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

2 **status**

3 **Authors;** Mark R. Antrobus [1, 2,*], Jon Brazier [1, 3], Peter Callus [1], Adam J. Herbert [4], Georgina K.

4 Stebbings [1], Stephen H. Day [5], Liam P. Kilduff [6], Mark A. Bennett [6], Robert M. Erskine [7, 11],

5 Stuart M. Raleigh [8], Malcolm Collins [9], Yannis P. Pitsiladis [10], Shane M. Heffernan [6], and Alun

6 G. Williams [1, 11]

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9 **Affiliations**

10 [1] Sports Genomics Laboratory, Department of Sport and Exercise Sciences, Manchester

11 Metropolitan University, Manchester M1 5GD, UK

12 [2] Sport and Exercise Science, University of Northampton, Northampton NN1 5PH, UK

13 [3] Department of Psychology and Sports Sciences, University of Hertfordshire, Hatfield AL10

14 9AB, UK

15 [4] School of Health Sciences, Birmingham City University, Birmingham, UK

16 [5] Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton WV1

17 1LY, UK

18 [6] Applied Sports Science Technology and Medicine Research Centre (A-STEM), Faculty of

19 Science and Engineering, Swansea University, Swansea SA1 8EN, UK

20 [7] Research Institute for Sport & Exercise Sciences, Liverpool John Moores University,

21 Liverpool L3 3AF, UK

22 [8] School of Health Sciences, Coventry University, Coventry, UK

23 [9] Division of Exercise Science and Sports Medicine, Department of Human Biology,

24 University of Cape Town, Cape Town, South Africa

25 [10] FIMS Reference Collaborating Centre of Sports Medicine for Anti-Doping Research,

26 University of Brighton, Brighton, UK

27 [11] Institute of Sport, Exercise and Health, University College London, London WC1E 6BT, UK

28

29 *Correspondence: mark.antrobus@northampton.ac.uk

30 **Abstract**

31 **Objective:**

32 Concussions are common match injuries in elite rugby and reports exist of reduced cognitive

33 function and long-term health consequences that can interrupt or end a playing career and

34 produce continued ill health. The aim of this study was to investigate the association between

35 elite rugby status and eight concussion-associated risk polymorphisms. We hypothesized that

36 concussion-associated risk genotypes and alleles would be underrepresented in elite rugby

37 athletes compared to non-athletes.

38 **Design:**

39 A case-control genetic association study.

40 **Setting:**

41 Institutional (university).

42 **Participants:**

43 Elite Caucasian male rugby athletes (n = 668, mean (standard deviation) height 1.85 (0.07) m,

44 mass 102 (12) kg, age 29 (7) yr) and 1015 non-athlete Caucasian men and women (48% men).

45 **Interventions:**

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

47 probes.

48 **Main Outcome Measure:**

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

50 **Results:**

51 The *COMT* rs4680 Met/Met (AA) genotype, Met allele possession and Met allele frequency

52 were lower in rugby athletes (24.8%, 74.6% and 49.7%, respectively) than non-athletes

53 (30.2%, 77.6%, and 54.0%; $P < 0.05$). The Val/Val (GG) genotype was more common in elite

54 rugby athletes than non-athletes (odds ratio 1.39, 95% confidence interval 1.04-1.86). No

55 other polymorphism was associated with elite athlete status.

56

57 **Conclusions:**

58 Elite rugby athlete status is associated with *COMT* rs4680 genotype that, acting

59 pleiotropically, could affect stress resilience and behavioral traits during competition,

60 concussion risk and/or recovery from concussion. Consequently, assessing *COMT* rs4680

61 genotype might aid future individualized management of concussion risk amongst athletes.

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

63 **Introduction**

64 Rugby is a full contact high velocity collision-based team sport comprised of two differing

65 codes, Rugby League (RL) and Rugby Union (RU). Both are characterized by multiple high-

66 intensity collisions (RL 24-47, RU 24-89 contact events per match) [1,2]. Contact events are

67 responsible for the prevalence of concussion in both codes of rugby [3–5]. Sport-related

68 concussion has been defined as a form of traumatic brain injury (TBI) induced by

69 biomechanical forces [6]. In the 2017-18 season of the English RU Premiership (the top tier of

70 competition in England) there was a reported incidence of ~18 concussions per 1000 match

71 hours (~0.7 concussion per match) [5]. In elite RL, concussion incidence ranges from ~15-28

72 concussions per 1000 player match hours [3,7].

73 Potential short- and long-term neurodegenerative consequences associated with concussion

74 include increased injury risk, migraines, sleep dysfunction, anxiety, cognitive impairment,

75 second impact syndrome, chronic post-concussion syndrome and forms of dementia [6,8–

76 16]. These consequences impact on continuance of an athletic career, causing temporary

77 suspension of play, early retirement and potential neuropathological consequences.

78 Concussion has a polygenic component due to the actions and interactions of multiple genes

79 [17]. Two common C/T single nucleotide polymorphisms (SNPs) at residues 112 (rs429358)

80 and 158 (rs7412) of the *apolipoprotein E* (*APOE*) gene have been associated with forms of TBI

81 [18,19] and in combination are termed ε2/ε3/ε4. *APOE* ε4 allele could be responsible for up

82 to 64% of the 'hazardous influence' of TBI [18] and athletes who possess the ε4 allele suffered

83 from prolonged physical (Cohen's *d* = 0.87) and cognitive (*d* = 0.60) symptomatic responses

84 to concussion [20]. A promoter region SNP of *APOE* (rs405509) has been associated with

85 quantitative impacts on APOE levels in brain tissue [21]. Carriers of the T allele (rs405509) had

86 a 3-8-fold greater risk of experiencing repeated concussions [22,23] and TT genotype carriers

87 experienced lower Glasgow Outcome Scale scores post-TBI [24]. In contrast, Abrahams et al.

88 [25] reported TT genotype (rs405509) was associated with a 45% reduced risk of concussion

89 and the T allele was associated with a rapid recovery (< 1 week) post-concussion in RU players.

90 *Microtubule associated protein tau* (*MAPT*) TT genotype (rs10445337) has been weakly

91 associated with a greater risk of repeated concussion [23,26]. Mutations in *MAPT* accelerate

92 aggregation of markers of neurotoxic hyperphosphorylated tau in response to repetitive

93 concussions by 20-60% in animal-based studies and are associated with neurodegenerative

94 diseases in humans [27,28]. The *nitric oxide synthase 3* (*NOS3*) -786T/C polymorphism

95 (rs2070744) has been associated with promoter region activity, reduced NO synthesis and

96 cerebral vasospasm [29]. Approximately 20-35% lower cerebral blood flow has been reported

97 in patients with severe TBI who carry the C allele [30].

98 The T allele (rs1800497) of the *ankyrin repeat and kinase domain-containing 1* (*ANKK1*) gene

99 has been associated with a 30-40% reduction in the expression of D2 receptors within the

100 ventral striatum [31,32]. Post-TBI T allele carriers perform worse in measures of learning,

101 working memory and response latencies [33–35]. A polymorphism (rs6265) of the *brain*

102 *derived neurotrophic factor (BDNF)* gene has been associated with neurocognitive

103 performance post-concussion; G allele carriers performed approximately 2-6 times better in

104 memory, executive function, attention and overall cognitive performance, both acutely and

105 6 months post-concussion compared to A allele carriers [36]. In addition, AA homozygotes

106 appear to be at higher risk of sustaining a concussion than GG homozygotes (~17% of AA

107 homozygotes suffered a concussion compared to ~4% of AG/GG) [37]. The G (Val) to A (Met)

108 missense variation at codon 158 (rs4680) in the *catechol-O-methyltransferase* (*COMT*) gene

109 appears to have multiple, pleiotropic effects. It is associated with behavioral traits and

110 executive function [38], with Lipsky et al. [39] reporting that Val homozygotes performed 40%

111 poorer on tests of executive function than Met homozygotes post-TBI. Significantly, Met-

112 carrying RU players are reportedly ~3-fold more likely to have a history of concussion [40].

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

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

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

136 **Methods**

137 *Participants*

138 As part of the ongoing RugbyGene project [46], a total of 1683 individuals were recruited and
139 gave written informed consent to participate in the present study. An *a priori* calculation for
140 80% power to detect a small effect size (w) of 0.1 required >785 participants and 0.12 required
141 >546 participants. The total sample comprised 668 Caucasian elite male rugby athletes (mean
142 (standard deviation) height 1.85 (0.07) m, mass 102 (12) kg, age 29 (7) yr) including 62.9%

143 British, 13.8% South African, 10.8% Irish, 8.9% Italian, and 3.6% of other nationalities, and

144 1015 Caucasian non-athletes (48% male, height 1.71 (0.11) m, mass 73 (13) kg, age 38 (22) yr)

145 including 91.8% British, 6.7% South African, 1.5% other nationalities. Male and female non-

146 athletes were suitable for genotype frequency comparison with the general population

147 because the gene variants analyzed in this study are not sex-linked, and genotype frequencies

148 did not differ between our male and female non-athletes. Athletes were considered elite if

149 they had competed regularly (>5 matches) since 1995 in the highest professional league in

150 the UK, Ireland or South Africa for RU, or the highest professional league in the UK for RL.

151 49.1% of the RU athletes had competed at international level for a "high performance union"

152 (Regulation 16, http://www.worldrugby.org) and 42% of RL athletes had competed

153 internationally. As the majority of athletes competed in RU, they were also divided into

154 forwards and backs for comparison. Ethical approval was granted by the ethics committees

155 of Manchester Metropolitan University, University of Glasgow, University of Cape Town and

156 University of Northampton, and all experimental procedures complied with the Declaration

157 of Helsinki [47].

158 *Procedures*

159 *Sample collection.* Procedures were consistent with those described previously [45,48,49].

160 Blood (70.4% of all samples), buccal swabs (15.4%) or saliva (14.2%) samples were obtained

161 (dependent upon environment, location and participant preference). Blood was drawn from

162 a superficial forearm vein into EDTA tubes, saliva samples were collected into Oragene DNA

163 OG-500 tubes (DNA Genotek, Ottawa, Ontario, Canada), and sterile buccal swabs (Whatman

164 OmniSwab, Springfield Mill, UK) were rubbed against the buccal mucosa of the cheek for ~30

165 s.

166 *DNA isolation and genotyping.* DNA isolation and genotyping were performed in the

167 Manchester, Glasgow and Cape Town laboratories. The majority of samples were processed

168 in the Manchester laboratory. There are some differences between protocols, summarized

169 below.

170 In Manchester and Glasgow, DNA isolation was performed with the QIAamp DNA Blood Mini

171 kit and spin column protocol (Qiagen, West Sussex, UK). Briefly, 200 μL of whole blood was

172 lysed and incubated, the DNA washed, and the eluate stored at 4°C. In Cape Town, using a

173   different protocol [50], samples were lysed and centrifuged, the DNA washed, and samples

174   stored at -20°C. DNA isolated in Cape Town was genotyped in Glasgow.

175   Genotyping for eight polymorphisms (see *Primers and probes*) was performed using two

176   protocols. Protocol one: Approximately 40% of samples were genotyped using a StepOnePlus

177   (Applied Biosystems, Paisley, UK) as previously described [48] with variations to

178   thermocycling conditions depending on reagents used. Protocol two: Approximately 60% of

179   samples were genotyped by combining 2 μL GTXpress Master Mix (2X) (Applied Biosystems),

180   0.2 μL 20X Fast GT Sample Loading Reagent (Fluidigm, Cambridge, UK), 0.2 μL H_2O and 1.6 μL

181   of purified DNA. Furthermore, 1.78 μL assay (20X) (Applied Biosystems), 1.78 μL 2X Assay

182   Loading Reagent (Fluidigm) and 0.18 μL ROX reference dye (Invitrogen, Paisley, UK) were

183   combined. An integrated fluid circuit controller RX (Fluidigm) mixed samples and assays using

184   a Load Mix (166x) script. PCR was performed using a real-time FC1 Cycler (Fluidigm) GT

185   192X24 Fast v1 protocol. The 192X24 microchip plate was placed into the EP1 Reader

186   (Fluidigm) for end-point analysis using Fluidigm SNP genotyping analysis software. Duplicates

187   of all samples were in 100% agreement for both protocols.

188   **Genotyping assays**

189   For *ANKK1* (rs1800497), *APOE* (rs429358, rs7412 and rs405509), *BDNF-AS* (rs6265), *COMT*

190   (rs4680), *MAPT* (rs10445337) and *NOS3* (rs2070744), the appropriate TaqMan assays were

191   utilised (Applied Biosystems). *APOE* ε2/ε3/ε4 data were derived from rs429358 and rs7412

192   [51]. The TaqMan assay context sequence for each polymorphism, with VIC/FAM highlighted

193   in **bold** and concussion-associated risk alleles underlined (although for some the prior

194   evidence of risk is controversial), were: *ANKK1* (rs1800497) TGGTC[**A/G**]AGGCA, *APOE*

195   (rs429358) ACGTG[**C/T**]GCGGC, *APOE* (rs7412) AGAAG[**C/T**]GCCTG, *APOE* (rs405509)

196   GTCTG[**G/T**]ATTAC, *BDNF-AS* (rs6265) TATCA[**C/T**]GTGTT, *COMT* (rs4680)

197   CTGGC[**A/G**]TGAAG, *MAPT* (rs10445337) TCACT[**C/T**]CCCGA, *NOS3* (rs2070744)

198   CTGGC[**C/T**]GGCTGA.

199   **Data Analysis**

200   SPSS for Windows version 26 (SPSS, Chicago, IL) software was used. Height and body mass

201   were compared between athletes and non-athletes using independent t-tests. Pearson's χ^2

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

207

208 **Results**

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

212 For *COMT* rs4680, the AA (Met/Met) genotype, proportion of A allele carriers and A allele
213 were underrepresented in all athletes (24.8%, 74.6% and 49.7%, respectively) and RU athletes
214 (24.3%, 73.1%, and 48.7%) compared with non-athletes (30.2%, 77.6%, and 54.0%, Table 1
215 and Fig. 1, $P \leq 0.05$). The GG (Val/Val) genotype was more common in all rugby athletes than
216 non-athletes (OR = 1.39, 95% confidence interval (CI) = 1.04-1.86), and more common in RU
217 athletes than non-athletes (OR = 1.49, 95% CI = 1.10-2.03). The AA genotype was
218 underrepresented in the subgroup of RU backs compared with the non-athletes (21.1% versus
219 30.3%, Table 1, $P \leq 0.05$), with the GG genotype more common in RU backs (OR = 1.62, 95%
220 CI = 1.07-2.48). However, there was no difference in genotype frequency between RU backs
221 and forwards ($P = 0.49$).

222 **Table 1**. Genotype and allele distribution of non-athletes and athletes, including athletes separated by code (RL
223 and RU) and into positional groups for RU. Data are genotype/allele count followed by percentage in
224 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.
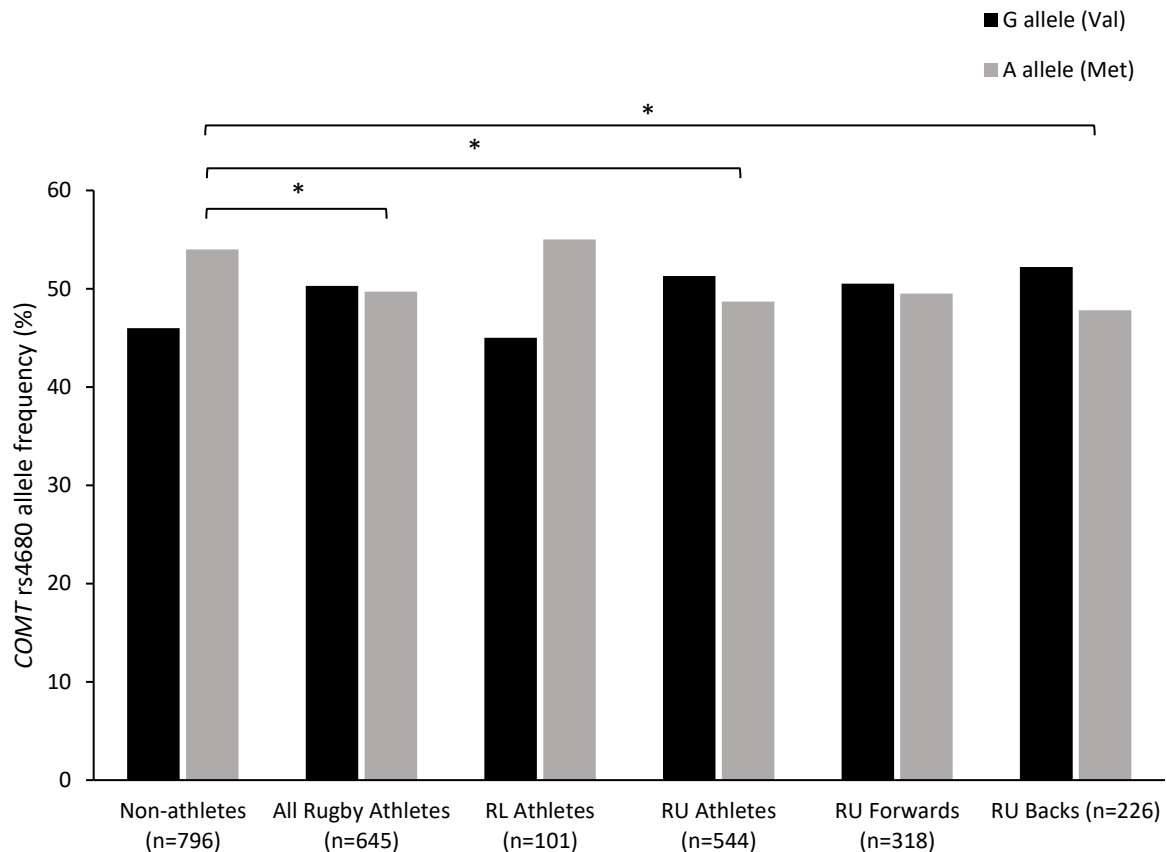226 Asterisks (*) indicate lower frequency than non-athletes ($P \leq 0.05$).

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* ≤ 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.

241 Similarly, there were no differences in genotype or allele frequency when comparing all

242 athletes with non-athletes for all other polymorphisms (*ANKK1* rs1800497, *BDNF-AS* rs6265,

243 *MAPT* rs10445337 and *NOS3* rs2070744) ($P > 0.05$; Table 1). Furthermore, no genotype

244 frequency or allele differences were observed between RU backs and forwards for all other

245 polymorphisms analyzed in this study (Table 1).

246

247 **Discussion**

248 The aim of this study was to investigate whether genotype frequency of eight suspected

249 concussion-associated polymorphisms differed between elite rugby athletes and a non-

250 athlete control population, and between RU playing positions. It was hypothesized that the

251 concussion-associated risk genotypes and alleles would be underrepresented in elite rugby

252 athletes compared to non-athletes, because of the interruption to competitive careers that

253 could result. The main finding was that *COMT* rs4680 genotype was associated with elite

254 rugby athlete status. However, the elite rugby athletes had ~1.4 times the odds of being

255 Val/Val (GG) genotype (previously associated with poorer cognitive function post-concussion)

256 than non-athletes, contradicting our original hypothesis. Nevertheless, *COMT* rs4680 has

257 pleiotropic effects as the two alleles have varying associations with history of concussion and

258 behavioral traits [40,43,44], some compatible with our observation.

259 Previously, *COMT* Val/Val (GG) homozygotes have been associated with poorer cognitive

260 function than Met/Met (AA) homozygotes. Specifically, following mild TBI non-verbal

261 cognitive function was affected [53] and following more severe TBI executive function was

262 affected [39]. This evidence led us to suspect that possessing the Met allele would contribute

263 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

14

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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