Journal of Science and Medicine in Sport 22 (2019) 22–28

Contents lists available at ScienceDirect

Journal of Science and Medicine in Sport

journal homepage: www.elsevier.com/locate/jsams

Original research

Toxic tau: The *TAU* gene polymorphisms associate with concussion history in rugby union players

Shameemah Abrahams[a], Sarah Mc Fie[a], Jon Patricios[b,c,d], Jason Suter[e], Alison V. September[a], Michael Posthumus[a,*]

[a] Division of Exercise Science and Sports Medicine, Department of Human Biology, Faculty of Health Science, University of Cape Town, South Africa
[b] Sports Concussion South Africa, South Africa,
[c] Section of Sports Medicine, University of Pretoria, South Africa
[d] Department of Emergency Medicine, University of the Witwatersrand, South Africa
[e] Cape Sports Medicine, Sports Science Institute, South Africa

ARTICLE INFO

Article history:
Received 3 October 2017
Received in revised form 25 April 2018
Accepted 13 June 2018
Available online 21 June 2018

Keywords:
MAPT
Tau
Sports concussion
Rugby
Genetic association

ABSTRACT

Objectives: Concussion is a brain injury that occurs when biomechanical forces are transmitted to the head region resulting in neurological deficits. The accumulation of tau protein in autopsies of athletes with multiple concussions implicates tau in concussion-associated neurodegeneration. The *TAU* rs2435211 (C > T) and rs2435200 (G > A) polymorphisms are involved in pathological tau expression and neurodegenerative disease risk. The aims of this study were to investigate the associations of *TAU* (rs2435211, rs2435200) polymorphisms with concussion history and sustaining multiple concussions in rugby.
Design: In total, 140 non-concussed controls and 163 previously concussed participants (all cases group, N = 163; clinically diagnosed, N = 140; multiple concussed, N = 87) were recruited from high school (N = 135, junior), club and professional rugby teams (N = 166, senior).
Methods: Participants were genotyped for *TAU* rs2435211 and rs2435200 polymorphisms.
Results: In seniors, the rs2435200 AA genotype was significantly over-represented in the control group compared to the multiple concussed subgroup ($P = 0.033$, control: 25%, N = 16, multiple concussed: 11%, N = 6; OR: 0.34, 95% CI 0.12–0.96). While the AG genotype was significantly under-represented in the control compared to multiple concussed ($P = 0.024$, control: 45%, N = 29, multiple concussed: 63%, N = 36; OR: 2.34, 95% CI 1.11–4.95). The inferred *TAU* (rs2435211 C > T–rs2435200 G > A) T-G haplotype was significantly under-represented in the control (19%, N = 12) compared to the all cases group (30%, N = 28, $P = 0.031$).
Conclusions: The *TAU*-associated neurodegenerative pathway was implicated as a potential pathophysiological mechanism underlying concussion in seniors. In future, the identification of *TAU* polymorphisms associated with concussion risk may assist clinical management and reduce risk of severe complications.
© 2018 Published by Elsevier Ltd on behalf of Sports Medicine Australia.

1. Introduction

Concussion is a closed brain injury in which biomechanical forces are transmitted to the head or neck resulting in neurological deficits including physical symptoms, balance, memory and cognitive impairments.[1,2] Concussions are common in high impact contact sports with an incidence range of 4–11 concussions/1000 player hours reported in elite Rugby Union ("rugby").[3] Although the underlying neurophysiological mechanisms of concussion are not clearly understood, several theories have been proposed.[4]

In response to a concussive event, cascades of cellular processes have been described.[4] These cellular processes can elicit pathophysiological disruption in the brain and lead to neurological impairments. Recently, elevated tau plasma levels were shown to occur immediately after brain injury and persist up to 90 days.[5] Toxic accumulation of tau (or tauopathy) was shown in autopsies of former contact sports players, with exposure to head impacts and a history of multiple concussions.[6–8] Tau is a structural brain protein that binds to microtubules and provides structural stability to neuronal tissue.[9] The hyperphosphorylated form of tau can lead to tau assembling into pathological helical filaments and neurofibrillary tangles which could lead to neurodegeneration.[10] Chronic traumatic encephalopathy (CTE), a neuropathological disorder, is also characterised by hyperphosphorylated tau deposits in

* Corresponding author.
E-mail address: mposthumus@me.com (M. Posthumus).

https://doi.org/10.1016/j.jsams.2018.06.012
1440-2440/© 2018 Published by Elsevier Ltd on behalf of Sports Medicine Australia.

neuronal and glial cells.[8] The neuropathology (e.g. tauopathy) and neurological deficits (e.g. poor motor control) characteristic of CTE were noted in individuals who either sustained multiple concussions or were exposed to head impacts.[8] Concussion is potentially linked to CTE[11] which was observed in 87% of a cohort of 202 former American football players.[6] Furthermore, contact sports players are exposed to high impact collisions and increased exposure to head impacts (i.e. boxing, American football and rugby). The sub-threshold concussive head impacts or subconcussive head impacts have been linked to risk of accumulative neurological damage including risk of neurodegenerative disorders such as CTE.[7,8,12] Therefore, the tau neuropathology observed in sports players with high exposure to multiple concussions and repetitive head impacts implicates tau in the pathophysiology underlying concussion.

Although there is limited research on the genetic contribution to concussion risk, genetic association studies have implicated certain genes.[13] A genetic association study correlates a physical trait with differences in DNA sequence between individuals. The DNA sequence is often referred to as the genotype and the physical trait as the phenotype. A single nucleotide polymorphism (SNP) is an example of a DNA sequence variation in which a single nucleotide base (A, T, C or G) is substituted for another base (allele) at a specific chromosomal location (locus). These individual SNPs are often used as potential genetic markers for a physical trait. In addition, haplotypes, which represent a series of DNA sequences or SNPs inherited together, are often used as a genetic tool to implicate a region on a chromosome to be correlated with a phenotype (e.g. concussion).

Tau-associated genetic markers may contribute to concussion pathophysiology. The microtubule-associated protein tau (MAPT) is encoded by the *MAPT* (or *TAU*) gene. The rs2435211 (C > T) and rs2435200 (G > A) intronic SNPs, within the *TAU* gene, are positioned within two kilobase pairs from regulatory glycosylation and microRNA motifs.[14,15] The rs2435211 SNP was previously associated with an increased level of tau protein and phosphorylated tau in the cerebrospinal fluid,[16] a marker of pathology in neurodegenerative diseases.[17] In addition, the *TAU* rs2435211 SNP was previously shown to interact with the rs3775423 SNP in the *SNCA* gene which encodes for α-synuclein, the characteristic pathology of Parkinson's disease.[18] The rs2435200 G allele was previously associated with an increased risk for Parkinson's disease.[19] Impaired movement and balance are hallmark symptoms in Parkinson's disease, which is also observed in patients with concussion and CTE.[1,8,20]

The role of the *TAU* rs2435211 and rs2435200 SNPs in *TAU* gene regulation and tau neuropathology implicates these SNPs in influencing tau protein regulation and tau binding to microtubules. Impairment of microtubule binding could destabilise the cytoskeletal scaffold supporting neurons, thereby resulting in neurons more susceptible to injury. Therefore, it is reasonable to hypothesise, that the genetic profile of the *TAU* gene of an individual may modulate their susceptibility to concussion. Senior sports players have a high exposure to cumulative head impacts over their sporting career[21] and may, as a result, be particularly susceptible to concussion-associated tauopathy. The aim of this novel, case-control genetic association study was to investigate the associations of *TAU* (rs2435211: C > T and rs2435200: G > A) SNPs with (1) sustaining a previous concussion (concussion history), and (2) the occurrence of multiple concussions in amateur-level club and professional (senior) rugby players. In addition, this study also investigated both SNPs, collectively, as an inferred haplotype with concussion history and sustaining multiple concussions. The *a priori* hypothesis is that *TAU* SNPs will be associated with concussion in senior rugby players.

2. Methods

This case-control genetic association study was performed according to the STrengthening the REporting of genetic association studies (STREGA) guidelines.[22] Ethics approval was obtained from the Human Research Ethics Committee of the Health Science Faculty, University of Cape Town and the Western Cape Education Department. Details on participant recruitment, concussion definition, concussion history and sports participation for this cohort was previously described.[23] Briefly, concussions diagnosed by a medical professional with one or more concussion symptoms reported were categorised as a "clinically diagnosed concussion"; whereas concussions not diagnosed by a medical professional but self-reporting one or more symptoms were categorised as a "suspected concussion". Symptoms were selected from the approved and validated list in the Sports Concussion Assessment Tool version 2 (SCAT2)[24] derived by the Concussion in Sport Group.[1] The main outcomes measured in this study were the genetic susceptibility of sustaining (1) minimum one previous concussion and (2) multiple concussions. Funders had no involvement in the paper.

In total, 420 participants from a previous cohort, which analysed *APOE* genetic variants and concussion susceptibility,[23] were recruited during the 2013–2015 rugby seasons in Cape Town and Johannesburg, South Africa. Participants were included in this study after completing the assent with parental consent (<18 years old, N = 148) or consent forms (≥18 years old, N = 272) and study questionnaire (N = 420). Participants were excluded based on criteria summarized in Supplementary Fig. S1; which included ancestry, sporting activity, sex, concussion mechanism and self-reporting neurological comorbidities. After all exclusions, a total of 303 white, male rugby players (aged 12–39 years old) were analysed; with 140 participants' self-reporting no concussion history (control group) and 163 participants with a history of clinically diagnosed and suspected concussions (all cases group). The clinically diagnosed concussed participants were also separately analysed as the clinically diagnosed subgroup (N = 140). As a consequence of the evidence for tauopathy in sports players with multiple concussions,[7,8] participants with two or more clinically diagnosed and suspected concussions were separately analysed as the multiple concussed subgroup (N = 87; Supplementary Fig. S1). Participants were collectively analysed and were stratified into seniors (amateur-level club, N = 116, and professional rugby players, N = 50), and independently analysed. The senior players were separately analysed to facilitate the identification of potential differences in genetic susceptibility at senior playing level. For example, senior contact sports players have a high and prolonged exposure to potentially pathology-induced, repetitive head impacts.[7,8,21] Junior players, in contrast, would have played fewer matches resulting in decreased exposure to repetitive impacts; therefore it is unlikely for concussion susceptibility to be modulated by repetitive impact-associated *TAU* polymorphisms in juniors. Therefore, the results of juniors when separately analysed were excluded from the results and included in supplementary material. All participants completed a study questionnaire detailing their concussion, sporting, and medical histories (Supplementary Fig. S2).

DNA was extracted from either a cheek swab or a 5 ml venous blood sample for genetic analysis and stored as previously described.[23] The selected SNPs, rs2435211 (C > T, Ch. 17:45985878) and rs2435200 (G > A, Ch. 17:45994485), within the *TAU* gene (Ch. 17q21.31) were either previously associated with Parkinson's disease risk or tau protein expression.[16,19] The rs2435211 SNP was located within intron six and rs2435200 SNP within intron nine of the *TAU* gene (Supplementary Fig. S3). Both SNPs had a minor allele frequency greater than 5% in the white European population (minor allele frequencies: rs2435211 T = 26%, rs2435200 A = 39%;

NCBI, http://www.ncbi.nlm.nih.gov/). The *TAU* SNPs were genotyped, at the Division of Exercise Science and Sports Medicine biochemistry laboratory, Faculty of Health Sciences, University of Cape Town, using fluorescence-based TaqMan® real time polymerase chain reaction (PCR) assays and the StepOnePlus™ Real Time PCR machine with software version 2.2.2 as per the manufacturer's protocol (Applied Biosystems, CA, USA). Standard SNP genotyping cycling conditions were used with slight modifications; holding stage at 95 °C for 10 min, 55 cycles of 92 °C for 15 s and 60 °C for 1 min. In a total of 8 μl, 2× pre-made TaqMan® SNP genotyping master mix reagent (Applied Biosystems, CA, USA), 0.2 μl of 40× target-specific catalogued assay (rs2435211: C_16017217_10, rs2435200: C_16017227_10) and 1 μl of DNA (6–77 ng/μl) were used. An average genotype call rate of 97% was noted, by two independent researchers, with the six control samples successfully repeated in all plates (three repeat samples of known genotypes and three samples without DNA).

All statistical analyses performed used the R language and environment for statistical computing.[25] The genetic susceptibility to sustaining at least one previous concussion (primary outcome) and multiple concussions (secondary outcome) were the two main outcomes tested. The current study's sample size of 160 cases is suitable to detect an allelic odds ratio of 2.0 at 80% statistical power and 5% significance level for concussion history as an outcome. However, in the secondary outcome (post hoc analyses) of the senior subgroup of 97 cases (haplotype analysis) and 57 cases (genotype analysis) reflected approximately 88% and 72% statistical power, respectively, for Type I error detection for concussion history as an outcome. A regression model was fitted for concussion history (case-control) as a function of demographic participant characteristics, and a separate analysis for genotypes as a function of participant characteristics. For the primary outcome (i.e. concussion history), all genotype, allele and haplotype frequency distributions were compared between participants without a concussion history (control group) and those with at least one previous concussion (all cases group and clinically diagnosed subgroup). For the secondary outcome, all analyses were compared between the control group and those with two or more previous concussions (multiple concussed subgroup), due to the evidence for tauopathy in sports players with multiple concussions.[7,8] The Hardy–Weinberg equilibrium (HWE) probabilities, genotype, allele and inferred haplotype frequency distribution differences, odds ratio (OR) and 95% confidence interval (CI) were determined using the Fisher's exact test, genetics, SNPassoc and haplo.stats packages in R. In the primary analysis, all analyses were adjusted for age as a possible confounding covariate. In the secondary analysis, all analyses were adjusted for mass and age as covariates for all participants and seniors, respectively. A hypothesis-driven approach was adopted with both polymorphisms located on a single gene, of which rs2435211 and rs2435200 ($D' = 0.804$, $r^2 = 0.183$) are in high linkage disequilibrium, and therefore correcting for multiple testing would be too conservative.[26] Statistical significance was set at $P < 0.05$.

3. Results

Of the total 163 concussed cases, 46% (N = 76) sustained one concussion, 33% (N = 54) sustained two concussions, 9% (N = 14) sustained three concussions and 12% (N = 19) sustained four concussions. When all participants were combined (Supplementary Table S1), the control group (N = 140) was significantly younger than the all cases group (N = 163) and the clinically diagnosed subgroup (N = 140) (**control vs. all cases**: $P = 0.001$; control: 18.8 ± 3.6 years old; all cases: 20.4 ± 4.5 years old; **control vs. clinically diagnosed**: $P = 0.004$; clinically diagnosed: 20.2 ± 4.2 years old). The control group (N = 136) weighed significantly less than the all cases group (N = 158), the clinically diagnosed subgroup (N = 135) and the MULTI subgroup (N = 86) (**control vs. all cases**: $P = 0.035$; control: 87.7 ± 16.1 kg; all cases: 91.8 ± 16.8 kg; **control vs. clinically diagnosed**: $P = 0.044$; clinically diagnosed: 91.8 ± 17.5 kg; **control vs. MULTI**: $P = 0.005$; MULTI: 94.0 ± 16.1 kg). However, adjusting for age removed the significance for the control group compared to all cases group and clinically diagnosed subgroup (**control vs. all cases**: $P = 0.647$; **control vs. clinically diagnosed**: $P = 0.677$). The control group (N = 133) had a lower BMI (body mass index) than the MULTI subgroup (N = 86) ($P = 0.029$, control: 26.6 ± 3.6 kg/m^2; MULTI: 27.7 ± 3.5 kg/m^2). The control group (N = 140) played fewer years of rugby compared to the MULTI subgroup (N = 87) ($P = 0.017$, control: 10.9 ± 4.1 years; MULTI: 12.3 ± 4.6 years). However, when adjusting for mass, the significance for BMI ($P = 0.994$) and rugby exposure ($P = 0.129$) were removed for the control group compared to the MULTI subgroup. Height (m) and non-rugby collision sport exposure (years) were not significantly different between groups (Supplementary Table S1).

In juniors, no significant differences were noted between groups for age, height, mass, BMI, rugby exposure and non-rugby collision sport exposure (Supplementary Table S1).

In seniors (Table 1), the control group (N = 66) was significantly younger than the all cases group (N = 100) and the MULTI subgroup (N = 63) (**control vs. all cases**: $P = 0.038$; control: 21.7 ± 3.3 years; all cases: 22.9 ± 3.9 years; **control vs. MULTI**: $P = 0.044$; MULTI: 23.1 ± 4.2 years old), however, the control group was not significantly different from the clinically diagnosed subgroup ($P = 0.094$). Height, mass, BMI, rugby exposure and non-rugby collision sport exposure were not significantly different between groups.

When all participants were combined (Supplementary Table S2), participants with the *TAU* rs2435211 TT genotype (N = 29) were significantly younger than those with either the CC (N = 150) or CT genotypes (N = 115) ($P = 0.028$; TT: 17.8 ± 2.9 years old; CC: 20.0 ± 4.1 years old; CT: 19.6 ± 4.3 years old). Participants with the TT genotype (N = 24) weighed significantly less than those with either the CC (N = 148) or CT genotypes (N = 113) ($P = 0.035$; TT: 83.6 ± 15.9 kg; CC: 91.8 ± 15.8 kg; CT: 88.2 ± 17.5 kg). BMI was significantly less for those with a TT genotype (N = 23) compared to those with either CC (N = 146) or CT genotypes (N = 110) ($P = 0.046$; TT: 26.0 ± 3.4 kg/m^2; CC: 27.4 ± 3.6 kg/m^2; CT: 26.4 ± 3.5 kg/m^2). However, when adjusting for age, the significance for mass ($P = 0.167$) and BMI ($P = 0.122$) were removed. No significant difference was noted for age, height, mass, BMI, rugby exposure and non-rugby collision sport exposure between rs2435200 genotypes for all participants (Supplementary Table S2).

No genotype associations were noted for age, height, mass, BMI, rugby exposure and non-rugby collision sport exposure when juniors and seniors were separately analysed (Supplementary Table S2).

When all participants were combined and when juniors were separately analysed (Supplementary Table S3), the rs2435211 and rs2435200 genotype and allele frequency distributions were not significantly different when the control group was compared to the all cases group, clinically diagnosed subgroup and MULTI subgroup.

For seniors (Table 2), the rs2435200 AA genotype was significantly over-represented in the control group compared to the MULTI subgroup (AA vs. GG + AG: age-adjusted $P = 0.033$; control: 25%, N = 16; MULTI: 11%, N = 6, OR: 0.34, 95% CI 0.12–0.96). Whereas, the rs2435200 AG genotype was significantly under-represented in the control group compared to the MULTI subgroup (AG vs. AA + GG: age-adjusted $P = 0.024$; control: 45%, N = 29; MULTI: 63%, N = 36, OR: 2.34, 95% CI 1.11–4.95). The rs2435211 SNP was not significantly different between groups in seniors. Both rs2435211 and rs2435200 SNPs were in HWE for the control and case groups ($P > 0.05$; Table 2).

Table 1
The demographic characteristics for all participants between the control group, all clinically diagnosed and suspected concussed cases (all cases), clinically diagnosed concussed cases only (clinically diagnosed) and those who sustained two or more previous concussions (multiple concussed).

	Control	All cases	*P*-value[a]	Clinically diagnosed	*P*-value[b]	Multiple concussed	*P*-value[c]
Senior							
N	(66)	(100)		(87)		(63)	
Age (yrs.)	21.7 ± 3.3 (66)	22.9 ± 3.9 (100)	**0.038**	22.6 ± 3.5 (87)	0.094	23.1 ± 4.2 (63)	**0.044**
Height (m)	1.84 ± 0.08 (65)	1.85 ± 0.08 (100)	0.452	1.86 ± 0.09 (87)	0.159	1.85 ± 0.09 (63)	0.799
Mass (kg)	96.2 ± 12.0 (65)	97.8 ± 14.0 (99)	0.436	98.2 ± 14.3 (86)	0.360	97.3 ± 13.9 (63)	0.636
BMI (kg/m^2)	28.3 ± 2.9 (65)	28.4 ± 2.9 (99)	0.835	28.5 ± 3.0 (86)	0.720	28.5 ± 2.7 (63)	0.801
Rugby exposure (yrs.)[d]	13.3 ± 4.2 (66)	12.6 ± 4.7 (100)	0.356	12.5 ± 4.7 (87)	0.286	13.2 ± 4.9 (63)	0.902
Non-rugby collision sport exposure (yrs.)[e]	6.2 ± 5.1 (5)	6.5 ± 5.1 (4)	0.932	6.0 ± 6.1 (3)	0.961	8.0 ± 5.0 (3)	0.643
Non-rugby contact sport exposure (yrs.)[f]	9.7 ± 4.7 (12)	9.5 ± 6.8 (26)	0.953	8.7 ± 4.8 (23)	0.587	10.3 ± 7.2 (19)	0.801
Non-contact sport exposure (yrs.)[g]	17.0 ± 9.0 (38)	15.9 ± 9.4 (52)	0.587	15.6 ± 9.9 (45)	0.506	16.0 ± 10.4 (39)	0.660
Total sport exposure (yrs.)[h]	23.5 ± 14.6 (66)	20.2 ± 14.5 (100)	0.154	23.1 ± 13.8 (87)	0.331	22.7 ± 14.1 (63)	0.751

All values are mean ± standard deviation with the number of participants with non-missing data (N) in parentheses. Statistical significant differences for the control group compared to [a]all cases group, [b]clinically diagnosed subgroup and [c]multiple concussed subgroup are highlighted ($P < 0.05$, regression analysis). [d]Self-reported number of years playing rugby. [e]Self-reported number of years playing other collision sports excluding rugby e.g. combative sports. [f]Self-reported number of years playing other contact sports excluding rugby e.g. soccer. [g]Self-reported number of years playing non-contact sports e.g. tennis, golf. [h]Total number of years playing all types of sport (collision + contact + non-contact). BMI — body mass index, yrs. — years.
Bold values indicate statistical significance, p < 0.05.

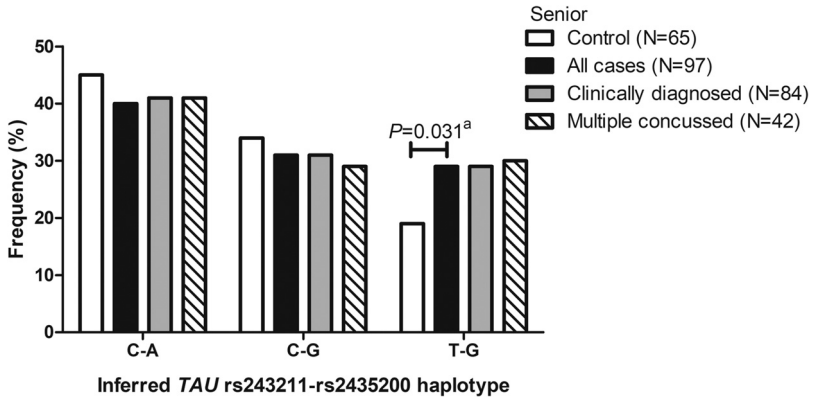

Fig. 1. The *TAU* rs2435211-rs2435200 inferred haplotype distributions.
The frequencies (%) between the control group, all clinically diagnosed and suspected concussed cases (all cases group), clinically diagnosed concussed cases only (clinically diagnosed subgroup) and those who sustained two or more previous concussions (multiple concussed subgroup) for the inferred *TAU* rs2435211 (C > T) and rs2435200 (G > A) haplotype in seniors (N = 162). Significant differences between groups are indicated (age-adjusted $P < 0.05$, additive model[a]).

The inferred *TAU* haplotype (rs2435211-rs2435200) was constructed using the genotype data (rs2435211: C > T, rs2435200: G > A). In total, three inferred haplotypes, above a frequency of 4%, were identified (Fig. 1). The C-A and C-G haplotypes were the most frequent haplotypes (30%–41%), while the T-G was the least frequent (24%–30%) observed for the control and case groups. No significant differences for the inferred *TAU* rs2435200-rs2435211 haplotype between groups were noted, when all participants were combined and when juniors were separately analysed (Supplementary Fig. S4).

In seniors (Fig. 1), the T-G haplotype was significantly underrepresented in the control group (19%, N = 12) compared to the all cases group (30%, N = 29, additive model: age-adjusted $P = 0.031$, hap.score = 2.16).

4. Discussion

Toxic tauopathy was reported in autopsies of individuals with repeated exposure to head impacts or a history of multiple concussions[7,8] A recent study also showed tau neuropathology in post-mortem brains of former American football players.[6] These findings are collectively not surprising, as dysregulation of the structural protein tau can result in destabilisation of the cytoskeletal scaffold thereby influencing the structural integrity of surrounding neurons[9] and potentially result in neurons being more prone to injuries, such as multiple concussive impacts. Thus, there is currently mounting evidence suggesting that *TAU*-associated neurodegenerative pathway should be considered more carefully when reviewing the biological mechanisms of concussion. The aims of this study were to investigate the association between genetic polymorphisms within the *TAU* gene (rs2435211: C > T and rs2435200: G > A SNPs) and risk of a previous concussion (concussion history), or sustaining multiple concussions in rugby players. The main and novel findings of this study included:

(1) The rs2435200 AA genotype was significantly associated with 66% reduced susceptibility (OR: 0.34, 95% CI 0.12–0.96) while the rs2435200 AG genotype was associated with 134% increased susceptibility (OR: 2.34, 95% CI 1.11–4.95) of sustaining multiple concussions in seniors.
(2) Although, the rs2435211 SNP was not independently associated with concussion history, the inferred T-G haplotype (rs2435211-rs2435200) was significantly associated with increased susceptibility for sustaining a concussion in seniors. The results for juniors, when separately analysed, were excluded due to the unlikelihood of cumulative impact-associated *TAU* SNPs influencing concussion in juniors, who have shorter career exposure to repetitive impacts.

In the current study, the rs2435200 AA genotype was significantly associated with a reduced susceptibility while the AG genotype was associated with increased susceptibility of sustain-

Table 2
The genotype, minor allele frequencies (%) and Hardy–Weinberg equilibrium test for the *TAU* rs2435211 and rs2435200 polymorphisms between the control group (control), all clinically diagnosed and suspected concussed cases (all cases), clinically diagnosed concussed cases only (clinically diagnosed) and those who sustained two or more previous concussions (multiple concussed).

		Control	All cases	OR (95% CI)	*P*-value[a]	Clinically diagnosed	OR (95% CI)	*P*-value[b]	Multiple concussed	OR (95% CI)	*P*-value[c]
Senior	N	(66)	(97)			(84)			(57)		
	CC	63.6 (42)	50.5 (49)	1.00	0.160[d]	52.4 (44)	1.00	0.281	49.1 (28)	1.00	0.348[d]
rs2435211	CT	31.8 (21)	41.2 (40)	1.67 (0.85–3.31)		38.1 (32)	1.45 (0.73–2.91)		42.1 (24)	1.68 (0.78–3.62)	
	TT	4.5 (3)	8.2 (8)	2.75 (0.67–11.22)		9.5 (8)	2.55 (0.63–10.25)		8.8 (5)	1.96 (0.42–9.16)	
	T allele	20.5 (27)	28.9 (56)	1.58 (0.91–2.78)	0.094	28.6 (48)	1.55 (0.88–2.78)	0.139	29.8 (34)	1.65 (0.89–3.10)	0.104
	HWE	1.000	1.000			0.593			0.765		
	N	(65)	(97)			(84)			(57)		
	GG	30.8 (20)	33.0 (32)	1.00	0.199[d]	32.1 (27)	1.00	0.179	26.3 (15)	1.00	**0.040**
rs2435200	AG	44.6 (29)	53.6 (52)	1.04 (0.50–2.16)		54.8 (46)	1.17 (0.56–2.47)		63.2 (36)	1.82 (0.77–4.27)	**(0.033, 0.024)**[d,e]
	AA	24.6 (16)	13.4 (13)	0.48 (0.19–1.23)		13.1 (11)	0.51 (0.19–1.33)		10.5 (6)	0.51 (0.16–1.62)	
	A allele	46.9 (61)	40.2 (78)	0.76 (0.47–1.22)	0.253	40.5 (68)	0.77 (0.47–1.25)	0.290	42.1 (48)	0.82 (0.48–1.41)	0.519
	HWE	0.456	0.298			0.263			0.114		

Genotype and allele frequencies are represented as percentages with the number of participants with non-missing data (N) shown in parentheses. Statistical significant differences for the control group compared to [a]all cases group, [b]clinically diagnosed subgroup and [c]multiple concussed subgroup are highlighted ($P < 0.05$). [d]Age-adjusted global *P*-values (likelihood test, Fisher's exact test) comparing genotype and allelic frequencies between control and case groups (minor vs. major: rs2435200, A vs. G; rs2435211, T vs. C). [e]Post-hoc: AA vs. GG + AG; AG vs. AA + GG. HWE are *P*-values from exact tests performed for the *TAU* polymorphisms. CI — confidence interval, HWE — Hardy–Weinberg equilibrium, OR — odds ratio.
Bold values indicate statistical significance, $p < 0.05$.

ing multiple concussions. To the author's knowledge, no previous study investigated rs2435200 (G > A) in multiple concussions or repeated head impacts. Concussion may modulate the development of neurodegenerative diseases due to clinical and potentially neuropathological similarities.[27] Interestingly, the previous association of the G allele with increased Parkinson's disease risk potentially implicates the G allele as an adverse allele[19] and is in alignment with the findings noted in this study; which identified the G allele to be associated with increased susceptibility for multiple concussions and by inference, the A allele with reduced susceptibility of sustaining multiple concussions.

Previous studies showed that the *TAU* rs2435211 T allele was associated with increased tau mRNA expression and protein levels in cerebrospinal fluid.[16,28] The presence of tau protein in the cerebrospinal fluid is a neuropathological indicator of neurodegenerative diseases including Alzheimer's disease.[6,8,17] The rs2435200 G allele was previously associated with the risk of Parkinson's disease.[19] These previous association studies implicate the rs2435211 T and rs2435200 G alleles in neurodegeneration. The biological association of the tau protein, rs2435211 T allele and rs2435200 G allele in neuropathology and neurodegenerative diseases, together with the functional supporting evidence, aligns with the current study's findings of an inferred T-G haplotype associated with increased concussion susceptibility. Although independent associations were not noted for these two functional SNPs, the inferred haplotype is highlighting a genomic interval of 8.6 kilobase pairs (NG_007398.1), which warrants further exploration. It is interesting to note, the close proximity of potential microRNA recognition binding sites (gene regulatory motifs)[15] to the rs2435211 and rs2435200 SNPs. Further interrogation is required to explore the association between potential genetic functional motifs underlying this genetic interval with concussion aetiology.

The findings of this study potentially implicate a *TAU*-associated neurodegenerative pathway as a mechanism for concussion aetiology (Supplementary Fig. S5). We propose that the rs2435211 T and rs2435200 G alleles increase *TAU* mRNA expression resulting in dysregulated tau protein production. Both tau protein dysregulation and inhibition of tau-microtubule binding can lead to tau aggregating into helical filaments and neurofibrillary tangles which promote neurotoxicity and possibly neuronal death.[10] These cellular processes can elicit the concussion signs and symptoms (e.g. memory impairments). Alternatively, the A allele of rs2435200 could decrease *TAU* mRNA expression leading to regulated tau production, which enables tau binding to microtubules and assisting in stabilising the cytoskeletal scaffold supporting neurons.

There is mounting evidence supporting the hypothesis that the repeated head impact exposure may result in cumulative production of the damaging tau protein deposits. The brain might not have sufficient time to recover from repeated high impact collisions and tau deposits accumulation over time, thereby increasing the player's vulnerability to sustaining multiple concussions. In addition, an accumulation of tau protein deposits, throughout a career-long exposure to head impacts, could elicit permanent neuronal damage and result in later life complications (e.g. dementia).[6] Notably, a greater proportion of the senior case group reported multiple previous concussions (63%, N = 63) compared to the juniors (38%, N = 24), which might explain the significant associations only observed in seniors with these *TAU* SNPs. The small proportion of juniors with multiple concussions may explain the lack of an association when all participants were combined (53%, N = 87 reported multiple concussions). Further inquiry is required in larger cohorts for the junior players.

In this study, the significant associations were only observed in seniors (amateur-level club and professional players). This observation is not unexpected as seniors, compared to juniors, have

played rugby for a longer period and consequently have potentially been exposed to higher match intensities more frequently.[21] The increased exposure to high intensity contact sport matches result in increased exposure to high impact collisions, including head impacts, as noted in seniors, and also reflected previously in other contact sports.[21] The rules of play for rugby do not differ between the junior players (school-level) and senior players (club to professional-level). However, the increased risk of cumulative impacts in senior players could be attributed to a longer playing season, faster game pace, increased aggression, and increased height and weight compared to junior players.[29]

For the current study, we focused on a history of concussion as the main outcome and not head impacts as this was not a longitudinal study on cumulative effects of concussion but rather a cross-sectional association study. The sample size for the association between the inferred rs2435211-rs2435200 haplotype and concussion history (Fig. 1) reflected approximately 88% statistical power for Type I error detection. The association is, therefore, unlikely to reflect a false positive. Whereas, the sample size for the association between rs2435200 SNP and multiple concussions (Table 2) had an approximate power of 72%, which is reduced from the standard power of 80%, and may reflect a Type I error. The findings, therefore, should be cautiously interpreted and require confirmation in a larger cohort preferably with a record of head impact exposure.

The limitations of this study were the inclusion of self-reported ancestry and suspected concussions (an average recall of concussion history of 2.5 ± 2.7 years after the concussion occurred for all participants and 3.3 ± 2.9 years for seniors). However, 86% (N = 140) of the cases from all participants and 87% (N = 87) of the senior cases were diagnosed by a medical professional. Concussion diagnosis often depends on self-reported concussion symptoms by athletes[30]; however, recall bias may influence the reliability of reporting on concussion history.

5. Conclusion

The novel finding presented in this study, although preliminary, supports the growing evidence that an increased vulnerability to a *TAU*-associated neurodegenerative response may increase an individual's risk of sustaining subsequent concussions and the resulting debilitating long-term deficits (including depression and dementia) as recently reported by Mez et al.[6] Therefore, in future, the identification of *TAU* polymorphisms associated with concussion susceptibility, with high certainty, may potentially assist in eventually improving clinical management strategies of vulnerable individuals to reduce the risk of severe later life complications and potentially improve quality of life.

Practical implications

- The *TAU* rs2435200 AA genotype may be associated with reduced susceptibility of sustaining multiple concussions in senior rugby players.
- The *TAU* rs2435200 AG genotype may be associated with increased susceptibility of sustaining multiple concussions in senior rugby players.
- The *TAU* rs2435211 SNP may not, independently, be associated with concussion history.
- The inferred *TAU* (rs2435211-rs2435200) T-G haplotype may be associated with increased susceptibility for sustaining a concussion in senior rugby players.

Acknowledgements

We would like to thank all the participants and respective authorities from the high schools, clubs, professional teams and medical practices for their time, effort and participation in our study. This study was funded by the South African National Research Foundation (A.V.S., grant number 90942), (M.P., grant numbers 93416:2015, 85534:2015). The South African National Research Foundation, the Deutscher Akademischer Austausch Dienst (DAAD) and the University of Cape Town funded S.A. and S.M.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jsams.2018.06.012.

References

1. Mccrory P, Meeuwisse W, Dvořák J et al. Consensus statement on concussion in sport — the 5th international conference on concussion in sport held in Berlin, October 2016. *Br J Sport Med* 2017; 51:838–847.
2. Guilmette TJ, Rasile D. Sensitivity, specificity, and diagnostic accuracy of three verbal memory measures in the assessment of mild brain injury. *Neuropsychology* 1995; 9(3):338–344.
3. Fuller CW, Taylor A, Raftery M. Epidemiology of concussion in men's elite Rugby-7s (Sevens World Series) and Rugby-15s (Rugby World Cup, Junior World Championship and Rugby Trophy, Pacific Nations Cup and English Premiership). *Br J Sports Med* 2015; 49(7):478–483.
4. Raghupathi R. Cell death mechanisms following traumatic brain injury. *Brain Pathol* 2004; 14(2):215–222.
5. Bogoslovsky T, Wilson D, Chen Y et al. Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid β up to 90 days after traumatic brain injury. *J Neurotrauma* 2017; 34(1):66–73.
6. Mez J, Daneshvar DH, Kiernan PT et al. Clinicopathological evaluation of chronic traumatic encephalopathy in players of American football. *JAMA* 2017; 318(4):360–370.
7. Omalu B, Bailes J, Hamilton RL et al. Emerging histomorphologic phenotypes of chronic traumatic encephalopathy in american athletes. *Neurosurgery* 2011; 69(1):173–183.
8. McKee AC, Stein TD, Nowinski CJ et al. The spectrum of disease in chronic traumatic encephalopathy. *Brain* 2013; 136(1):43–64.
9. Avila J, Lucas JJ, Perez M et al. Role of tau protein in both physiological and pathological conditions. *Physiol Rev* 2004; 84(2):361–384.
10. Bramblett GT, Goedert M, Jakes R et al. Abnormal tau phosphorylation at Ser 396 in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding. *Neuron* 1993; 10(6):1089–1099.
11. Johnson VE, Stewart JE, Begbie FD et al. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain* 2013; 136(1):28–42.
12. Breedlove KM, Breedlove EL, Robinson M et al. Detecting neurocognitive and neurophysiological changes as a result of subconcussive blows in high school football athletes. *Athl Train Sports Health Care* 2014; 6(X):1–9.
13. Panenka W, Gardner A, Dretsch M et al. Systematic review of genetic risk factors for sustaining a mild traumatic brain injury. *J Neurotrauma* 2017; 7, neu.2016.4833.
14. Smet-Nocca C, Wieruszeski J-M, Chaar V et al. The thymine–DNA glycosylase regulatory domain: residual structure and DNA binding. *Biochemistry* 2008; 47(25):6519–6530.
15. Wahid F, Shehzad A, Khan T et al. MicroRNAs: synthesis, mechanism, function, and recent clinical trials. *Biochim Biophys Acta* 2010; 1803:1231–1243.
16. Elias-Sonnenschein LS, Helisalmi S, Natunen T et al. Genetic loci associated with alzheimer's disease and cerebrospinal fluid biomarkers in a Finnish case-control cohort. *PLoS One* 2013; 8(4):e59676.
17. Mazanetz MP, Fischer PM. Untangling tau hyperphosphorylation in drug design for neurodegenerative diseases. *Nat Rev Drug Discov* 2007; 6(6):464–479.
18. Biernacka JM, Armasu SM, Cunningham JM et al. Do interactions between *SNCA*, *MAPT*, and *LRRK2* genes contribute to Parkinson's disease susceptibility? *Parkinsonism Relat Disord* 2011; 17(10):730–736.
19. Chung SJ, Armasu SM, Biernacka JM et al. Common variants in *PARK* loci and related genes and Parkinson's disease. *Mov Disord* 2011; 26(2):280–288.
20. Fahn S. Description of Parkinson's disease as a clinical syndrome. *Ann N Y Acad Sci* 2003; 991:1–14.
21. Reynolds BB, Patrie J, Henry EJ et al. Comparative analysis of head impact in contact and collision sports. *J Neurotrauma* 2016; 34(1):38–49.
22. Little J, Higgins JPT, Ioannidis JPA et al. STrengthening the REporting of genetic association studies (STREGA)—an extension of the STROBE statement. *Genet Epidemiol* 2009; 33(7):581–598.

23. Abrahams S, McFie S, Patricios J et al. An association between polymorphisms within the *APOE* gene and concussion aetiology in rugby union players. *J Sci Med Sport* 2017; 21(2):117–122, pii:S1440-2440-6.
24. Putukian M, Echemendia R, Dettwiler-danspeckgruber A et al. Prospective clinical assessment using sideline concussion assessment tool-2 testing in the evaluation of sport-related concussion in college athletes. *Clin J Sport Med* 2015; 25(1):36–42.
25. R Core Team. *R: A Language and Environment for Statistical Computing*, Vienna, Austria, R Foundation for Statistical Computing, 2017.
26. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 2004; 74(4):765–769.
27. Acosta SA, Tajiri N, de la Pena I et al. Alpha-synuclein as a pathological link between chronic traumatic brain injury and Parkinson's disease. *J Cell Physiol* 2015; 230(5):1024–1032.
28. Kauwe JSK, Cruchaga C, Mayo K et al. Variation in *MAPT* is associated with cerebrospinal fluid tau levels in the presence of amyloid-beta deposition. *Proc Natl Acad Sci U S A* 2008; 105(23):8050–8054.
29. Lee AJ, Garraway WM. Epidemiological comparison of injuries in school and senior club rugby. *Br J Sports Med* 1996; 30(3):213–217.
30. Erlanger D, Kaushik T, Cantu R et al. Symptom-based assessment of the severity of a concussion. *J Neurosurg* 2003; 98(3):477–484.