Inflammatory and apoptotic signalling pathways and concussion severity: a genetic association study

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ABSTRACT

The objective was to investigate the relationship between *IL-1B* rs16944, *IL-6* rs1800795, and *CASP8* rs3834129 genetic polymorphisms and concussion severity. Rugby players from high school, senior amateur, and professional teams completed a concussion severity questionnaire and donated a DNA sample. Participants (n = 163) were split into symptom severity groups around the median number and duration of symptoms. The frequency of participants with high symptom counts (more than five symptoms) increased across the *IL-1B* (C/C: 35%; C/T: 51%; T/T: 56%; P = 0.047) and the *IL-6* (C/C: 31%; C/G: 44%; G/G: 58%; P = 0.027) genotypes. The C–C inferred interleukin allele construct frequency, created from combining the *IL-1B* and *IL-6* genotype data, was lower in participants reporting a high symptom count (18%), compared to those with a low symptom count (fewer than six symptoms, 36%, P = 0.002). Similarly, the C–C inferred interleukin allele construct frequency was lower in those reporting prolonged symptom duration (more than one week, 16%), as opposed to short symptom duration (less than one week, 34%, P = 0.015). This study provides evidence of novel inflammatory pathway genetic associations with concussion severity, which supports the hypothesis implicating neuroinflammation in the development of concussion symptoms.

KEYWORDS Concussion; genetics; interleukin; neuroinflammation; rugby

Introduction

Concussion is a common head injury in collision sports that is defined as a traumatic brain injury (TBI) induced by biomechanical forces, which causes an onset of neurological signs and symptoms that usually resolve spontaneously (McCrory et al., 2017). In the majority of athletes, concussion symptoms resolve within a week to 10 days. However, for a subset comprising approximately 10 to 15% of athletes, symptoms may last for weeks to months post concussion (Cancelliere et al., 2014; Mccrea et al., 2014). This can result in significant absence from school, work, and sporting participation.

Currently, there are few prognostic factors to determine those most at risk for increased concussion severity (Cancelliere et al., 2014; McCrory et al., 2017), measured as the number or duration of symptoms and deficits (Erlanger et al., 2003; Makdissi et al., 2010). The individual variability associated with concussion severity (Cancelliere et al., 2014; Mccrea et al., 2014) suggests a possible genetic influence. Exploring the genetic factors that modulate concussion severity may help to i) identify those at risk for heightened or prolonged symptoms, ii) provide further information on the underlying pathophysiology of concussion, and iii) improve management and/or treatment strategies. To date there have been a limited number of studies investigating the role of genetic polymorphisms in concussion (Abrahams, Mc Fie, Patricios, Posthumus, & September, 2014; Panenka et al., 2017).

The neuroinflammatory response is initiated following concussion (Patterson & Holahan, 2012; Smith et al., 2013) and this was suggested to influence concussion symptom presentation and duration (Blaylock & Maroon, 2011; Lai & Todd, 2008). Furthermore, a review of the post-concussion inflammatory response suggested that the post-concussion syndrome could be referred to as a "post-inflammatory brain syndrome" due the significant role of inflammation in the concussion pathophysiology and the overlap of common symptoms between inflammatory disorders and concussion (Rathbone, Surejini, Jiang, Rathbone, & Kumbhare, 2015). Interleukin 1β (IL-1B) and interleukin 6 (IL-6) are the two interleukins primarily expressed following brain injury (Harrison, Rowe, O'Hara, Adelson, & Lifshitz, 2014; Hopkins & Rothwell, 1995) and both play an important role in mediating the neuroinflammatory response (Bélanger, Allaman, & Magistretti, 2011). IL-1B up-regulates the expression of IL-6 (Norris, Tang, Sparacio, & Benveniste, 1994; Woodroofe et al., 1991) and both interleukins act to stimulate the neuroinflammatory response (Basu, Krady, & Levison, 2004; Erta, Quintana, & Hidalgo, 2012). Altered IL-1B and IL-6 expression and protein levels have been associated with neuroinflammatory variation (Almolda et al., 2014; Rothwell, 2003) and clinical outcomes following brain injury (Clausen et al., 2011; Singhal et al., 2002; Winter, Pringle, Clough, & Church, 2004).

The *IL-1B* gene contains a single nucleotide polymorphism (SNP) (*IL-1B* rs16944 C>T) that modifies IL-1B availability

(Santtila, Savinainen, & Hurme, 1998) and this polymorphism was associated with unfavourable clinical consequences six months after moderate to severe TBI (Uzan et al., 2005). In addition, a SNP (*IL-6* rs1800795 G>C) in the *IL-6* gene was previously implicated in modulating the expression of IL-6 (Fishman et al., 1998; Gaudino et al., 2003; Hulkkonen, Pertovaara, Antonen, Pasternack, & Hurme, 2001; Stivala, Mazzarino, & Malaponte, 2006). Due to prior associations between IL-1B and IL-6 protein levels and outcome following brain injury (Clausen et al., 2011; Singhal et al., 2002; Winter et al., 2004), it can be hypothesized that functional genetic polymorphisms within the *IL-1B* and *IL-6* genes may be appropriate candidates to test for an association with concussion severity.

In addition to the inflammatory response, neuronal apoptosis is thought to be induced and influences the pathology of concussion (Conti, Raghupathi, Trojanowski, & McIntosh, 1998; Rink et al., 1995). Caspase 8 is an initiator caspase that was specifically implicated in regulating the apoptotic pathways subsequent to brain injury (Zhang, Graham, Kochanek, & Clark, 2003). Interestingly, an insertion/deletion polymorphism (*CASP8* rs3834129 Ins>Del) in the promoter region of *CASP8* was shown to alter *CASP8* transcription (Sun et al., 2007). It is therefore rational to propose that this functional polymorphism may modulate the apoptotic pathways and subsequent neuronal dysfunction, and thereby potentially also influence concussion severity.

Consequently, the aims of this study were to investigate the relationship between *IL-1B* rs16944 C>T, *IL-6* rs1800795 G>C, and *CASP8* rs3834129 Ins>Del genetic polymorphisms and concussion severity.

Methods

The study followed a case-control genetic association design and is presented in accordance with the "Strengthening the Reporting of Genetic Association Studies" (STREGA) statement for reporting the results of genetic association studies (Little et al., 2009).

Participant recruitment

The Human Research Ethics Committee of the University of Cape Town granted ethical approval for this study and the Western Cape Education Department and relevant school administrators gave permission to conduct research at schools. All participants gave written informed consent and in the case of minors, written informed assent from the minor and informed consent from a parent or legal guardian were obtained.

Apparently healthy male rugby players, from high schools (junior), senior amateur-level clubs, and senior professional teams, were invited to participate in the study between January 2013 and August 2015. Four hundred and eighty-seven rugby players agreed to participate and completed the consent and/or assent forms. Participants were then required to (i) complete the study questionnaire containing; personal details and medical, sporting and concussion history questionnaires, and (ii) donate a buccal swab for DNA collection. The senior participants completed the consent form and study questionnaire in a single session. For the junior participants, consent was first required from both parent and junior before the study questionnaire was completed at a subsequent time point. Junior participants who had completed consent and assent forms but did not submit the study questionnaire were excluded (n = 67).

Participants were excluded if they had previously been diagnosed with meningitis (n = 6), epilepsy (n = 4), stroke (n = 2), or had sustained a non rugby-related concussion (n = 24). Known siblings of enrolled participants were excluded (n = 2). Participants were grouped according to self-reported ancestry due to potential population stratification effects, which may significantly confound the results of genetic association studies (Thomas & Witte, 2001). Only participants reporting the same ancestry were compared and included in this study. Based on the exclusion criteria, 303 participants (aged 12 to 39 years old) completed the study. However, only participants reporting a previous concussion were included in the analysis (n = 163; 63 junior + 100 senior players, age: 20.4 ± 4.5 years).

Medical and sporting history

Participants were asked to disclose any current or previously diagnosed medical conditions and describe details of their lifetime sporting participation, including the total years of participation, highest level of play, and playing position.

Concussion history

Concussion was defined according to the 2012 Zurich Consensus statement (McCrory et al., 2013), namely a direct, or indirect, blow to the head during a rugby-related activity, that resulted in a set of clinical signs and symptoms that may or may not have involved loss of consciousness. The self-reported concussion history questionnaire required participants to provide details of their four most recent concussions. Specifically, information was collected on the date and mechanism of concussion injury, whether the concussion was diagnosed by a medical professional, the occupation of that medical professional (medical doctor, physiotherapist, nurse, paramedic), the symptoms experienced, and the duration of symptoms. Concussions diagnosed by a medical professional, and qualified with one or more concussion symptoms, were termed "diagnosed concussions". Concussions that were not diagnosed by medical professional, but were described in conjunction with concussion symptoms, were defined as "suspected concussions".

The details of the most recent concussion were used in the concussion symptom severity analysis. The analysed concussion events occurred, on average, 2.5 ± 2.7 (n = 142) years before recruitment. Participants selected their experienced symptoms from the 22-item post-concussion scale (Lovell & Collins, 1998), a validated concussion symptom checklist (Chen, Johnston, Collie, McCrory, & Ptito, 2007) that was extracted from the Sports Concussion Assessment Tool (3rd edition) (Guskiewicz et al., 2013). Participants also selected the duration of symptoms from four time categories, namely less than one week, one to two weeks, two to four weeks, and more than four weeks. Of the 163 participants reporting a previous concussion, 153 (94%) completed the details on the

number of symptoms experienced and 133 (82%) reported the duration of symptoms on their most recent concussion.

For the statistical analysis, participants were dichotomized into symptom severity groups around the median number of reported symptoms (five symptoms) and the median reported symptom duration (less than one week). Fifty-four percent (n = 83) of participants reported five or fewer symptoms and were termed as having a "low" symptom count. The remaining 46% (n = 70) of participants reported more than five symptoms and were thus termed as having a "high" symptom count. Similarly, participants with a symptom duration of less than one week were defined as having a "short" symptom duration (62%, n = 82), while participants reporting a symptom duration of more than one week were defined as having a "prolonged" symptom duration (38%, n = 51).

Furthermore, all participants with both symptom count and duration data (n = 132) were divided into four quadrants, by symptom count and duration medians. Quadrant one included participants with a low symptom count and a short symptom duration; quadrant two comprised of participants with a high symptom count and a short symptom duration; quadrant three included participants with a low symptom count and a prolonged symptom duration; and quadrant four contained participants with a high symptom count and a prolonged symptom duration.

DNA extraction

A buccal cheek swab was collected from participants using the Isohelix® SK-2 (Cell projects Ltd, Kent, UK) or the Epicentre® Catch All™ (Epicentre® Biotechnologies, WI, USA) buccal swabs and DNA extracted as per the manufacturers' instructions.

Genotyping

DNA was genotyped, for the *IL-1B* rs16944 C>T and *IL-6* rs1800795 G>C polymorphisms, using standard polymerase chain reaction (PCR) and restriction length fragment polymorphism analysis as previously described (September et al., 2011). The *CASP8* rs3834129 Ins>Del variant was genotyped using a custom designed fluorescent-based Taqman® real time PCR assay and the StepOnePlus™ Real Time PCR system as per the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). Negative controls (no DNA added) were included in each experiment to detect PCR DNA contamination and samples of known genotypes were included on each 96-well PCR or TAQMAN reaction plate. Two independent investigators confirmed all genotypes. All laboratory work was completed at the Division of Exercise Science and Sports Medicine, Department of Human Biology, within the University of Cape Town.

Statistics

Statistical analysis was performed using the language and environment R statistical program (R Core Team, R, 2013) and STATISTICA version 13 (StatSoft Inc., Tulsa, OK, USA). A one-way analysis of variance and a Pearson's Chi-squared test were used to observe differences in participant characteristics data between symptom count (low versus high) and symptom duration (short versus prolonged) groups.

The genetics and SNPassoc packages in R (González et al., 2007; Warnes & Leisch, 2006) were used to calculate the genotype frequency distributions. A Pearson's Chi-squared test was used to compare differences in genotype frequencies between symptom count and symptom duration groups and calculate the resultant odds ratios (OR's) with 95% confidence intervals (95% CI's). Genotype frequency analyses were adjusted for age and previous concussions as possible confounding covariates. In addition, a Chi-squared test for trend was used to investigate the distribution of participants with a high symptom count or prolonged symptom duration across each of the genotype groups.

Inferred interleukin allele constructs were created from the *IL-1B* rs16944 C>T and *IL-6* rs1800795 G>C genotype data using the haplo.score package in R (Schaid, Rowland, Tines, Jacobson, & Poland, 2002). A Pearson's Chi-squared test was then used to compare differences in the frequencies of inferred interleukin allele constructs between symptom count and symptom duration groups. A Chi-squared test for trend was used to observe the distribution of specific inferred interleukin allele constructs, which displayed significant associations with symptom count and duration, across the previously defined symptom count and duration quadrants. For the analysis quadrants two and three were combined. *P* values less than 0.05 were accepted as statistically significant.

Results

Participant characteristics

Participants reporting a high symptom count had a greater proportion of participants with a prolonged symptom duration (22%, n = 15), in comparison to participants with a low symptom count (54%, n = 35, $p < 0.001$; Table 1). A greater proportion of the high symptom count group reported losing consciousness (44%, n = 31) than the low symptom count group (19%, n = 16, $p < 0.001$). There was however, no difference in the proportion reporting loss of consciousness between symptom duration groups ($P = 0.968$; Table 1). The prolonged symptom duration group tended to have a higher proportion of their concussions diagnosed by medical professionals (90%, n = 46) compared to the short symptom duration group (77%, n = 63, $P = 0.051$). There was no significant difference in the proportion of diagnosed concussions between the symptom count groups ($P = 0.176$; Table 1).

There were no differences between symptom count and symptom duration groups in the proportion of junior participants (symptom count: $P = 0.505$; symptom duration: $P = 0.207$), the age at concussion (symptom count: $P = 0.551$; symptom duration: $P = 0.467$), the proportion of participants with a previous concussion (symptom count: $P = 0.963$; symptom duration: $P = 0.297$), the number of previous concussions (symptom count: $P = 0.312$; symptom duration: $P = 0.070$), the proportion of participants with a self-reported learning disorder (symptom count: $P = 0.470$; symptom duration: $P = 0.800$), or the proportion of participants with a self-reported migraine history (symptom count: $P = 0.094$; symptom duration: $P = 1.000$; Table 1).

Table 1. The characteristic of South African rugby participants in the low symptom count (five or fewer symptoms), high symptom count (more than five symptoms), short symptom duration (less than one week), and prolonged symptom duration (more than one week) symptom severity groups.

	Symptom count			Symptom duration		
	Low	High	*P* value[a]	Short	Prolonged	*P* value[b]
n	**83**	**70**		**82**	**51**	
Junior	44 (31)	33 (27)	0.505	34 (28)	45 (23)	0.207
Age at concussion	18.2 ± 4.3 (75)	17.8 ± 3.6 (66)	0.551	18.1 ± 4.4 (73)	17.5 ± 2.9 (50)	0.467
Previous concussion	52 (43)	51 (36)	0.963	48 (39)	57 (29)	0.297
Number of previous concussions	0.9 ± 1.1 (83)	0.7 ± 0.9 (70)	0.312	0.7 ± 0.9 (82)	1.0 ± 1.1 (51)	0.070
Loss of consciousness	19 (16)	44 (31)	**<0.001**	32 (26)	31 (16)	0.968
Diagnosed by a medical professional	77 (64)	86 (60)	0.176	77 (63)	90 (46)	0.051
Learning disorder	4 (3)	7 (5)	0.470	5 (4)	6 (3)	0.800
Migraine history	0 (0)	4 (3)	0.094	2 (2)	2 (1)	1.000
Prolonged symptom duration	22 (15)	54 (35)	**<0.001**	n/a	n/a	n/a
High symptom count	n/a	n/a	n/a	37 (30)	70 (35)	**<0.001**

Data are expressed as mean ± standard deviation or the percentage of participants with the number of participants with non-missing data (n) in parentheses. Unadjusted *P* values for [a]low versus high symptom count groups and [b]short versus prolonged symptom duration groups are displayed. Statistically significant differences ($P < 0.05$) between symptom count and symptom duration groups are displayed in bold.

Symptom count

There were significant general linear trends in the frequency of high symptom count (more than five symptoms) participants across the *IL-1B* rs16944 (C/C: 35%, n = 21; C/T: 51%, n = 37; T/T: 56%, n = 10; $P = 0.047$) and the *IL-6* rs1800795 genotype groups (C/C: 31%, n = 9; C/G: 44%, n = 34; G/G: 58%, n = 23; $P = 0.027$, Table 2). Although tendencies existed, the proportion of participants with high symptom count did not differ between the individual *IL-1B* rs16944 ($P = 0.103$) or *IL-6* rs1800795 ($P = 0.058$) genotype groups. The frequency of participants reporting a high symptom count was similar between the *CASP8* rs3834129 genotype groups ($P = 0.684$, Table 2).

Table 2. The proportion (%) of participants reporting a high symptom count (more than five symptoms) and a prolonged symptom duration (more than one week) for each *IL-1B* rs16944 C>T, *IL-6* rs1800795 G>C, and *CASP8* rs3834129 Ins>Del genotype group.

	High symptom count	*P* value[a]	Prolonged symptom duration	*P* value[b]
IL-1B rs16944 (n)	**45 (68)**		**37 (48)**	
C/C	35 (21)	0.103	32 (18)	0.383
C/T	51 (37)	**0.047***	37 (21)	0.153*
T/T	56 (10)		53 (9)	
IL-6 rs1800795 (n)	**45 (66)**		**37 (47)**	
C/C	31 (9)	0.058	23 (6)	0.083
G/C	44 (34)	**0.027***	42 (27)	0.108*
G/G	58 (23)		38 (14)	
CASP8 rs3834129 (n)	**46 (62)**		**38 (45)**	
Ins/Ins	48 (19)	0.684	30 (10)	0.548
Ins/Del	48 (32)	0.468*	38 (24)	0.184*
Del/Del	38 (11)		48 (11)	

Data are the proportion, expressed as a percentage, of participants reporting a high symptom count or prolonged symptom duration in each *IL-1B* rs16944, *IL-6* rs1800795, and *CASP8* rs3834129 genotype group, with the number of participants with non-missing data (n) in parentheses. *P* values, calculated from Pearson's Chi-squared tests and adjusted for age of concussion and the number of previous concussions, are displayed for differences in the percentage of participants reporting a [a]high symptom count and a [b]prolonged symptom duration between the *IL-1B* rs16944, *IL-6* rs1800795, and *CASP8* rs3834129 genotype groups. *Unadjusted *P* values, calculated from a Chi-squared test of trend comparing the percentage of participants reporting [a]high symptom count and [b]prolonged symptom duration across the *IL-1B* rs16944, *IL-6* rs1800795, and *CASP8* rs3834129 genotype groups are also displayed. Statistically significant differences ($P < 0.05$) between groups are displayed in bold.

Symptom duration

Although not significantly different, there was a trend for the frequency of participants with prolonged symptom duration (more than one week) to differ between *IL-6* rs1800795 genotype groups (C/C: 23%, n = 6; C/G: 42%, n = 27; G/G: 38%, n = 14, $P = 0.083$; Table 2). The proportion of participants reporting prolonged symptom duration was similar between *IL-1B* rs16944 ($P = 0.383$) and *CASP8* rs3834129 genotypes ($P = 0.548$; Table 2). There were no significant general linear trends observed with any of the genotypes.

Inferred interleukin allele constructs

All four inferred interleukin allele constructs (C–C, T–C, C–G, and T–G), created from *IL-1B* rs16944 C>T and *IL-6* rs1800795 G>C genotype data, were expressed at a frequency above 4% in all analyses (C–C: 28%; T–C: 18%; C–G: 36%; T–G: 18%). Significant differences were noted in the distribution of the inferred interleukin allele constructs between symptom count ($P = 0.019$) and symptom duration groups ($P = 0.041$). More specifically, the C–C allele construct was significantly under-represented in participants reporting a high symptom count (18%), compared to those with a low symptom count (36%, $P = 0.002$). Similarly, the C–C inferred interleukin allele construct was also under-represented in those reporting prolonged symptom duration (16%), as opposed to short symptom duration (34%, $P = 0.015$). In contrast, the alternate T–G allele construct was over-represented in the high symptom count individuals (15%) compared to the low symptom count individuals (20%, $P = 0.027$). There was a significant general linear trend for the C–C allele construct distribution to decrease across the quadrants (quadrant 1: 39%; quadrant 2 + 3: 25%; quadrant 4: 11%; $P = 0.005$; Figure 1).

Discussion

To the best of our knowledge, this was the first study to investigate the role of genetic polymorphisms involved in neuroinflammatory or apoptotic pathways in concussion severity. Significant associations were noted between the functional neuroinflammatory-related *IL-1B* rs16944 C>T and *IL-6* rs1800795 G>C polymorphisms, but not the apoptosis-linked *CASP8* rs3834129 Ins>Del polymorphism. Specifically, the results suggested that

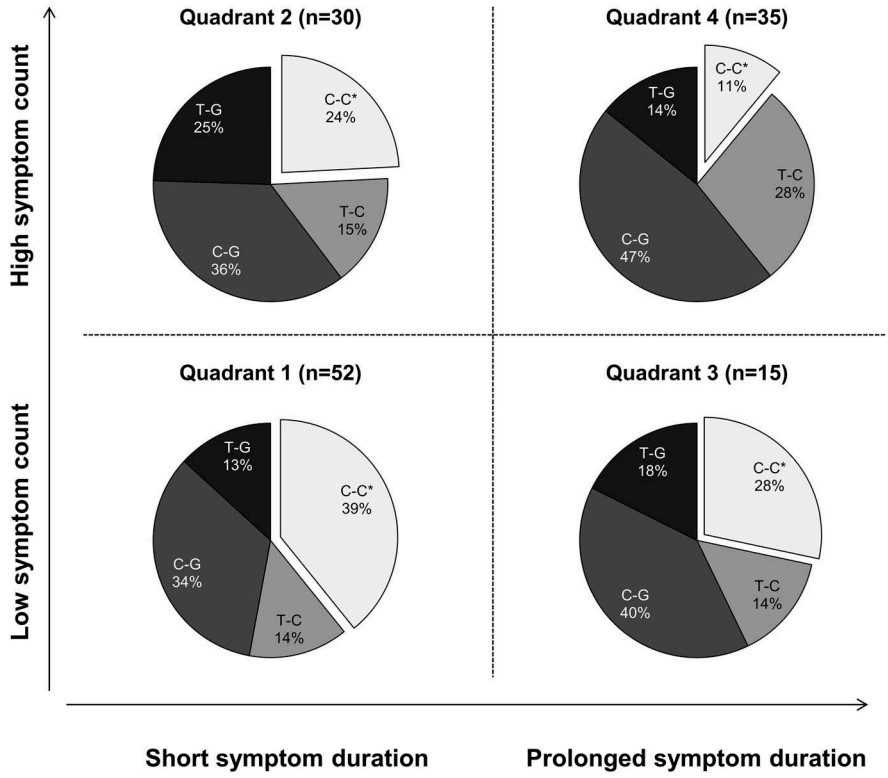

Figure 1. The frequency distribution of the inferred interleukin allele constructs (C–C, T–C, C–G, and T–G), created from *IL-1B* rs16944 C>T and *IL-6* rs1800795 G>C genotype data, in each of the four symptom number and duration quadrants. Quadrant one: participants reporting low symptom count (five or fewer symptoms) and short symptom duration (less than one week); quadrant 2: participants with high symptom count (more than five symptoms) and short symptom duration; quadrant three: participants with low symptom count and prolonged symptom duration (more than one week); quadrant four: participants with high symptom count and prolonged symptom duration. *There was a significant general linear trend for the C–C inferred interleukin allele construct distribution to decrease across the quadrants (quadrant 1: 39%; quadrant 2 + 3: 25%; quadrant 4: 11%; $P = 0.005$).

the *IL-1B* rs16944 C allele and the *IL-6* rs1800795 C allele were linked to reduced concussion symptom severity compared to the *IL-1B* rs16944 T allele and the *IL-6* rs1800795 G allele, respectively. Furthermore, the associations observed with the inferred interleukin allele construct suggest that the *IL-1B* rs16944 and *IL-6* rs1800795 polymorphisms might have had an additive modulating effect on concussion symptom severity.

IL-1B and IL-6 act as pro-inflammatory cytokines, stimulating the neuroinflammatory response following brain injury (Basu et al., 2004; Erta et al., 2012). Previous functional evidence associated both the *IL-1B* rs16944 C and *IL-6* rs1800795 C alleles with reduced protein expression levels compared to the *IL-1B* rs16944 T and *IL-6* rs1800795 G alleles, respectively (Miñambres et al., 2003; Santtila et al., 1998). Consequently, carriers of the *IL-1B* rs16944 C and *IL-6* rs1800795 C alleles would presumably exhibit decreased IL-1B or IL-6 production and signalling, which would result in a decreased neuroinflammatory response. Conversely, carriers of the *IL-1B* rs16944 T and *IL-6* rs1800795 G alleles would potentially display increased IL-1B or IL-6 signalling and an elevated neuroinflammatory response.

Interestingly, diminishing IL-1B and IL-6 signalling was found to improve outcomes following experimental neurotrauma (Loddick & Rothwell, 1996; Relton, Martin, Thompson, & Russell, 1996; Yang, Gangidine, Pritts, Goodman, & Lentsch, 2013). While, direct administration of IL-1B and IL-6 exacerbated the deficits following several types of experimental TBI (Almolda et al., 2014; Toulmond & Rothwell, 1995). The excitotoxic effects of IL-1B and IL-6 overexpression have previously been attributed to a heightened neuroinflammatory response (Almolda et al., 2014; Chao, Hu, Ehrlich, & Peterson, 1995; Lee, Dickson, Liu, & Brosnan, 1993).

Although neuroinflammation is necessary to promote healing in the central nervous system, a prolonged or overactive response can have detrimental effects on the health of nervous tissue (Lenzlinger, Morganti-Kossmann, Laurer, & McIntosh, 2001; Morganti-Kossmann, Rancan, Stahel, & Kossmann, 2002). For example, increased blood brain barrier disruption resulted in prolonged inflammatory stimulation, oedema, and cell death (Schlosberg, Benifla, Kaufer, & Friedman, 2010), while increased neuronal apoptosis caused disruption of white matter networks and neurological difficulties (Kraus et al., 2007). Furthermore, activated microglia and astroglia release several neurotoxic chemicals, including nitric oxide, which can be damaging to the health of surrounding neurons (Brown & Neher, 2010).

Therefore in this cohort, it is reasonable to hypothesize from the collective previous functional evidence that the preliminary findings of this genetic association study noted between the *IL-1B* rs16944 C and *IL-6* rs1800795 C alleles and reduced concussion symptom severity may be a reflection of reduced neuroinflammation. However, as this study did not directly measure neuroinflammatory markers, further research is required to characterize the neuroinflammatory response following concussion in each of the *IL-1B* rs16944 and *IL-6* rs1800795 genotype groups and to observe the relationship with concussion symptom presentation and resolution.

In addition to the neuroinflammatory response, apoptosis is proposed to contribute to the pathophysiology of concussion (Conti et al., 1998; Raghupathi et al., 2002; Rink et al., 1995). Apoptosis is thought to increase the degree of neuronal network disruption following brain injury, resulting in more severe symptoms (Kraus et al., 2007). In this study, no associations were noted between the functional *CASP8* rs3834129 Ins>Del polymorphism and symptom severity. Despite this, apoptotic pathways still warrant further investigation as modulators of concussion severity, due to their role in concussion pathophysiology.

The novel findings of this study highlight the role of the neuroinflammatory response in the development and resolution of concussion symptoms. This information, once validated by repeat studies in independent larger samples sets together with further functional evidence, may have several implications to concussion treatment and management strategies.

Biomarkers for concussion severity may be useful to empirically identify those at risk for prolonged deficits. Presently, no such biomarker has been validated (Jeter et al., 2013; Papa, Ramia, Edwards, Johnson, & Slobounov, 2014). Considering the suggested involvement of the neuroinflammatory response, biological correlates of neuroinflammation may be considered as potential biomarkers for concussion severity. In support, the plasma level of C-reactive protein, indicative of systemic inflammation, measured at hospital admittance, was found to correlate with persistent post-concussive symptoms and was thus advocated as a potential biomarker for prolonged concussion deficits (Su et al., 2014). Further investigation and characterization of neuroinflammatory-linked biomarkers may therefore be of interest.

Currently, there are no effective pharmaceutical treatments for concussion (Schneider et al., 2013). Characterization of the neuroinflammatory response induced by concussion was suggested to be a vital step toward the development of pharmaceutical treatments for concussion (Patterson & Holahan, 2012). The associations observed between the pro-inflammatory interleukin genes and concussion severity suggests that diminishing the neuroinflammatory response may possibly reduce the number and duration of symptoms. Administration of $_{L}$-serine for seven days following TBI in an animal model resulted in decreased IL-1B, IL-6, TNF-α, and interleukin-10 protein levels in conjunction with improved neurological deficit scores and reduced neuronal loss (Zhai et al., 2015). Interestingly, a survey of physicians treating sport-related concussion revealed that 54% prescribed non-steroidal anti-inflammatory drugs (Kinnaman, Mannix, Dawn Comstock, & Meehan, 2013), despite an absence of evidence on the efficacy of these pharmaceutical agents on concussion injuries (Schneider et al., 2013). Further research is required to identify whether modulation of the neuroinflammatory response, via anti-inflammatory agents, may reduce concussion symptom presentation and duration.

In recent years, several studies have examined the influence of genetic polymorphisms on concussion symptom severity. Within these studies, a number of genes have been investigated, including the *apolipoprotein E* (*APOE*) (Merritt & Arnett, 2016; Merritt, Rabinowitz, & Arnett, 2016), *microtubule associated protein tau (Tau)* (Terrell et al., 2013), *glutamate ionotropic receptor NMDA type subunit 2A* (*GRIN2A), neurofilament heavy (NEFH)* (McDevitt et al., 2015), and *solute carrier family 17 member 7 (SLC17A7)* (Madura et al., 2016) genes. *APOE* is implicated in neuronal and white matter structural integrity (Heise, Filippini, Ebmeier, & Mackay, 2011; Nathoo, Chetty, Van Dellen, & Barnett, 2003; Persson et al., 2006) and the *APOE* ε4 isoform has been associated with greater concussion symptom scores than other isoforms (Merritt & Arnett, 2016). However, no differences were noted in the post-concussion neurocognitive scores between the different *APOE* isoforms in a subsequent study of the same cohort (Merritt et al., 2016). An investigation of *Tau* polymorphisms, suggested to influence neuronal degeneration (Frost & Feany, 2015; Mietelska-Porowska, Wasik, Goras, Filipek, & Niewiadomska, 2014), reported that the *Tau* rs10445337 T/T genotype group displayed greater post-concussion complex reaction time changes from baseline compared to the other *Tau* rs10445337 genotypes (Terrell et al., 2013). McDevitt et al. (2015) reported that athletes with a glutamate receptor gene polymorphism (*GRIN2A* rs3219790 long allele) were more likely to take longer than 60 days to return to play following a concussion (McDevitt et al., 2015). While no significant association was noted between the *NEFH* rs165602 polymorphism, implicated in neuronal integrity, and concussion symptom severity or return to play (McDevitt et al., 2011). The *SLC17A7* rs74174284 C/C genotype had worse motor speed at the first post-concussion assessment, while carriers of the G allele were six times more likely to experience prolonged symptom durations (Madura et al., 2016).

Although, these studies provide some interesting early evidence for the role of genetic polymorphisms in concussion severity, the literature is generally limited by small sample sizes, variability in the concussion severity outcome measures, and the absence of supporting replication studies. Further adequately powered investigations are thus required to elucidate the role of these and novel genetic polymorphisms to further the field of investigation.

Although this study produced novel findings, there were several limitations. The main limitation was that the concussion severity measures were retrospectively self-reported by participants. Concussion management routinely relies upon the self-reported symptom presentation of athletes (Cancelliere et al., 2014; Erlanger et al., 2003; Makdissi et al., 2010). However, self-reported concussion histories may be subject to recall bias and therefore are potentially less reliable (Kerr, Marshall, & Guskiewicz, 2012; Kerr et al., 2015). Another limitation was that participants recalled concussions that occurred a variable length of time before the study, which might have influenced the accuracy of symptom reporting. Future studies should prospectively follow the medically documented symptom presentation and resolution of athletes for more accurate measures of concussion severity. The small sample size was a further limitation. Due to the sample size, high school, amateur and professional rugby players were analysed as a single cohort and therefore age or experience related trends might have been missed. Further studies should aim to include larger cohorts in order to perform more detailed analyses and interpret findings with greater confidence.

In conclusion, this study provides preliminary evidence of two novel genetic associations with sport-related concussion symptom severity. This information conveys insight into the underlying pathophysiology of concussion and further supports the hypothesis implicating neuroinflammation in the development of

concussion symptoms. In addition, with further investigation, these findings may in the future aid in the development of biologically based concussion management or treatment strategies.

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