Cytokine Gene Polymorphisms and Outcome after Traumatic Brain Injury

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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 that 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 years, mean age 37) was used. 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 unfavorable outcome compared with 31% of non-carriers (adjusted odds ratio 1.67, confidence interval 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 tumor necrosis factor alpha plays an important role in this process.

Key words: cytokines; genetics; head injury; inflammation; traumatic brain injury

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 and associates³ demonstrated that age of more than 40 years, pre-existing physical disability, and a history of brain illness were independent predictors of poor outcome after mild brain injury. In 35% of patients without any of these factors, however, they still failed to achieve a good outcome. This suggests that other features, such as genetic variability, may determine a person'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 outcome⁵ in the short term potentially by promoting neuronal death, edema, 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 that attempts to correlate cytokine gene polymorphisms with outcome after TBI, and studies that have been performed have been of small cohorts.^{7–10}

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 persons 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

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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 more common of the two variants is termed allele 1.

Each person inherits one copy of each gene from either parent and therefore may have one of three possible genotypes (1.1, 1.2, or 2.2). A specific reference number identifies the entry in the online SNP database hosted by the United States National Center for Biotechnology Information (e.g., dbSNP ID 1800629). Eleven SNPs in four 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 that 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,^{11–13} and in clinical studies, raised levels of TNF- α in plasma and cerebrospinal fluid (CSF) 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 knockout 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 (IL1 α and IL1 β) 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^{22–24} 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) $-\beta$ 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 6 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; 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 that had been taken for routine clinical analysis was stored and analyzed 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 anonymized 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 (GCS),²⁹ and pupil reaction. CT scan findings were according to the scheme of Marshall and colleagues (1991).³⁰ Operative findings were obtained from the clinical records. Six months after injury, patient outcome was assessed by the Glasgow Outcome Scale (GOS).^{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 To perform multiple SNP assays on the limited amount of DNA available, a pre-amplification step with the GenomiPhi kit (GE Healthcare) was used. This uses the bacteriophage Phi29 polymerase to exponentially amplify linear DNA template by strand displacement.³⁴

The 11 SNPs in four 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-labeled oligonucleotide melting from matched or mismatched target, monitored in an Idaho Technology (Salt Lake City, UT) 384-well Odyssey. Detection used reduction of opposed G-base quenching of fluorescence during a thermal ramp. Polymerase chain reaction (PCR) was performed on 5 μ L GenomiPhi amplified template for each sample. The PCR reaction mix consisted of 1x PCR buffer (Promega), 200 μ M dNTPs (Promega), 100 nM forward/reverse primer, 500 nM reverse/forward primer, 200 nM FITC-labeled probe, 200 nM DABCYL-labeled probe, 1.5/2.0 mM MgCl₂, 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 94°C for 2 min, then 50 cycles of 94°C for 20 sec, the appropriate annealing temperature for 30 sec and 72°C for 30 sec, followed by a final 2 min at 72°C. After thermal cycling, the samples were overlaid with 5 μ L Chill-Out™ wax (Genetic Research Instrumentation) to prevent evaporation during analysis. Analysis was performed in a 384-well Odyssey (Idaho Technology, Salt Lake City, UT). Samples were melted from 35°C to 70°C. LightTyper software (Roche Diagnostics Ltd) was used to analyze the fluorescence change during melting. Results were then manually checked using in-house software.

Based on 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 that span the -308 region of the TNFA promoter (forward: 5'-aggcaataggttttgagggcat-3' and reverse: 5'-tcctcctgctccgattccg-3'). The PCR products were then digested with the restriction enzyme Nco I 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 ultraviolet transillumination.

Analysis

Clinical outcome at 6 months was determined using the GOS. Outcome was then dichotomized into unfavorable (death, vegetative state, severe disability) or favorable (moderate disability or good recovery). This approach had been used when this cohort was analyzed in respect to potential association with APOE genotype.²⁸ Secondary complications were assessed according to their presence or absence: "seizures" were defined as seizures necessitating treatment at any stage during admission, "raised ICP" was raised intracranial pressure necessitating 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 the Pearson chi-square test. Logistic regression analysis was performed using the covariates of age (modeled as a continuous variable using cubic smoothing splines), GCS motor response categorized into four groups, pupil reactivity categorized into three groups, initial CT scan findings into three groups, traumatic subarachnoid hemorrhage, hypoxia, hypotension, and APOE genotype (presence or absence of the ϵ 4 allele). Statistical analyses were performed using SAS 9.2 for Windows.

Results

Association between cytokine gene SNPs and outcome at six 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 on 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 samples²⁸ (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 GOS at six months

After repeating and extending TNFA -308 SNP typing with the Nco I 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 the first hospital or on admission to the study hospital ($n=1$), pupil reactivity was not available either on admission to the first hospital or on admission to the study hospital ($n=21$), or CT scan was not performed ($n=6$) were excluded.

The remaining 937 patients had missing values for traumatic subarachnoid hemorrhage on admission CT scan ($n=7$), hypoxia in the first 24 hours ($n=73$), hypotension in the 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, sex, initial GCS score (reflecting the severity of injury), CT characteristics of the injuries, presence of hypoxia and hypotension, and APOE ϵ 4 allele carriage (Table 2).

In our previous studies investigating APOE genotype, outcome was dichotomized into unfavorable (death, vegetative survival, or severe disability at 6 months) and favorable (moderate disability or good recovery).^{28,36} Analyzed in this manner, TNFA -308 allele 2 carriers were more likely to have an unfavorable 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%). Allele 2 carriers who survived, however, were more likely to have severe disability (25% vs. 18%) and less likely to have a good outcome (37% vs. 43%).

TABLE 1. DISTRIBUTION OF TNFA -308 GENOTYPES AND ALLELE CARRIAGE RATES

<i>TNFA -308 genotype and allele carriage rates</i>	
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

TNF, tumor necrosis factor.

TABLE 2. CHARACTERISTICS OF ACUTELY HEAD-INJURED PATIENTS BY POSSESSION OF TNFA -308 ALLELE 2

	<i>Non-carriers of allele 2</i>	<i>Carriers of allele 2</i>	<i>All patients</i>
Number (%) of patients	595 (64)	342 (36)	937 (100)
Sex:			
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)
Localizing	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 ϵ 4 allele:			
Non-carrier	395 (66)	233 (68)	628 (67)
Carrier	200 (34)	109 (31)	309 (33)

SD, standard deviation; GCS, Glasgow Coma Scale; CT, computed tomography; NVP, no visible pathology; SAH, subarachnoid hemorrhage.

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 toward 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 unfavorable 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 ICP; and IL1B –31 and severe sepsis.

The strengths of a study of this nature in this cohort have been outlined previously.²⁸ These include the large number of subjects in the cohort, prospective recruitment from consecutive admissions to a regional center, 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 TNF α plays a pivotal role in the initiation and control of the inflammatory response after TBI.^{37,38} TNF α has been shown to be upregulated in the CNS after TBI,^{11–13,39} and raised levels of TNF α have been demonstrated in plasma and CSF after TBI.^{14,15} The TNFA -308 polymorphism sits within the promoter

TABLE 3. GLASGOW OUTCOME SCALE AT SIX MONTHS BY POSSESSION OF TNFA ALLELE 2—NUMBER (%) OF PATIENTS

	<i>Non-carriers of 2 allele</i>	<i>Carriers of 2 allele</i>	<i>All patients</i>
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 WITH 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 ϵ 4 allele and interaction between APOE ϵ 4 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.

TNF, tumor necrosis factor; CI, confidence interval; GCS, Glasgow Coma Scale, CT, computed tomography, SAH, subarachnoid hemorrhage.

region of the gene and alters transcription, allele 2 being associated with significantly higher expression of TNF α .^{40,41} Association between the TNFA -308 polymorphism and outcome after TBI is therefore biologically plausible because the genetic variation is in a position to influence expression of TNF α in response to a stimulus such as TBI.

Experimental studies show that TNF α 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 TNF α receptor subtypes,^{44,45} so TNF α 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 TNF α , in the CSF with outcome after TBI in relatively small cohorts without demonstrating evidence of a correlation between high TNF α levels and poor outcome.^{46,47} It has been shown, however, that serum and CSF TNF α 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-1 β and IL-6 correlating with poor outcome⁵⁰ or without a clear relationship to outcome.⁵¹

The relationship between levels of TNF α in the CSF, which is obviously relatively accessible for sampling, and the time course and localization of its production and utilization 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 TNF α 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 TNF α and clinical follow-up, would be required to test this hypothesis.

Although the association between the TNFA -308 SNP and 6-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 skepticism 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 needed 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 colleagues⁸ identified a possible association between the -511 and +3953 SNPs within the IL1 β gene and outcome after head injury. The study was small, involving 69 patients admitted to a neurosurgical unit after TBI. Fourteen of 25 (56%) patients 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 of 28 (71.4%) patients 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 6 months. This also confirms the lack of an association between the IL1 -889 SNP and overall 6-month outcome identified in a small study by Tanriverdi and coworkers.⁹ In a further small study ($n=151$), post-traumatic brain hemorrhage 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

TABLE 5. SECONDARY COMPLICATIONS AFTER TRAUMATIC BRAIN INJURY 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)	

ICP, intracranial pressure.

current study has confirmed the lack of association between three IL6 SNPs and 6-month outcome after TBI.

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 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 TNF α 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,⁵¹ 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.

Acknowledgments

We thank the staff and patients at the Institute of Neurological Sciences, Southern General Hospital, Glasgow. Hilda Fiddes and Elaine Stewart collected the clinical data. Initial genotyping was performed in the Division of Human Genetics, University of Southampton. Thanks also to Dr. Willie Stewart and Prof. David Graham, Institute of Neurological Sciences, Southern General Hospital, Glasgow, for facilitation of the study.

This work was supported by the Medical Research Council (grant number G9601296N) which funded establishment of the Head Injury Prospective Cohort (1996–1999) and APOE genotyping; and a Fausto Iannotti Scholarship (Mr Bob Barber) that funded Ryan Waters.

Author Disclosure Statement

No competing financial interests exist.

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