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Concussion-associated gene variant COMT rs4680 is associated with elite rugby athlete status

Concussion-associated gene variant *COMT* rs4680 is associated with elite rugby athlete status

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Abstract

Objective:

Concussions are common match injuries in elite rugby and reports exist of reduced cognitive function and long-term health consequences that can interrupt or end a playing career and produce continued ill health. The aim of this study was to investigate the association between elite rugby status and eight concussion-associated risk polymorphisms. We hypothesized that concussion-associated risk genotypes and alleles would be underrepresented in elite rugby athletes compared to non-athletes.

Design:

A case-control genetic association study.

Setting:

Institutional (university).

Participants:

Elite Caucasian male rugby athletes (n = 668, mean (standard deviation) height 1.85 (0.07) m, mass 102 (12) kg, age 29 (7) yr) and 1015 non-athlete Caucasian men and women (48% men).

Interventions:

Genotype was the independent variable, obtained via PCR of genomic DNA using TaqMan probes.

Main Outcome Measure:

Elite athlete status, with groups compared using χ^2 and odds ratio.

Results:

The *COMT* rs4680 Met/Met (AA) genotype, Met allele possession and Met allele frequency were lower in rugby athletes (24.8%, 74.6% and 49.7%, respectively) than non-athletes (30.2%, 77.6%, and 54.0%; $P < 0.05$). The Val/Val (GG) genotype was more common in elite

rugby athletes than non-athletes (odds ratio 1.39, 95% confidence interval 1.04-1.86). No other polymorphism was associated with elite athlete status.

Conclusions:

Elite rugby athlete status is associated with *COMT* rs4680 genotype that, acting pleiotropically, could affect stress resilience and behavioral traits during competition, concussion risk and/or recovery from concussion. Consequently, assessing *COMT* rs4680 genotype might aid future individualized management of concussion risk amongst athletes.

Key words; rugby, genetics, concussion, brain, polymorphism, behavior

Introduction

Rugby is a full contact high velocity collision-based team sport comprised of two differing codes, Rugby League (RL) and Rugby Union (RU). Both are characterized by multiple high-intensity collisions (RL 24-47, RU 24-89 contact events per match) [1,2]. Contact events are responsible for the prevalence of concussion in both codes of rugby [3–5]. Sport-related concussion has been defined as a form of traumatic brain injury (TBI) induced by biomechanical forces [6]. In the 2017-18 season of the English RU Premiership (the top tier of competition in England) there was a reported incidence of ~18 concussions per 1000 match hours (~0.7 concussion per match) [5]. In elite RL, concussion incidence ranges from ~15-28 concussions per 1000 player match hours [3,7].

Potential short- and long-term neurodegenerative consequences associated with concussion include increased injury risk, migraines, sleep dysfunction, anxiety, cognitive impairment, second impact syndrome, chronic post-concussion syndrome and forms of dementia [6,8–16]. These consequences impact on continuance of an athletic career, causing temporary suspension of play, early retirement and potential neuropathological consequences.

Concussion has a polygenic component due to the actions and interactions of multiple genes [17]. Two common C/T single nucleotide polymorphisms (SNPs) at residues 112 (rs429358) and 158 (rs7412) of the *apolipoprotein E* (*APOE*) gene have been associated with forms of TBI [18,19] and in combination are termed ε2/ε3/ε4. *APOE* ε4 allele could be responsible for up

to 64% of the 'hazardous influence' of TBI [18] and athletes who possess the ε4 allele suffered from prolonged physical (Cohen's *d* = 0.87) and cognitive (*d* = 0.60) symptomatic responses to concussion [20]. A promoter region SNP of *APOE* (rs405509) has been associated with quantitative impacts on APOE levels in brain tissue [21]. Carriers of the T allele (rs405509) had a 3-8-fold greater risk of experiencing repeated concussions [22,23] and TT genotype carriers experienced lower Glasgow Outcome Scale scores post-TBI [24]. In contrast, Abrahams et al. [25] reported TT genotype (rs405509) was associated with a 45% reduced risk of concussion and the T allele was associated with a rapid recovery (< 1 week) post-concussion in RU players. *Microtubule associated protein tau* (*MAPT*) TT genotype (rs10445337) has been weakly associated with a greater risk of repeated concussion [23,26]. Mutations in *MAPT* accelerate aggregation of markers of neurotoxic hyperphosphorylated tau in response to repetitive concussions by 20-60% in animal-based studies and are associated with neurodegenerative diseases in humans [27,28]. The *nitric oxide synthase 3* (*NOS3)* -786T/C polymorphism (rs2070744) has been associated with promoter region activity, reduced NO synthesis and cerebral vasospasm [29]. Approximately 20-35% lower cerebral blood flow has been reported in patients with severe TBI who carry the C allele [30].

The T allele (rs1800497) of the *ankyrin repeat and kinase domain-containing 1* (*ANKK1*) gene has been associated with a 30-40% reduction in the expression of D2 receptors within the ventral striatum [31,32]. Post-TBI T allele carriers perform worse in measures of learning, working memory and response latencies [33–35]. A polymorphism (rs6265) of the *brain derived neurotrophic factor (BDNF)* gene has been associated with neurocognitive performance post-concussion; G allele carriers performed approximately 2-6 times better in memory, executive function, attention and overall cognitive performance, both acutely and 6 months post-concussion compared to A allele carriers [36]. In addition, AA homozygotes appear to be at higher risk of sustaining a concussion than GG homozygotes (~17% of AA homozygotes suffered a concussion compared to ~4% of AG/GG) [37]. The G (Val) to A (Met) missense variation at codon 158 (rs4680) in the *catechol-O-methyltransferase* (*COMT*) gene appears to have multiple, pleiotropic effects. It is associated with behavioral traits and executive function [38], with Lipsky et al. [39] reporting that Val homozygotes performed 40% poorer on tests of executive function than Met homozygotes post-TBI. Significantly, Met-carrying RU players are reportedly ~3-fold more likely to have a history of concussion [40].

However, in addition, the *COMT* warrior/worrier theory describes the Val allele as advantageous for stress resilience (warrior) and the Met allele advantageous for cognitive function (worrier) [41,42]. Indeed, mixed martial arts professional fighter status is associated with Val/Val genotype [43], potentially due to better performance in threatening environments [44]. Thus, *COMT* could also influence rugby player behaviors, including those that affect risk of concussion.

Given the biological and clinical associations with the polymorphisms introduced here, possession of the risk alleles might limit an individual's ability to withstand exposure to the environment of competitive rugby due to an elevated risk of repeated concussions and greater risk of delayed recovery and consequent neurological impairment. Such individuals would be more likely than their peers to miss training, selection and competitive events important for career progression. Indeed, Heffernan et al. [45] previously reported an association between injury risk-associated *COL5A1* (rs12722 and rs3196378) polymorphisms and elite rugby status based on the same premise regarding career progression.

The primary aim of this study, therefore, was to investigate whether genotype frequency of suspected concussion-associated polymorphisms differed between elite rugby athletes and a non-athlete control population, and between RU playing positions. Based on published associations of the polymorphisms with concussion risk and poorer outcome following brain injury, and the interruption to competitive careers that could result, it was hypothesized that the concussion-associated risk genotypes and alleles would be underrepresented in elite rugby athletes compared to non-athletes. In other words, it was hypothesized that rugby athletes would have greater genetic resistance to concussion than non-athletes, because that would have facilitated their prolonged participation in a high-risk environment.

Methods

Participants

As part of the ongoing RugbyGene project [46], a total of 1683 individuals were recruited and gave written informed consent to participate in the present study. An *a priori* calculation for 80% power to detect a small effect size (w) of 0.1 required >785 participants and 0.12 required >546 participants. The total sample comprised 668 Caucasian elite male rugby athletes (mean (standard deviation) height 1.85 (0.07) m, mass 102 (12) kg, age 29 (7) yr) including 62.9%

British, 13.8% South African, 10.8% Irish, 8.9% Italian, and 3.6% of other nationalities, and 1015 Caucasian non-athletes (48% male, height 1.71 (0.11) m, mass 73 (13) kg, age 38 (22) yr) including 91.8% British, 6.7% South African, 1.5% other nationalities. Male and female non-athletes were suitable for genotype frequency comparison with the general population because the gene variants analyzed in this study are not sex-linked, and genotype frequencies did not differ between our male and female non-athletes. Athletes were considered elite if they had competed regularly (>5 matches) since 1995 in the highest professional league in the UK, Ireland or South Africa for RU, or the highest professional league in the UK for RL. 49.1% of the RU athletes had competed at international level for a "high performance union" (Regulation 16, http://www.worldrugby.org) and 42% of RL athletes had competed internationally. As the majority of athletes competed in RU, they were also divided into forwards and backs for comparison. Ethical approval was granted by the ethics committees of Manchester Metropolitan University, University of Glasgow, University of Cape Town and University of Northampton, and all experimental procedures complied with the Declaration of Helsinki [47].

Procedures

Sample collection. Procedures were consistent with those described previously [45,48,49]. Blood (70.4% of all samples), buccal swabs (15.4%) or saliva (14.2%) samples were obtained (dependent upon environment, location and participant preference). Blood was drawn from a superficial forearm vein into EDTA tubes, saliva samples were collected into Oragene DNA OG-500 tubes (DNA Genotek, Ottawa, Ontario, Canada), and sterile buccal swabs (Whatman OmniSwab, Springfield Mill, UK) were rubbed against the buccal mucosa of the cheek for ~30 s.

DNA isolation and genotyping. DNA isolation and genotyping were performed in the Manchester, Glasgow and Cape Town laboratories. The majority of samples were processed in the Manchester laboratory. There are some differences between protocols, summarized below.

In Manchester and Glasgow, DNA isolation was performed with the QIAamp DNA Blood Mini kit and spin column protocol (Qiagen, West Sussex, UK). Briefly, 200 μL of whole blood was lysed and incubated, the DNA washed, and the eluate stored at 4°C. In Cape Town, using a

different protocol [50], samples were lysed and centrifuged, the DNA washed, and samples stored at -20°C. DNA isolated in Cape Town was genotyped in Glasgow.

Genotyping for eight polymorphisms (see *Primers and probes*) was performed using two protocols. Protocol one: Approximately 40% of samples were genotyped using a StepOnePlus (Applied Biosystems, Paisley, UK) as previously described [48] with variations to thermocycling conditions depending on reagents used. Protocol two: Approximately 60% of samples were genotyped by combining 2 μL GTXpress Master Mix (2X) (Applied Biosystems), 0.2 μL 20X Fast GT Sample Loading Reagent (Fluidigm, Cambridge, UK), 0.2 μL H_2O and 1.6 μL of purified DNA. Furthermore, 1.78 μL assay (20X) (Applied Biosystems), 1.78 μL 2X Assay Loading Reagent (Fluidigm) and 0.18 μL ROX reference dye (Invitrogen, Paisley, UK) were combined. An integrated fluid circuit controller RX (Fluidigm) mixed samples and assays using a Load Mix (166x) script. PCR was performed using a real-time FC1 Cycler (Fluidigm) GT 192X24 Fast v1 protocol. The 192X24 microchip plate was placed into the EP1 Reader (Fluidigm) for end-point analysis using Fluidigm SNP genotyping analysis software. Duplicates of all samples were in 100% agreement for both protocols.

Genotyping assays

For *ANKK1* (rs1800497), *APOE* (rs429358, rs7412 and rs405509), *BDNF-AS* (rs6265), *COMT* (rs4680), *MAPT* (rs10445337) and *NOS3* (rs2070744), the appropriate TaqMan assays were utilised (Applied Biosystems). *APOE* ε2/ε3/ε4 data were derived from rs429358 and rs7412 [51]. The TaqMan assay context sequence for each polymorphism, with VIC/FAM highlighted in **bold** and concussion-associated risk alleles underlined (although for some the prior evidence of risk is controversial), were: *ANKK1* (rs1800497) TGGTC[**A**/**G**]AGGCA, *APOE* (rs429358) ACGTG[**C**/**T**]GCGGC, *APOE* (rs7412) AGAAG[**C**/**T**]GCCTG, *APOE* (rs405509) GTCTG[**G**/**T**]ATTAC, *BDNF-AS* (rs6265) TATCA[**C**/**T**]GTGTT, *COMT* (rs4680) CTGGC[**A**/**G**]TGAAG, *MAPT* (rs10445337) TCACT[**C**/**T**]CCCGA, *NOS3* (rs2070744) CTGGC[**C**/**T**]GGCTGA.

Data Analysis

SPSS for Windows version 26 (SPSS, Chicago, IL) software was used. Height and body mass were compared between athletes and non-athletes using independent t-tests. Pearson's χ^2

tests compared genotype and allele frequencies between athletes and non-athletes and between positional subgroups. Twenty-four comparisons per SNP (18 for *APOE* ε2/ε3/ε4) were subjected to Benjamini-Hochberg corrections [BH;52] to control false discovery rate and corrected probability values are reported. Odds ratios (OR) were calculated to estimate effect size. Alpha was set at 0.05.

Results

Genotype frequencies were in Hardy-Weinberg equilibrium for all polymorphisms in the non-athlete and athlete groups. Athletes (all male) were taller and heavier ($P < 0.05$) than the male non-athletes.

For *COMT* rs4680, the AA (Met/Met) genotype, proportion of A allele carriers and A allele were underrepresented in all athletes (24.8%, 74.6% and 49.7%, respectively) and RU athletes (24.3%, 73.1%, and 48.7%) compared with non-athletes (30.2%, 77.6%, and 54.0%, Table 1 and Fig. 1, $P \leq 0.05$). The GG (Val/Val) genotype was more common in all rugby athletes than non-athletes (OR = 1.39, 95% confidence interval (CI) = 1.04-1.86), and more common in RU athletes than non-athletes (OR = 1.49, 95% CI = 1.10-2.03). The AA genotype was underrepresented in the subgroup of RU backs compared with the non-athletes (21.1% versus 30.3%, Table 1, $P \leq 0.05$), with the GG genotype more common in RU backs (OR = 1.62, 95% CI = 1.07-2.48). However, there was no difference in genotype frequency between RU backs and forwards ($P = 0.49$).

Table 1. Genotype and allele distribution of non-athletes and athletes, including athletes separated by code (RL and RU) and into positional groups for RU. Data are genotype/allele count followed by percentage in parentheses.

Polymorphism	Genotype	All Rugby Athletes	RL Athletes	RU Athletes	RU Forwards	RU Backs	Non-athletes
***ANKK1* rs1800497**	GG	417 (65.2)	59 (58.4)	358 (66.5)	208 (66.1)	150 (66.1)	475 (65.2)
	GA	198 (31.0)	37 (36.6)	161 (29.9)	99 (31.4)	64 (28.2)	223 (30.6)
	AA	24 (3.8)	5 (5.0)	19 (3.5)	8 (2.5)	13 (5.7)	31 (4.2)
	Total	639	101	538	315	227	729
	G allele	1032 (80.8)	155 (76.7)	877 (81.5)	515 (81.7)	364 (80.2)	1173 (80.5)

	A allele	258 (20.2)	47 (23.3)	199 (18.5)	115 (18.3)	90 (19.8)	285 (19.5)
	G allele carriers	615 (96.2)	96 (95.1)	519 (96.5)	307 (97.5)	214 (94.3)	698 (95.7)
	A allele carriers	222 (34.7)	42 (41.6)	180 (33.5)	107 (34.0)	77 (33.9)	254 (34.8)
***APOE* rs405509**	GG	163 (25.8)	23 (23.0)	140 (26.3)	75 (24.4)	66 (28.8)	191 (26.2)
	GT	308 (48.7)	51 (51.0)	257 (48.3)	154 (50.2)	105 (45.9)	344 (47.3)
	TT	161 (25.5)	26 (26.0)	135 (25.4)	78 (25.4)	58 (25.3)	193 (26.5)
	Total	632	100	532	307	229	728
	G allele	634 (50.2)	97 (48.5)	537 (50.5)	304 (49.5)	237 (51.7)	726 (49.9)
	T allele	630 (49.8)	103 (51.5)	527 (49.5)	310 (50.5)	221 (48.3)	730 (50.1)
	G allele carriers	471 (74.5)	74 (74.0)	397 (74.6)	229 (74.6)	171 (74.7)	535 (73.5)
	T allele carriers	469 (74.2)	77 (77.0)	392 (73.7)	232 (75.6)	163 (71.2)	537 (73.8)
***APOE* ε2/ε3/ε4**							
	ε2/ε2	1 (0.2)	0 (0.0)	1 (0.2)	1 (0.3)	0 (0.0)	4 (0.6)
	ε2/ε3	74 (11.2)	14 (13.7)	60 (10.8)	32 (10.0)	28 (11.8)	88 (12.7)
	ε2/ε4	11 (1.7)	1 (1.0)	10 (1.8)	7 (2.2)	3 (1.3)	19 (2.7)
	ε3/ε3	393 (59.7)	51 (50)	342 (61.5)	199 (62.0)	146 (61.3)	404 (58.5)
	ε3/ε4	159 (24.2)	32 (31.4)	127 (22.8)	73 (22.7)	54 (22.7)	159 (23.0)
	ε4/ε4	20 (3.0)	4 (3.9)	16 (2.9)	9 (2.8)	7 (2.9)	17 (2.5)
	Total	658	102	556	321	238	691
	ε4 allele carriers	190 (28.9)	37 (36.3)	153 (27.5)	89 (27.7)	64 (26.9)	195 (28.2)
	Non- ε4 allele carriers	468 (71.1)	65 (63.7)	403 (72.5)	232 (72.3)	174 (73.1)	496 (71.8)
***BDNF-AS* rs6265**							
	GG	432 (67.5)	74 (73.3)	358 (66.4)	206 (65.4)	154 (67.6)	530 (66.3)
	GA	185 (28.9)	23 (22.7)	162 (30.1)	98 (31.1)	66 (28.9)	241 (30.1)
	AA	23 (3.6)	4 (4.0)	19 (3.5)	11 (3.5)	8 (3.5)	29 (3.6)
	Total	640	101	539	315	228	800
	G allele	1049 (82.0)	171 (84.7)	878 (81.4)	510 (81.0)	374 (82.0)	1301 (81.3)
	A allele	231 (18.0)	31 (15.3)	200 (18.6)	120 (19.0)	82 (18.0)	299 (18.7)
	G allele carriers	617 (96.4)	97 (96.0)	520 (96.5)	304 (96.5)	220 (96.5)	771 (96.4)
	A allele carriers	208 (32.5)	27 (26.7)	181 (33.6)	109 (34.6)	74 (32.5)	270 (33.8)
***COMT* rs4680**							
	GG	164 (25.4)	18 (17.8)	146 (26.8)	86 (27.0)	60 (26.5)	178 (22.4)
	GA	321 (49.8)	55 (54.5)	266 (48.9)	149 (46.9)	116 (51.3)	377 (47.4)
	AA	160 (24.8)*	28 (27.7)	132 (24.3)*	83 (26.1)	50 (22.1)*	241 (30.2)
	Total	645	101	544	318	226	796
	G allele	649 (50.3)	91 (45.0)	558 (51.3)	321 (50.5)	236 (52.2)	733 (46.0)

	A allele	641 (49.7)*	111 (55.0)	530 (48.7)*	315 (49.5)	216 (47.8)*	859 (54.0)
	G allele carriers	485 (75.2)	73 (72.3)	412 (75.7)	235 (73.9)	176 (77.9)	555 (69.7)
	A allele carriers	478 (74.6)	83 (82.2)	398 (73.1)	232 (73.0)	166 (73.5)	618 (77.6)
***MAPT* rs10445337**							
	TT	384 (59.6)	54 (53.5)	330 (60.8)	201 (63.8)	133 (57.4)	465 (63.9)
	TC	230 (35.7)	40 (39.6)	190 (35.0)	102 (32.4)	88 (37.9)	229 (31.5)
	CC	30 (4.7)	7 (6.9)	23 (4.2)	12 (3.8)	11 (4.7)	34 (4.7)
	Total	644	101	543	315	232	728
	T allele	998 (77.5)	148 (73.3)	850 (78.3)	504 (80.0)	354 (76.3)	1159 (79.6)
	C allele	290 (22.5)	54 (26.7)	236 (21.7)	126 (20.0)	110 (23.7)	297 (20.4)
	T allele carriers	614 (95.3)	94 (93.1)	520 (95.8)	303 (96.2)	221 (95.3)	694 (95.3)
	C allele carriers	260 (40.4)	47 (46.5)	213 (39.2)	114 (36.2)	99 (42.7)	263 (36.1)
***NOS3* rs2070744**							
	TT	239 (37.6)	36 (35.6)	203 (37.9)	115 (37.0)	91 (39.9)	282 (38.7)
	CT	303 (47.6)	50 (49.5)	251 (46.9)	145 (46.6)	106 (46.5)	323 (44.3)
	CC	94 (14.8)	15 (14.9)	81 (15.2)	51 (16.4)	31 (13.6)	124 (17.0)
	Total	636	101	535	311	228	729
	T allele	781 (61.4)	122 (60.4)	657 (61.4)	375 (60.3)	288 (63.2)	887 (60.8)
	C allele	491 (38.6)	80 (39.6)	413 (38.6)	247 (39.7)	168 (36.8)	571 (39.2)
	T allele carriers	542 (85.2)	86 (85.1)	454 (84.9)	260 (83.6)	197 (86.4)	605 (83.0)
	C allele carriers	397 (62.4)	65 (64.4)	332 (62.1)	196 (63.0)	137 (60.1)	447 (61.3)

225 The genotype and allele carrier data represent the additive, dominant and recessive models, respectively.
226 Asterisks (*) indicate lower frequency than non-athletes ($P \leq 0.05$).

227

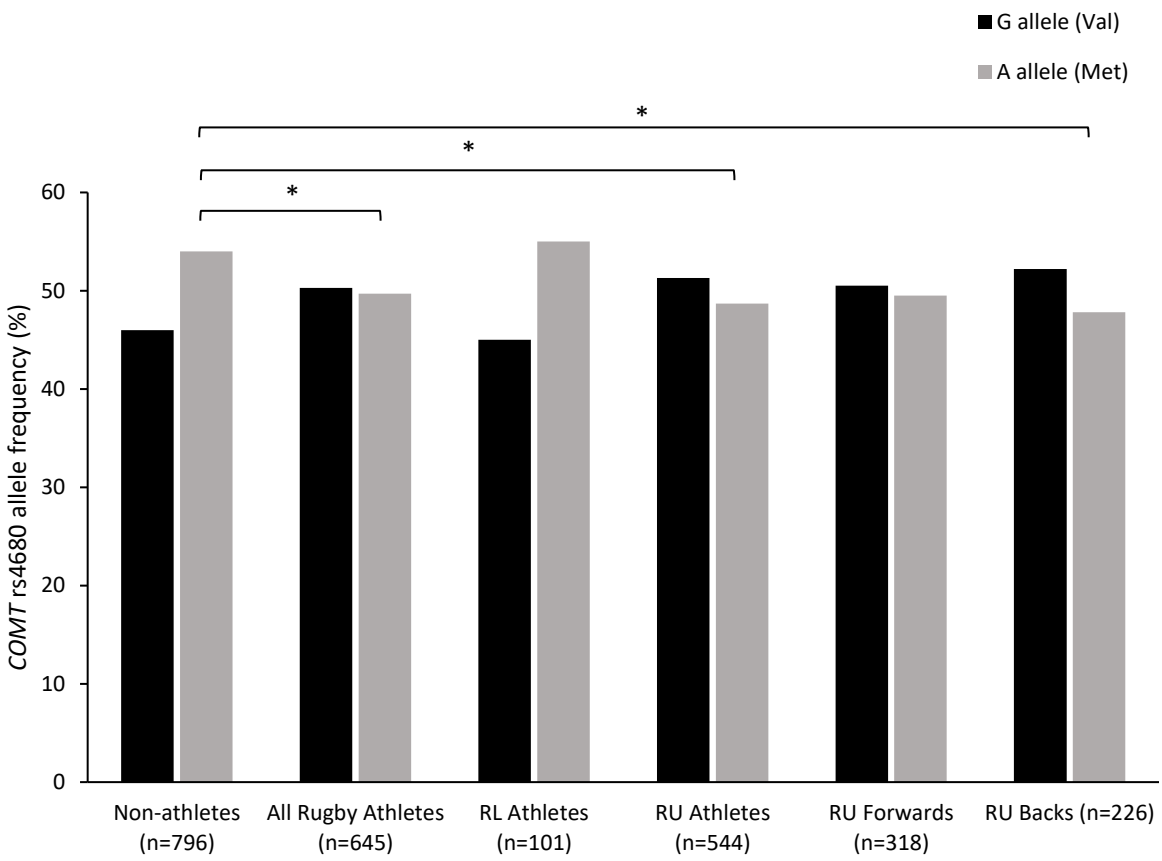

Figure 1. Allele frequency of *COMT* rs4680 for non-athletes and athlete groups. G allele = black, A allele = grey. Asterisks (*) indicate G (Val) allele more common and A (Met) allele less common in athletes than non-athletes ($P \leq 0.05$). RU, rugby union; RL, rugby league.

There were no differences in *APOE* ε2/ε3/ε4 genotype or ε4 allele possession frequency when comparing all ($P = 0.19$, $P = 0.71$), RU ($P = 0.28$, $P = 0.71$), RU forwards ($P = 0.62$, $P = 0.85$) and RU backs ($P = 0.62$, $P = 0.65$) with non-athletes (Table 1). Furthermore, no *APOE* ε2/ε3/ε4 genotype frequency or ε4 allele possession differences were observed between RU backs and forwards ($P = 0.87$, $P = 0.83$, respectively). There were no differences in *APOE* rs405509 genotype or allele frequency when comparing all groups of athletes to non-athletes (Table 1). In addition, no *APOE* rs405509 differences in genotype or allele frequency were observed between RU backs and forwards.

Similarly, there were no differences in genotype or allele frequency when comparing all athletes with non-athletes for all other polymorphisms (*ANKK1* rs1800497, *BDNF-AS* rs6265, *MAPT* rs10445337 and *NOS3* rs2070744) ($P > 0.05$; Table 1). Furthermore, no genotype frequency or allele differences were observed between RU backs and forwards for all other polymorphisms analyzed in this study (Table 1).

Discussion

The aim of this study was to investigate whether genotype frequency of eight suspected concussion-associated polymorphisms differed between elite rugby athletes and a non-athlete control population, and between RU playing positions. It was hypothesized that the concussion-associated risk genotypes and alleles would be underrepresented in elite rugby athletes compared to non-athletes, because of the interruption to competitive careers that could result. The main finding was that *COMT* rs4680 genotype was associated with elite rugby athlete status. However, the elite rugby athletes had ~1.4 times the odds of being Val/Val (GG) genotype (previously associated with poorer cognitive function post-concussion) than non-athletes, contradicting our original hypothesis. Nevertheless, *COMT* rs4680 has pleiotropic effects as the two alleles have varying associations with history of concussion and behavioral traits [40,43,44], some compatible with our observation.

Previously, *COMT* Val/Val (GG) homozygotes have been associated with poorer cognitive function than Met/Met (AA) homozygotes. Specifically, following mild TBI non-verbal cognitive function was affected [53] and following more severe TBI executive function was affected [39]. This evidence led us to suspect that possessing the Met allele would contribute to the attainment of elite status via quicker or more complete recovery following TBI after

264 the inevitable high-intensity contacts that occur during rugby. Thus, better cognitive function
265 post-concussion would facilitate rugby athletes' prolonged participation in the high
266 concussion-risk environment of competitive rugby. However, the Val/Val genotype was
267 overrepresented in elite rugby athletes (25.4%), and RU athletes separately (26.8%),
268 compared to non-athletes (22.4%) (Figure 1). *COMT* encodes an enzyme that methylates and
269 in turn deactivates catechol-based neurotransmitters such as synaptic dopamine [39].
270 Optimal cognitive function is affected by the prefrontal cortex's (PFC) sensitivity to dopamine
271 [54], which makes *COMT* a strong candidate to influence inter-individual variability in
272 cognitive function post-concussion. Chen *et al*. [55] noted Met/Met carriers had ~33%
273 decreased COMT activity (higher dopamine activity) compared to Val/Val carriers (lower
274 dopamine activity); heterozygotes had intermediate activity.

275 Furthermore, Mc Fie at al. [40] recently reported Met carriers in a cohort of youth and
276 professional South African RU players were ~3-fold more likely to have a history of
277 concussion. Elevated dopamine could increase impulsivity and risk taking, meaning Met allele
278 carriers could place themselves at increased risk of sustaining a concussion [56,57]. We found
279 the Met allele was underrepresented in elite rugby athletes (49.7%), RU athletes (48.7%), RU
280 forwards (49.5%) and RU backs (47.8%) compared to non-athletes (54.0%). Our findings are
281 therefore compatible with Mc Fie et al. [40], because lower risk of concussion via the Val allele
282 would provide less disruption to rugby training and selection, increasing the chance of long-
283 term career success. However, further replication studies are warranted to support this
284 hypothesis.

285 Professional fighters have been reported to have a higher frequency of Val/Val genotype
286 (52%) than non-athletes (20%) [43]. The higher COMT activity in the PFC of Val/Val carrying

287 professional fighters (compared to the MET carriers) is in line with the COMT warrior/worrier
288 U-shaped curve theory of excessive or insufficient dopamine in the PFC impairing cognitive
289 performance [41,42]. Previous findings indicate that Val/Val carriers performing under
290 stressful conditions would have an increased resilience to stress and be more able to cope
291 with perceived threats [41,43]. Met allele carriers have an increased reactivity to aversive
292 stimuli and relative greater cognitive performance capacity than Val carriers [41,42,58,59].
293 This greater executive function and working memory of Met allele carriers is attributed to the
294 effects of higher levels of extracellular dopamine in the PFC [41,42], although some
295 differences between sexes might also exist [60]. In addition, the Met allele has been
296 associated with experiencing anxiety and pain sensitivity characteristics unfavorable for elite
297 rugby competition in some studies [61,62] but not all [63–65].

298 For *APOE* ε2/ε3/ε4 there were no differences in ε4/ε4 genotype or ε4 allele frequency
299 between elite rugby athletes and non-athletes (Table 1). Previous findings indicate that ε4
300 allele carriers experience more severe cognitive and physical symptoms following TBI [18–
301 20], so we hypothesized that the ε4 allele would be underrepresented in elite rugby athletes
302 compared to non-athletes. However, the present data do not support that hypothesis, despite
303 elite rugby being an environment of high risk of concussion [3–5,7,66,67]. Nevertheless, it is
304 noteworthy that 28.9% of elite rugby athletes were ε4 carriers, including several of ε4/ε4
305 genotype (3.0% of all athletes), who may be at elevated risk of cognitive and physical
306 impairments post-concussion compared to non-carriers [18–20].

307 Similarly, we found no association between any other polymorphism examined in this study
308 and elite rugby athlete status. However, based on previous biological and clinical data
309 regarding those polymorphisms, our study confirms the existence of a considerable number

of athletes who appear to have a genetic predisposition for sustaining repetitive concussions and/or poorer outcomes post-concussion. For example, ~20% of elite rugby athletes could be at risk of poorer cognitive performance post-concussion due to possession of either the A allele of *ANNK1* rs1800497 or the A allele of *BDNF* rs6265 [33–36]. In addition, 60% of elite rugby athletes possess the *MAPT* rs10445337 TT genotype which could suggest a greater risk of repeated concussion [23,26] and potential risk of neurodegenerative disease [27,28]. Similarly, 60% of elite rugby athletes could experience reduced cerebral blood flow post-concussion due to possession of the *NOS3* rs20707044 C allele [29,30].

Conclusion

A considerable number of elite rugby athletes possess several concussion-associated risk alleles that should be explored further in conjunction with concussion injury data. In addition, the Val allele and Val/Val genotype of the *COMT* rs4680 polymorphism were more common in elite rugby athletes than non-athletes, suggesting an advantage for attaining elite competitive status. Based on this observation and prior literature, we propose that elite rugby athletes possessing the Val allele of *COMT* (rs4680) could be at lower risk of experiencing concussions, potentially due to greater stress resilience and reduced anxiety in threatening competitive environments. However, they might also be at increased risk of poorer cognitive function post-concussion. Consequently, we recommend continued careful monitoring of brain injury in rugby, tight adherence to return-to-play procedures, and the development of more sensitive methods for early detection of neurodegeneration, particularly in those athletes potentially at higher risk.

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