findings of many other studies (Alexandre et al. 1983, Bishara et al. 1992, Ellenberg et al. 1996), suggest that the dominating role of GCS in assessing TBI severity should be questioned, at least concerning long-term outcome. We claim that many cases of poor recovery from a seemingly mild TBI are due to misinterpretations of the actual severity when using only GCS as a measure. If the duration of PTA is not properly assessed and the presence of TAI lesions is missed due to late imaging or insensitive techniques, the real nature of the injury may be underestimated.

Reliable outcome prediction of TBI remains difficult despite major progress in cerebral monitoring and imaging techniques. Hence, there is a need for supplementary tests to enable early prediction, both to select appropriate management strategies and to determine the need for prolonged follow-up. Our study supports the view that an early MRI has a key role in assessing the injury severity and prognosis. The more detailed information offered by MRI, especially with the newest more sensitive imaging techniques such as diffusion-weighted imaging, diffusion tensor imaging and susceptibility-weighted imaging (Arfanakis et al. 2002, Babikian et al. 2005, Ezaki et al. 2006), particularly in patients with relatively normal CT, may be a more reliable tool with which to manage the patients and communicate more accurately with their families. MRI allows the detection of diffuse white matter brain damage or axonal brain injury with a much greater accuracy than conventional CT. Most MRI studies have failed to identify consistent relationships between the pattern of neuropsychological impairments and the site of focal structural lesions, again suggesting the importance of diffuse damage for the outcome.

A combination of clinical and imaging variables seems to be the best approach to predict the outcome in mild as well as more severe TBI. The combined use of multiple predicting variables probably more accurately reflects the multifactorial pathophysiology that leads to the development of posttraumatic neuropsychological and neuropsychiatric impairment (Boake et al. 1996). Generalisation of the present results is restricted by the small sample size, which is why these findings should be replicated in larger trials. Because of the relatively homogeneous sample population (mostly mild TBIs), the lack of variability may have limited the power of the analyses. Moreover, the prognostic role of MRI may be largely determined by the sequences used.

7. SUMMARY AND CONCLUSIONS

1. The apoE ε4 was not associated with the development of hippocampal or ventricular atrophy after TBI. If apoE ε4 allele is associated with an unfavourable outcome after TBI as proposed, this association may involve mechanisms other than those responsible for the development of brain atrophy.

2. After three decades, TBI patients with the apoE ε4 allele showed significantly poorer general cognitive level than those without this allele. This decline was wholly accounted for by a subgroup of these patients who had developed incident or clinical dementia, while the majority of the apoE ε4 positive patients showed no decline at all. The other outcome measures describing vocational, physical, or subjective symptom outcome did not show significant relationships with the apoE genotype. A portion of the TBI patients with the apoE ε4 allele seem to be at risk of long-term cognitive decline.

3. The presence of apoE ε4 was not associated with MRI changes during the first year after TBI. This suggests that if the apoE ε4 is associated with an unfavourable outcome after TBI, the processes responsible for the repair of visible lesions are not dependent on apoE genotype.

4. In multivariate models, the duration of PTA and acute MRI were the best predictors of one-year outcome in TBI, whereas the prognostic values of GCS and apoE were modest. The dominating role of GCS in assessing TBI severity should be questioned.

Most published literature supports the view that the apoE ε4 allele adversely affects outcome after TBI in the first six months post injury. Its effects over a much longer period are less clear. In general the populations studied have been small. The assessment of outcome has been variable, in terms of both measurement tools and time of assessement, making comparison of different studies difficult. Early MRI is important for the detection of traumatic lesions. Both the number and extent of lesions diminish significantly with time. The findings of our prospective study on acute injuries are in agreement with our earlier study, suggesting that the processes responsible for the development of atrophy or for the repair of visible lesions are not dependent on apoE. These findings merit further confirmation with a larger sample size and with new imaging techniques. Future research will continue to elucidate the pathophysiology of apoE in neuronal repair. As clinical studies continue, more information may be obtained about the ability of the apoE genotype to predict long-term outcome in brain injury. In the case of TBI, we may perhaps be better able to prognosticate functional recovery and survival.

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Heli Hiekkanen

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