Cytokine gene polymorphisms and outcome after traumatic brain injury

[1]Ryan J. Waters

[2]Gordon D. Murray

[3]Graham M. Teasdale

[3]Janice Stewart

[4]Ian Day

[2]Robert J. Lee

[5]James A. R. Nicoll

[1]Wessex Neurological Centre,
University Hospital Southampton NHS Foundation Trust
Southampton
UK

[2]Centre for Population Health Sciences
University of Edinburgh Medical School
Edinburgh

[3]Institute of Neurological Sciences
Southern General Hospital
University of Glasgow
Glasgow
UK

[4]Bristol Genetic Epidemiology Laboratories
School of Social and Community Medicine
Bristol
UK

[5]Clinical and Experimental Sciences
University of Southampton
Southampton General Hospital
Southampton
UK

Correspondence to:

James AR Nicoll
Clinical and Experimental Sciences
Mailpoint 813, Level E
Southampton General Hospital
Southampton
SO16 6YD
UK

J.Nicoll@soton.ac.uk

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Abstract

Clinical outcome after traumatic brain injury (TBI) is variable and cannot easily be predicted. There is increasing evidence to suggest there may be genetic influences on outcome. Cytokines play an important role in mediating the inflammatory response provoked within the central nervous system after TBI. This study was designed to identify associations between cytokine gene polymorphisms and clinical outcome 6 months after head injury.

A prospectively identified cohort of patients (*n*=1096, age range 0-93, mean age 37) was utilised. Clinical outcome at 6 months was assessed using the Glasgow Outcome Scale. In an initial screen of 11 cytokine gene single nucleotide polymorphisms (SNPs) previously associated with disease susceptibility or outcome (TNFA -238 and -308, IL6 -174, -572 and -597, IL1A -889, IL1B -31, -511 and +3953 and TGFB -509 and -800) TNFA -308 was identified as having a likely association. The TNFA -308 SNP was further evaluated and a significant association was identified, with 39% of allele 2 carriers having an unfavourable outcome compared with 31% of non-carriers (adjusted OR 1.67, CI 1.19-2.35, *p*=0.003). These findings are consistent with experimental and clinical data suggesting that neuroinflammation has an impact on clinical outcome after TBI and that TNFα plays an important role in this process.

Keywords: traumatic brain injury, head injury, genetics, cytokines, inflammation

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Introduction

Outcome from head injury is variable and cannot be predicted purely on the basis of clinical features or radiological findings [1,2]. It has been observed that apparently similar patients will have different outcomes despite similar injuries and clinical treatment. Thornhill *et al*. [3] demonstrated that age of over 40 years, pre-existing physical disability and a history of brain illness were independent predictors of poor outcome after mild brain injury. However 35% of patients without any of these factors still failed to achieve a good outcome. This suggests that other features, such as genetic variability, may determine an individual's response to head injury [4].

Clinical outcome after traumatic brain injury (TBI) is in part determined by secondary processes, occurring after the initial injury. A systemic and neuroinflammatory response is provoked after TBI and the magnitude and duration of this response may influence clinical outcome [5] in the short term potentially by promoting neuronal death, oedema and pyrexia and in the long term by influencing repair processes. The response is primarily mediated by cytokines [6] and the production of these cytokines may be altered by variation of the DNA sequence within their genes (gene polymorphisms). Understanding the role of cytokine gene polymorphisms in determining outcome after head injury may have important implications in understanding the neuroinflammatory response after brain injury, for prognosis after injury and possibly future treatment of patients who sustain head injury. To date there is little information attempting to correlate cytokine gene polymorphisms with outcome after TBI and studies that have been performed have been of small cohorts [7,8,9,10].

In this study we screened a panel of cytokine gene polymorphisms in a much larger patient cohort than has previously been published. A single nucleotide polymorphism

(SNP) is a variation between people in the DNA sequence at a single nucleotide position in a particular gene. Definition of the SNP includes whether it is within the coding sequence of the gene (designated +) and is therefore in a position to potentially alter the amino acid sequence of the protein encoded by the gene or is in the promoter region of the gene (designated -) which regulates gene expression and therefore could potentially alter the amount of protein produced. The specific nucleotide position relative to the start of the coding sequence of the gene is denoted by a number (e.g. -308, +3953). The nucleotide base variation is indicated (e.g. G/A means G or A) and the commoner of the two variants is termed allele 1. Each individual inherits one copy of each gene from either parent and therefore may have one of 3 possible genotypes (1.1, 1.2 or 2.2). A specific reference number identifies the entry in the online SNP database hosted by the US National Center for Biotechnology Information (e.g. dbSNP ID 1800629). Eleven SNPs in 4 cytokine genes were identified from the literature as having a potential influence on the neuroinflammatory response to acute brain injury as follows.

Tumour Necrosis Factor-α (TNF-α, protein; TNFA, gene) is a classical pro-inflammatory cytokine which plays an important role in initiating and controlling the inflammatory response. In the central nervous system (CNS) TNF-α is produced primarily by microglia and astrocytes. TNF-α is known to be upregulated in the CNS after TBI [11,12,13] and in clinical studies raised levels of TNF-α in plasma and cerebrospinal fluid have been demonstrated in patients after TBI [14,15]. Experimental studies have shown evidence that TNF-α may both exacerbate neuronal injury and be neuroprotective. For example, studies of TNF knock out mice have shown that deficiency of TNF-α is beneficial early after trauma but causes deleterious effects in the long term [16]. In human studies variation in the TNF-α promoter region has been associated with susceptibility to cerebral malaria [17], death from septic shock [18] and meningococcal disease [19]. The -308 (G/A; dbSNP ID 1800629) and -238 (G/A,

dbSNP ID: 361525) SNPs within the promoter region of the TNFA gene have been most extensively explored and therefore were investigated in this study.

Interleukin-1 (IL1), acting together with TNF-α, has a major role in the production of inflammation and the generation of pyrexia. The IL1 family consists of two agonists of the IL1 receptor (IL1α and IL1β) and an antagonist (IL1ra). Microglia are the main source of IL1 in the CNS and there is rapid IL1 upregulation after acute CNS injury, including TBI [20], with evidence that it exacerbates neuronal damage [21]. Four IL1 SNPs have been shown to influence transcription and/or have been associated with human CNS disease [22,23,24] and were therefore included in this study: IL1A -889 (C/T; dbSNP ID 1800587), IL1B -31 (C/T; dbSNP ID 1143627), IL1B -511 (G/T; dbSNP ID 16944) and IL1B +3953 (C/T; 1143634).

Interleukin-6 (IL6) plays an important role in the acute phase response. IL6 production, in the CNS primarily by microglia, is stimulated by TNF and IL1 and high levels have been associated with better outcome in human TBI [25,26]. SNPs identified in the promoter region of IL6 influence transcription through complex interactions [27] and therefore the following SNPs were included in this study: IL6 -174 (G/C; 1800795), -572 (G/C; 1800796) and -597 (G/A; 1800797).

Transforming Growth Factor (TGF) -β has a role in the CNS in cell proliferation, differentiation and inflammation. Microglia and astrocytes are the main source of TGF after injury and it has a mainly anti-inflammatory action, limiting microglial activation and having neuroprotective effects. Two SNPs located in the promoter region are associated with higher concentrations of TGF in the plasma and therefore were included in this study: TGFB -509 (C/T; 1800469) and TGFB -800 (G/A; 1800468).

Using a previously identified large cohort of patients who had sustained TBI [28] we have addressed the hypothesis that cytokine gene polymorphisms, potentially by altering the magnitude and duration of the neuroinflammatory response, influence clinical outcome at six months post head injury.

Methods

Sample

The participants in this study were recruited from consecutive head injury admissions to the regional Neurosurgical Unit for the West of Scotland at the Institute of Neurological Sciences, Glasgow from 1996 to 1999. Appropriate consent was obtained and the patients were originally entered into a study of the relationship between *APOE* genotype and outcome after head injury and details of this cohort are as outlined previously [28]. Consent for inclusion in the study had been obtained from the patient's next of kin or carer and a buccal swab sample was taken for genotyping. In patients who died rapidly or if no responsible person was available a buccal swab was not performed but the residue of a blood sample which had been taken for routine clinical analysis was stored and analysed subsequently after consent had been obtained. Survivors were invited to approve use of clinical outcome data at late follow up. The ethical committee gave permission for anonymised genotyping for the small number of patients who were not able to give consent and who had no responsible relative.

Information was extracted from the case notes concerning the patient's demographic features, age, cause of injury, clinical severity of brain damage in the acute stage indicated by the Glasgow Coma Scale [29]and pupil reaction. CT scan findings were according to the scheme of Marshall et al (1991) [30]. Operative findings were obtained

from the clinical records. Six months after injury patient outcome was assessed by the Glasgow Outcome Scale.[31,32]

Ethical approval had been obtained from the Local Research Ethics Committee of the Southern General Hospital, Glasgow, at the time of the original study. It was also agreed, and patient consent obtained at the time, that further genetic testing could be performed on the cohort at a later date for other genes of potential relevance to outcome. Further ethical approval was obtained for this study from both the Southampton and South West Hampshire and the Southern General Hospital Local Research Ethics Committees.

Genotyping

Genotyping was performed on buccal swabs or blood samples. Collection and preparation of the buccal swabs was performed as detailed previously [33]. In order to perform multiple SNP assays on the limited amount of DNA available a pre-amplification step was utilised using the GenomiPhi kit (GE Healthcare). This utilises the bacteriophage Phi29 polymerase to exponentially amplify linear DNA template by strand displacement [34].

The 11 SNPs in 4 cytokine genes chosen for investigation for the reasons stated above were: TNFA -238 (G/A;dbSNP ID: 361525) and -308 (G/A; 1800629), IL6 -174 (G/C; 1800795), -572 (G/C; 1800796) and -597 (G/A; 1800797), IL1A -889 (C/T; 1800587), IL1B -31 (C/T; 1143627), -511 (G/T; 16944) and +3953 (C/T; 1143634) and TGFB -509 (C/T; 1800469) and -800 (G/A; 1800468). Genotypes were determined using fluorescence-labelled oligonucleotide melting from matched or mismatched target, monitored in an Idaho Technology (Salt Lake City, Utah, USA) 384-well Odyssey. Detection utilised reduction of opposed G-base quenching of

fluorescence during a thermal ramp. PCR was performed on 5µl GenomiPhi amplified template for each sample. The PCR reaction mix consisted of 1x PCR buffer (Promega), 200 µM dNTPs (Promega), 100nM forward/reverse primer, 500nM reverse/forward primer, 200nM FITC-labelled probe, 200nM DABCYL-labelled probe, 1.5/2.0mM MgCl and 0.4 units of *Taq* polymerase (Promega) per reaction. Heat cycling was performed on an MJ Research PTC-225 DNA Engine Tetrad® (Genetic Research Instrumentation) using a protocol of 94ºC for 2 minutes, then 50 cycles of 94ºC for 20 seconds, the appropriate annealing temperature for 30 seconds and 72ºC for 30 seconds, followed by a final 2 minutes at 72ºC. After thermal cycling the samples were overlaid with 5µl Chill-Out™ wax (Genetic Research Instrumentation) to prevent evaporation during analysis. Analysis was carried out in a 384-well Odyssey (Idaho Technology, Salt Lake City, Utah, USA). Samples were melted from 35°C to 70°C. LightTyper software (Roche Diagnostics Ltd) was used to analyse the fluorescence change during melting. Results were then manually checked using in-house software.

Based upon results from this screen (described below), genotyping for the TNFA -308 SNP (rs1800629) was performed using a PCR protocol similar to that used previously for APOE genotyping of this cohort [28], except that primers for PCR were used which span the -308 region of the TNFA promoter (forward: 5'-aggcaataggttttgaggggcat-3' and reverse: 5'-tcctccctgctccgattccg-3'). The PCR products were then digested with the restriction enzyme Nco 1 giving fragment sizes of 87bp and 20bp [35]. The digestion products were separated according to size by polyacrylamide gel electrophoresis, stained with ethidium bromide and viewed and photographed by u.v. transillumination.

Analysis

Clinical outcome at 6 months was determined using the Glasgow Outcome Scale (GOS). Outcome was then dichotomised into unfavourable (death, vegetative state, severe disability) or favourable (moderate disability or good recovery). This approach had been used when this cohort was analysed in respect of potential association with APOE genotype [28]. Secondary complications were assessed according to their presence or absence: 'seizures' were defined as seizures requiring treatment at any stage during admission, 'raised ICP' was raised intracranial pressure requiring treatment during admission and 'infection' was life-threatening infection at any point during admission. The proportions experiencing each complication in the non-carriers and carriers of TNFA -308 allele 2 were compared using Pearson Chi-Square test. Logistic regression analysis was performed using the covariates of age (modelled as a continuous variable using cubic smoothing splines), GCS motor response categorised into 4 groups, pupil reactivity categorised into 3 groups, initial CT scan findings into 3 groups, traumatic subarachnoid haemorrhage, hypoxia, hypotension and APOE genotype (presence or absence of the ε4 allele). Statistical analyses were performed using SAS 9.2 for Windows.

Results

Association between cytokine gene SNPs and outcome at 6 months

The combination of the multi-SNP assay methodology and the available DNA samples (derived mainly from buccal swabs) emerged as suboptimal for this type of analysis and the results were therefore treated as a preliminary screen. There was variability in terms of the number of samples successfully typed, ranging between 90.8% (TNFA -238) and 55.4% (IL6 -174). Overall 746 samples (68.8%) had 9 or more of the 11 results available. The relationship (unadjusted) between SNP genotype and outcome at 6 months was assessed for each of the 11 cytokine SNPs. In the initial screen, no SNPs reached statistical significance at the 0.05 level,

although the TNFA -308 and TGFB -800 polymorphisms were: -308; *p*=0.07, -800; *p*=0.1. Based upon this preliminary screen, the TNFA -308 SNP was then selected for a more detailed analysis using PCR methodology which was known to be reliable when applied to these samples [28] (see below).

Association between cytokine gene SNPs and secondary complications

Associations (unadjusted) between the cytokine SNPs and secondary complications were explored. Possession of at least one copy of the rarer T allele of IL1A -889 showed a weak association with increased seizure occurrence (CI 0.479-0.999, *p*=0.049). There was also evidence of an association with raised intracranial pressure and: the rarer alleles at the –889 position in IL1A (*p*=0.01) and +3953 in IL1B (*p*=0.027). IL1B -31 was associated with serious infection occurring after TBI (*p*=0.027) with the rarer C allele associated with a lower likelihood of infection.

Association between the TNFA -308 SNP and Glasgow Outcome Scale at six months

After repeating and extending TNFA -308 SNP typing with the Nco1 restriction digest method there were 965 patients in the initial cohort identified who had an acute head injury, were included in the original APOE study, consented, and had both APOE genotype and TNF -308 SNPtype available.

Twenty eight patients for whom GCS motor response was recorded as untestable either on admission to first hospital or on admission to study hospital (n = 1), pupil reactivity was not available either on admission to first hospital or on admission to study hospital (n = 21), or CT scan was not done (n = 6) were excluded.

The remaining 937 patients had missing values for traumatic subarachnoid haemorrhage on admission CT scan (n = 7), hypoxia in first 24 hours (n = 73), hypotension in first 24 hours (n = 72) and values for these were imputed.

TNFA genotype was 1.1 for 595 (63.5%), 1.2 for 298 (31.8%), and 2.2 for 44 (4.7%) of the 937 patients (Table 1).

The characteristics of patients who were TNFA -308 allele 2 carriers were similar to non-carriers with respect to age, gender, initial GCS (reflecting the severity of injury), CT characteristics of the injuries, presence of hypoxia and hypotension and APOE ε4 allele carriage (table 2).

In our previous studies investigating APOE genotype, outcome was dichotomised into unfavourable (death, vegetative survival or severe disability at 6 months) and favourable (moderate disability or good recovery) [28,36]. Analysed in this manner, TNFA -308 allele 2 carriers were more likely to have an unfavourable outcome than non-carriers (39% vs. 31%) (table 3).

The proportion of head injured patients who died was similar for allele 2 carriers and non-carriers (13% vs. 12%). However allele 2 carriers who survived were more likely to have severe disability (25% vs. 18%) and less likely to have a good outcome (37% vs. 43%).

The association between possession of the TNFA -308 allele 2 and poor outcome 6 months after head injury (table 4) strengthens when controlling for age, motor response in the Accident and Emergency department and pupil reactivity (p=0.003) and remains when the analysis includes APOE genotype (p=0.007).

Association between TNFA -308 SNP and secondary complications

Possession of the TNFA -308 allele 2 does not appear to influence the complications of pyrexia during the first 24 hours of admission, raised intracranial pressure during admission or seizure occurrence during admission after TBI. There is a non-significant trend towards a lower likelihood of life-threatening infection in allele 2 carriers during admission after TBI (Table 5).

Discussion

This study is the first to show a significant association between the TNFA -308 polymorphism and overall 6 month outcome after TBI. Thus, TNFA -308 allele 2 carriers were more likely to have an unfavourable outcome compared with non-carriers. There were also relatively weak associations between SNPs in other cytokine genes and some of the secondary complications of head injury: IL1A -889 and seizures; both IL1A -889 and IL1B +3953 and raised intracranial pressure; and IL1B -31 and severe sepsis.

The strengths of a study of this nature in this cohort have previously been outlined [28]. These include the large number of subjects in the cohort, prospective recruitment from consecutive admissions to a regional centre and follow-up achieved in a very high proportion of initial participants.

The association between TNFA -308 allele 2 and poor outcome may reflect that TNFα plays a pivotal role in the initiation and control of the inflammatory response after TBI [37,38]. TNFα has been shown to be upregulated in the CNS after TBI [11,12,13,39] and raised levels of TNFα have been demonstrated in plasma and cerebrospinal fluid after TBI [14,15]. The TNFA -308 polymorphism sits within the promoter region of the gene and alters transcription, allele 2 being associated with significantly higher

expression of TNFα [40,41]. Association between the TNFA -308 polymorphism and outcome after TBI is therefore biologically plausible as the genetic variation is in a position to influence expression of TNFα in response to a stimulus such as TBI. Experimental studies show that TNFα has a role in mediating neuronal death in the acute phase and in promoting neuronal repair and plasticity in the longer term [42,43], with differential effects according to activation of specific TNFα receptor subtypes [44,45], so TNFα could influence outcome in both the short and longer term after human TBI in potentially complex ways. A number of groups have investigated the relationship between cytokine protein measurements, including TNFα, in the CSF with outcome after TBI in relatively small cohorts without demonstrating evidence of a correlation between high TNFα levels and poor outcome [46,47]. However, it has been shown that serum and CSF TNFα, correlate with the occurrence of the secondary complications of intracranial hypertension and decreased cerebral perfusion pressure [48,49]. Alterations in other cytokines have also been studied after TBI with increases in IL-1β and IL-6 correlating with poor outcome [50] or without a clear relationship to outcome [51].

The relationship between levels of TNFα in the CSF, which is obviously relatively accessible for sampling, and the timecourse and localisation of its production and utilisation in the brain in response to the multiple primary and secondary forms of pathology that occur after TBI is currently unclear and may not be a simple relationship. In post mortem human brain tissue both TNF mRNA and protein are detectable within a few minutes of TBI [52] whereas the rise in TNF in the CSF is delayed and sustained over a matter of days to weeks [38]. Despite these complexities, the results of this study generate the simple hypothesis that carriers of TNFA -308 allele 2 have increased expression of TNFα in response to TBI which in turn is responsible for their worse outcome. Further studies of patients with TBI genotyped for the TNFA -308 polymorphism, involving measurement of serum, CSF,

and ideally brain [39,53] levels of TNFα and clinical follow up would be required to test this hypothesis.

Although the association between the TNFA –308 SNP and six-month outcome after TBI has not previously been demonstrated, other studies have implicated the TNFA –308 SNP as having a role in other conditions in which neuroinflammation is important, such as Alzheimer's disease [54,55] and the risk of lacunar infarction within the brain [56]. However, some have expressed scepticism in the utility of single gene association observations, including specifically TNFA –308 [57]. The gene sits within the MHC cluster on chromosome 6, and is closely related to the lymphotoxin-α and -β genes. The probability is that TNFA SNPs form part of a haplotype that stretches across these closely related genes, and therefore it is difficult to ascribe an apparent association to a single causative SNP within this region [58]. Further genetic studies would be required to identify haplotype associations within this extended region to delineate the role of other SNPs within these closely related genes.

Some previous relatively small studies have explored cytokine polymorphisms and TBI. Uzan and colleagues [8] identified a possible association between the –511 and +3953 SNPs within the IL1β gene and outcome after head injury. The study was small, involving 69 patients admitted to a neurosurgical unit after TBI. Fourteen out of twenty-five patients (56%) with the +3953 T allele had a poor outcome compared with only 8/44 (18.1%) in those patients who did not possess the T allele. Twenty out of twenty eight patients (71.4%) with the -511 T allele had a poor outcome compared with 2/41 (4.8%) without the rarer allele. These findings have not been confirmed in our substantially larger study and none of the IL1 SNPs studied in this cohort showed an association with overall outcome at six months. This also confirms the lack of an association between the IL1 –889 SNP and overall six month outcome identified in a

small study by Tanriverdi *et al*. [9]. In a further small study (n=151), post-traumatic brain haemorrhage was associated with possession of IL1RN allele 2 [10]. Brain microdialysis and CSF studies have suggested that IL6 may have a neuroprotective effect after severe TBI [25,26] although a previous study of the influence of the IL6 -174 SNP on outcome after TBI did not identify any association [7]. The current study has confirmed the lack of association between three IL6 SNPs and six month outcome after TBI.

In conclusion, of the 11 cytokine gene SNPs investigated in this study the TNFA -308 G/A SNP was identified as having a significant association with outcome at 6 months after TBI. The magnitude of the effect of was small, but points to the need for further research into the role of neuroinflammatory processes in secondary complications and outcome of head injury, including particularly TNFα for which inhibitors are already available [59]. In response to TBI there is a complex upregulation of cytokine gene expression, as demonstrated by multiplex analysis [60], and interactions occur between the different cytokines. This raises the possibility that although any single cytokine SNP has a small effect, possession of different combinations of alleles across the range of cytokine genes may have an additive larger effect. The findings of this study merit confirmation and extension in other large cohorts of patients with TBI.

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Table 1. Distribution of TNFA -308 genotypes and allele carriage rates.

TNFa -308 genotype and allele carriage rates	
1.1 genotype	595 (63.5%)
1.2 genotype	298 (31.8%)
2.2 genotype	44 (4.7%)
Allele 2 carriers	342 (36.5%)
Allele 2 non-carriers	595 (63.5%)
Total	937

Table 2 - Characteristics of acutely head-injured patients by possession of TNFA -308 allele 2

	Non-carriers of allele 2	Carriers of allele 2	All patients
Number (%) of patients	595 (64)	342 (36)	937 (100)
Gender:			
Male	483 (81)	281 (82)	764 (82)
Female	112 (19)	61 (18)	173 (18)
Age (years):			
Mean (SD)	34.9 (21.6)	35.7 (21.6)	35.2 (21.6)
Range	<1 - 87	<1 - 85	<1 – 87
GCS motor response:			
Obeys	343 (58)	215 (63)	558 (60)
Localising	111 (19)	60 (18)	171 (18)
Withdraws or abnormal flexion	71 (12)	27 (8)	98 (10)
Extension or no response	70 (12)	40 (12)	110 (12)
Pupil reactivity:			
Both reactive	530 (89)	298 (87)	828 (88)
One reactive	20 (3)	10 (3)	30 (3)
Neither reactive	45 (8)	34 (10)	79 (8)
CT classification:			
Diffuse injury, NVP	150 (25)	79 (23)	229 (24)
Diffuse injury	235 (40)	127 (37)	362 (39)
Diffuse injury + swelling/shift	45 (8)	21 (6)	66 (7)
Mass lesion	165 (28)	115 (34)	280 (30)
Traumatic SAH:			
No	467 (78)	256 (75)	723 (77)
Yes	128 (22)	86 (25)	214 (23)
Hypoxia:			
No	452 (76)	246 (72)	698 (74)
Yes	143 (24)	96 (28)	239 (26)
Hypotension:			
No	534 (90)	305 (89)	839 (90)
Yes	61 (10)	37 (11)	98 (10)
APOE e4 allele:			
Non-carrier	395 (66)	233 (68)	628 (67)
Carrier	200 (34)	109 (31)	309 (33)

Table 3 - Glasgow Outcome Scale at six months by possession of TNFA allele 2 - number (%) of patients

	Non-carriers of 2 allele	Carriers of 2 allele	All patients
Dead	72 (12)	44 (13)	116 (12)
Vegetative state	4 (1)	2 (1)	6 (1)
Severe disability	106 (18)	87 (25)	193 (21)
Moderate disability	155 (26)	84 (25)	239 (26)
Good recovery	258 (43)	125 (37)	383 (41)

Table 4 – Glasgow Outcome Scale score of 1 - 3 (dead, vegetative state, or severe disability) at six months in carriers of TNFA allele 2 compared to non-carriers of TNFA allele 2 – odds ratio from logistic regression

	Odds ratio (95% CI)	**p-value**
Unadjusted	1.44 (1.09, 1.91)	0.010
Adjusted for age, GCS motor response and pupil reactivity	1.67 (1.19, 2.35)	0.003
Adjusted for age, GCS motor response, pupil reactivity, CT classification, traumatic SAH, hypoxia and hypotension	1.55 (1.09, 2.21)	0.015
Adjusted for age, GCS motor response, pupil reactivity, CT classification, traumatic SAH, hypoxia, hypotension, APOE ε4 allele and interaction between APOE ε4 allele and age	1.63 (1.14, 2.34)	0.007

Age included as a continuous predictor using cubic smoothing spline and GCS motor response, pupil reactivity, CT classification, traumatic SAH, hypoxia, hypotension included as categorical predictors

Table 5 – Secondary complications after TBI by possession of TNFA allele 2 – number (%) of patients

	Non-carriers of allele 2	Carriers of allele 2	p-value
Hyperthermia in first 24 hours:			
No	554 (98)	324 (99)	0.14
Yes	13 (2)	3 (1)	
Raised ICP during admission:			
No	550 (90)	311 (88)	0.51
Yes	63 (10)	41 (12)	
Seizures during admission:			
No	504 (82)	298 (85)	0.33
Yes	109 (18)	54 (15)	
Life-threatening infection during admission:			
No	567 (92)	336 (95)	0.071
Yes	46 (8)	16 (5)	

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