The association between *COMT* rs4680 and 5-HTTLPR genotypes and concussion history in South African rugby union players

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Acknowledgements
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 and the University of Cape Town funded S.A. and S.M.

Abstract

The objective was to investigate the relationship between *Catechol O-methyltransferase* (*COMT)* rs4680 and serotonin-transporter-linked polymorphic region (5-HTTLPR) genotypes with concussion history and personality traits. Rugby players ("all levels": n=303), from high schools ("junior", n=137) and senior amateur and professional teams ("senior", n=166), completed a concussion history questionnaire, Cloninger's Tridimensional Personality Questionnaire, and donated a DNA sample. Based on self-reported concussion history, participants were allocated into control (non-concussed, n=140), case (all) (previous suspected or diagnosed concussions, n=163), or case (diagnosed only) (previous diagnosed concussion, n=140) groups. The *COMT* rs4680 Val/Val genotype was over-represented in controls in all levels (*P*=0.013, OR:2.00, 95% CI:1.15–3.57; control:31%, case all:17%) and in juniors (*P*=0.003, OR:3.57, 95% CI:1.45–9.09; control:37%, case all:14%). The junior Val/Val participants displayed increased "anticipatory worry" (*P*=0.023). The 5-HTTLPR low expressing group was under-represented in controls when all levels were considered (*P*=0.032; OR:2.02, 95% CI:1.05–3.90; control:13%, case all:26%) and in juniors (*P*=0.021; OR:3.36, 95% CI:1.16–9.72; control:11%, case all:25%). The junior 5-HTTLPR low and intermediate expressing groups displayed decreased "harm avoidance" (*P*=0.009), "anticipatory worry" (*P*=0.041), and "fear of uncertainty" (*P*<0.001). This study provides preliminary indication that personality associated genetic variants can influence concussion in rugby.

Key words: concussion, rugby, personality, COMT, SLC6A4

Introduction

Concussion is defined as “a complex pathophysiological process affecting the brain induced by traumatic biomechanical forces” (McCrory et al., 2013). Rugby union, hereafter referred to as rugby, is a sport characterised by frequent high impact collisions and is associated with a relatively high concussion risk compared to other team sports (Koh, Cassidy, & Watkinson, 2003). In South African youth rugby, concussions occurred at an incidence of 6.8/1000 player-match-hours, approximately one concussion every five matches, and made up one third of all time-loss injuries (Mc Fie et al., 2016). Furthermore, a study reported that over 60% of New Zealand high school rugby players have a history of concussion (Sye, Sullivan, & McCrory, 2006), while 45% of adult Irish professional rugby players reported a concussion during a single monitored season (Fraas, Coughlan, Hart, & McCarthy, 2014).

The long-term effects of numerous concussion injuries are currently unknown (Raftery, 2014). However, a history of multiple concussions was associated with increased risk of permanent neurological dysfunctions (Baugh et al., 2012; Mckee et al., 2009), common mental disorders (Gouttebarge, Aoki, Lambert, Stewart, & Kerkhoffs, 2017), and mental health disturbances, including increased aggression and impulsivity (Kerr et al., 2014), in retired athletes. The relative frequency and the potential long-term effects of concussion, underline the need to identify factors that may modulate the risk of concussion in rugby.

Recently, there has been exploration into the role of genetic variation in concussion susceptibility (Panenka et al., 2017). A systematic review described three potential mechanisms by which genetic polymorphisms may influence concussion risk (Panenka et al., 2017). Firstly, genetic modulation of the underlying neurophysiology or neuroanatomy could influence an individual's resilience to neurotrauma. Secondly, genetic polymorphisms that influence the susceptibility to comorbid conditions, for example attention deficit with hyperactivity disorder, may potentially modulate concussion risk. Thirdly, genetic variation may influence an individual's personality or behavioural profile, which may increase the risk of concussion.

It has been proposed that an athlete's personality might affect their concussion susceptibility. Specifically, increased concussion risk was observed in rugby players with high impulsivity scores (Hollis et al., 2009), collegiate athletes with elevated attention impulsivity, motor impulsivity, and total risk-taking impulsivity (Beidler, 2016), and ice-hockey players who claimed to play in order get rid of excess aggression (Gerberich et al., 1987). Personality may influence how a rugby player approaches or engages in contact situations, and thereby modify their concussion susceptibility.

An individual's personality is the result of a complex interplay between genetic and environmental factors (Bouchard, 1994). Personality is in part determined by the subtle differences in neural connections, neural architecture, and neurotransmission within certain key areas of the brain, including the prefrontal cortex (Davidson, 2001; Kennis, Rademaker, &

Geuze, 2013). Polymorphisms involved in the regulation of neurotransmitter systems are the most frequently investigated candidates in genetic association studies of personality, due to their potential roles in modulating neurotransmission (Balestri, Calati, Serretti, & De Ronchi, 2014). Within those studies, two of the most routinely examined functional polymorphisms are the *catechol-O-methyltransferase* (*COMT*) rs4680 single nucleotide polymorphism (SNP) and the serotonin-transporter-linked polymorphic region (5-HTTLPR) (Balestri et al., 2014).

COMT is an enzyme responsible for the degradation of several catecholamines, including dopamine, epinephrine, and norepinephrine, from the synaptic cleft in the prefrontal cortex. A functional G>A substitution SNP, rs4680 or Val158Met, in the *COMT* gene causes an amino acid change from Valine (Val) to Methionine (Met) at codon 158. The Val allele has a three to four fold greater enzyme activity compared to the Met allele, and therefore, presumably has greater clearance of dopamine from the synaptic cleft (J. Chen et al., 2004).

This rs4680 polymorphism has a significant effect on dopaminergic neurotransmission in the prefrontal cortex (Meyer-Lindenberg et al., 2006) and has been associated with a variety of cognitive, psychiatric, behavioural, and personality measures (Montag, Jurkiewicz, & Reuter, 2012; Stein, Fallin, Schork, & Gelernter, 2005). Elevated dopamine signaling has been proposed to increase impulsive (Dalley & Roiser, 2012; Pine, Shiner, Seymour, & Dolan, 2010), risk-taking (Riba, Krämer, Heldmann, Richter, & Münte, 2008), and

novelty seeking (NS) (C R Cloninger, 1986) traits. In support, the *COMT* rs4680 Met allele, characterised by decreased dopamine degradation, was associated with increased impulsivity (Soeiro-De-Souza, Stanford, Bio, Machado-Vieira, & Moreno, 2013), risk-taking (Amstadter et al., 2012), and NS scores (Montag et al., 2012). Considering *COMT* rs4680's involvement in impulsive and risk-taking personality traits, it is reasonable to propose that the *COMT* rs4680 SNP may influence concussion susceptibility in sport.

The 5-HTTLPR polymorphism, within the serotonin transporter gene (*SLC6A4*), is a 43 base pair insertion or deletion, resulting in a short allele (S), with 14 repeat units, or a long allele (L) comprising 16 repeat units (Heils et al., 1996). The S allele results in reduced serotonin transporter expression and function (Heils et al., 1996; Lesch et al., 1996). Furthermore, within the 5-HTTLPR is an A>G SNP (*SLC6A4* rs25531) that modulates the functional effect of the 5-HTTLPR by interfering with a putative transcription factor binding site, such that the L_G allele has significantly lower expression than the L_A allele (Hu et al., 2006).

Serotonin signaling plays a significant role in personality and behaviour (Carver & Miller, 2006; Nordquist & Oreland, 2010) and the 5-HTTLPR and *SLC6A4* rs25531 variants have previously been associated with several personality measures (Balestri et al., 2014; Lee & Prescott, 2014). Increased serotonergic transmission is linked to heightened harm avoidance (HA) (C Robert Cloninger, 1987), while decreased serotonin production, achieved via tryptophan depletion, resulted in increased impulsivity measures in a number

of studies (Dalley & Roiser, 2012). The 5-HTTLPR S allele, presumably with higher serotonin signaling, has been associated with increased HA (Lesch et al., 1996), impulsivity (Sonuga-Barke et al., 2011; Walderhaug, Herman, Magnusson, Morgan, & Landro, 2010), and risk-taking measures (Stoltenberg, Lehmann, Anderson, Nag, & Anagnopoulos, 2011). Hence, it was of interest to investigate whether the 5-HTTLPR and *SLC6A4* rs25531 variants might also influence concussion susceptibility in sport.

To the best of the authors' knowledge there have been no published investigations into the role of personality-associated genetic markers and concussion susceptibility in sport. Therefore, the aim of the study was to investigate the relationship between *COMT* rs4680 and 5-HTTLPR genotypes, concussion history, and personality traits in a rugby-playing population.

Methods

Participant recruitment

The investigation followed a case-control genetic association study design and the study is presented in accordance with the "Strengthening the Reporting of Genetic Association Studies" (STREGA) statement for reporting the results of genetic association studies (Little et al., 2009). Ethical approval for the study was obtained from the Human Research Ethics Committee of the University of Cape Town. The Western Cape Education Department, and

relevant school and club administrators, granted permission to conduct research at the respective high schools and clubs. All participants gave written informed consent and, in the case of minors, informed assent from the minor and informed consent from a parent or legal guardian was obtained. Participants were assigned to control or case groups after recruitment, based on their self-reported concussion history.

A total of 693 apparently healthy male rugby players, from high schools, senior amateur-level clubs, and professional teams, were invited to participate in the study between January 2013 and August 2015. Four hundred and eighty-seven rugby players agreed to participate and completed the consent and/or assent forms. Participants were then required to (i) complete the study questionnaire containing; personal details, medical, and sporting history questionnaire, a concussion history questionnaire, and the Tridimensional Personality Questionnaire (TPQ) (C Robert Cloninger, 1987), and (ii) donate a non-invasive, self-administered buccal swab for DNA collection. The adult participants completed the consent form and study questionnaire in a single session. For the junior (under-18) participants, consent was first required from both parent and junior before the study questionnaire was completed at a subsequent time point. Junior participants who had completed consent and assent forms, but did not submit the study questionnaire were excluded (n=67).

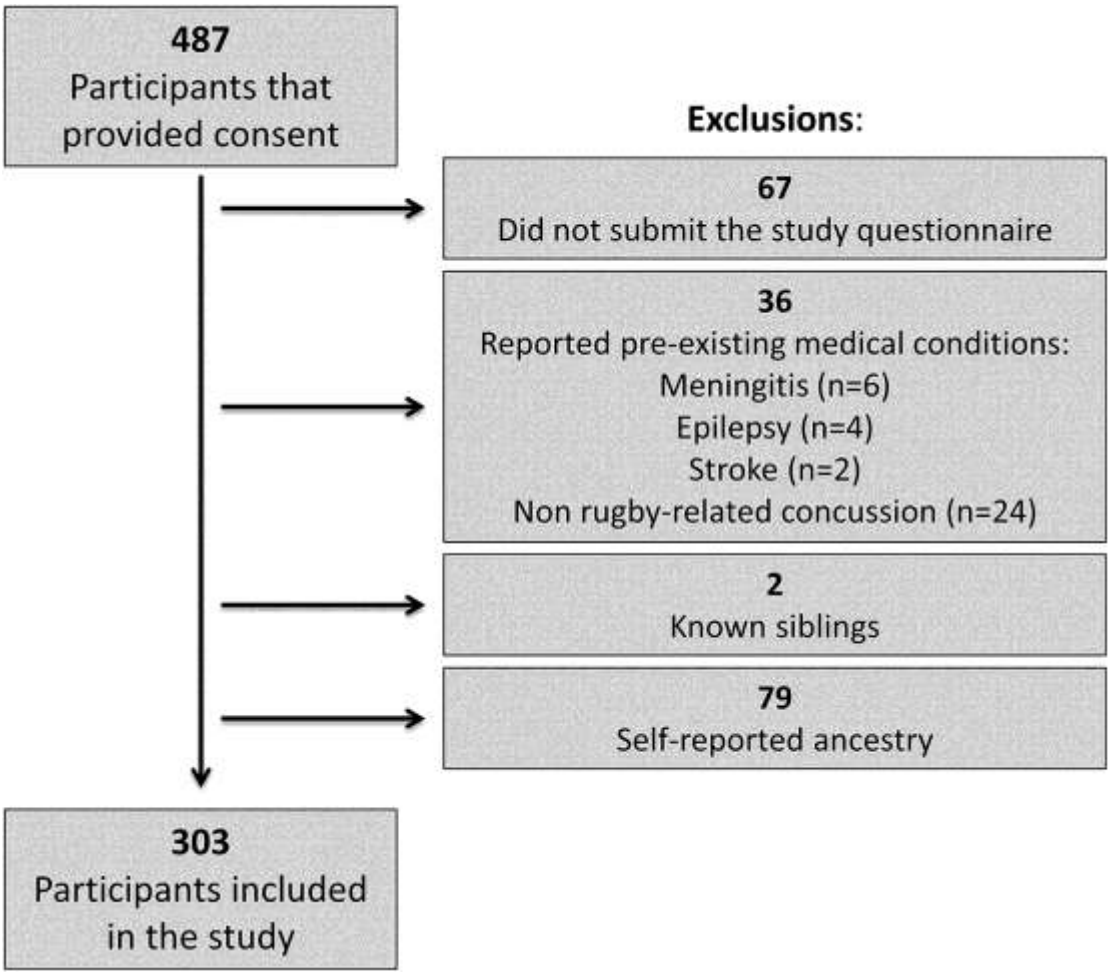

Figure 1: A diagramatic description of the participant exclusion criteria applied in the study.

The participant exclusions are summarised in Figure 1. Participants were excluded if they had previously been diagnosed with meningitis (n=6), epilepsy (n=4), stroke (n=2), or had sustained a non rugby-related concussion (n=24). Known siblings of enrolled participants were excluded (n=2). Participants were grouped according to self-reported ancestry due to potential population stratification effects, which may significantly confound the results of genetic association studies (Thomas & Witte, 2001). Because of sample size and statistical power, only participants of self-reported white ancestry were included and participants of other ethnicities were excluded (n=79).

A total of 303 participants were included in the study (aged 12 – 39 years old). Due to the proposed effect of age on personality traits (Fresán, Robles-García, López-Avila, & Cloninger, 2011) and the difference in rugby exposure between levels of play, analysis was completed on “all levels” (all participants combined: n=303, age: 19.7 ± 4.2 years), and additionally in a “junior” (high school players: n=137, age: 16.3 ± 1.3 years) and a “senior” (114 senior amateur club and 52 senior professional players: n=166, age: 22.4 ± 3.7 years) cohort.

Concussion history

Concussion was defined according to the Zurich Consensus statement (McCrory et al., 2013). Briefly, for this study, concussion was defined as a direct, or indirect blow, to the head during a rugby-related activity, that resulted in a set of clinical signs and symptoms that may or may not have involved loss of consciousness. The self-reported concussion history questionnaire required participants to provide details of their four most recent concussions. Specifically, information was collected on the date and mechanism of concussion injury, whether the concussion was diagnosed by a medical professional, the occupation of that medical professional (medical doctor, physiotherapist, nurse, paramedic), the symptoms experienced, and the duration of symptoms. Participants selected the relevant symptoms from a concussion symptom checklist, which was constructed from the Sports Concussion Assessment Tool (3rd edition) (Guskiewicz et al., 2013).

Concussions that were reported to have been diagnosed by a medical professional (doctor, physiotherapist, nurse, paramedic), and qualified with one or more concussion symptoms, were termed “diagnosed concussions”. Concussions that were not diagnosed by medical professional, but were described in conjunction with concussion symptoms, were defined as “suspected concussions”.

In this study, the control group included all individuals reporting no suspected or diagnosed concussions, while the “case (all)” group included all participants with a minimum of one suspected or diagnosed concussion. The case subgroup, “case (diagnosed only)”, only included participants with one or more diagnosed concussions, thus excluding suspected concussions. Of the 303 participants that met the inclusion criteria, 140 participants were assigned to the control group (junior: n=74; senior: n=66) and 163 participants assigned to the case (all) group (junior: n=63; senior: n=100). Of the 163 case (all) participants, 140 were additionally allocated into the case (diagnosed only) subgroup (junior: n=53; senior: n=87).

Medical and sporting history

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

TPQ

Cloninger's TPQ assesses three dimensions of personality, namely NS, HA, and reward dependence (RD), each with four subscales (Table I) (C Robert Cloninger, 1987). NS is defined as "a heritable tendency toward frequent exploratory activity and intense excitement in response to novel stimuli" and is indicative of risk-taking and impulsive behavior. HA is the "heritable tendency to avoid aversive situations" and is described as a measure of the perceived risk and anxiety of a given situation. RD is "a tendency to respond intensely to signals of reward" (C Robert Cloninger, 1987).

Participants without a self-reported English language comprehension did not complete the TPQ (n=18). Within the TPQ, there were two validation items (blank questions with true and false answer boxes) to test for carelessness and inaccuracy of responses. If one, or both, of these validation items were answered the participant was removed from the TPQ analysis (n=43). Of the 303 participants, 243 (80%) completed the TPQ and were included in the analysis (junior: n=93, 69%; senior n=150, 90%).

DNA extraction

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

Table I

Personality characteristics for high and low scoring for the novelty seeking (NS), harm avoidance (HA), and reward dependence (RD) dimensions, and each of the four corresponding dimension subscales, as measured by the Tridimensional Personality Questionnaire (TPQ).

Table adapted from Cloninger et al. 1987.

Dimension	Low	High	Items (n)
NS	Deliberate and reserved	Risk taking and impulsive	33
NS1	Stoic rigidity	Increased exploratory excitability	8
NS2	Reflection	Impulsive	8
NS3	Reserved	Extravagant	7
NS4	Regimented	Disorderly	10
HA	Carefree and confident	Cautious and worried	34
HA1	Uninhibited optimism	Increased anticipatory worry	10
HA2	Confident	Increased fear of uncertainty	7
HA3	Gregarious	Shy with strangers	7
HA4	Vigourous	Increased fatigability and asthenia	10
RD	Detached and independent	Sentimental and social	29
RD1	Insensitive	Sentimental	5
RD2	Irresolute	Persistent	8
RD3	Detached	Attached	11
RD4	Independent	Dependent	5

Genotyping

DNA was genotyped, for the *COMT* rs4680, 5-HTTLPR, and *SLC6A4* rs25531 genetic polymorphisms, using standard polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis as previously described (van Breda, Collins, Stein, & Rauch, 2015; Wendland, Martin, Kruse, Lesch, & Murphy, 2006).

Negative controls (no DNA added) and two samples of known genotypes (positive controls) were included in each 96-well PCR plate to detect potential DNA contamination. Two independent investigators confirmed all genotypes. All laboratory work was completed at the Division of Exercise Science and Sports Medicine within the University of Cape Town.

Statistics

Power analysis was calculated using QUANTO version 1.2.4 (http://biostats.usc.edu/software) to determine adequate sample size. The minimum sample of control and case pairs calculated to be sufficient to detect an odds ratio of 2.0, at 80% statistical power and 5% significance level, was between 71 and 74 for both *COMT* rs4680 (minor allele frequency of 0.4 to 0.5) and the 5-HTTLPR (minor allele frequency 0.4 to 0.5). Statistical analysis was performed using the language and environment R statistical program (R Core Team, 2013), STATA® version 13 (Stata Corporation, College Station, TX, USA), and STATISTICA version 13 (StatSoft Inc., Tulsa, OK, USA). All

figures were created using GraphPad Prism statistical software version 5.03 (GraphPad Software, San Diego, CA, USA).

The Shapiro Wilk test was used to investigate whether the data sets were normally distributed. All participant characteristic data and TPQ dimension scores were normally distributed. A One-way Analysis of Variance (ANOVA), with Tukey's *post-hoc* test, was used to observe differences in participant characteristics data between groups.

The genetics and SNPassoc packages in R (González et al., 2007; Warnes & Leisch, 2006) were used to calculate the Hardy-Weinberg equilibrium (HWE) probabilities and genotype or allele frequency distributions. Using the SNPassoc package, a Pearson's Chi-squared test was used to compare differences in genotype and allele frequencies between control and case groups and calculate the resultant odds ratios (OR) with 95% confidence intervals (95% CI). Genotype and allele frequency analyses were adjusted for age as a possible confounding covariate. Based on the previously reported effect of the rs25531 SNP on serotonin transporter gene transcription levels (Hu et al., 2006), the S_A/S_A (n=49; 18%) genotypes were classified as the low expressing group ("low": n=49; 18%); the S_A/L_A (n=129, 47%), S_A/L_G (n=18, 7%), L_A/L_G (n=21, 8%), and L_G/L_G (n=0, 0%) were grouped as the intermediate expressing group ("intermediate": n=168, 62%); and L_A/L_A (n=56, 21%) genotypes were termed the high expressing group ("high": n=56, 21%). The combined 5-HTTLPR and *SLC6A4* rs25531 genotype will hereafter be referred to as the 5-HTTLPR genotype.

A Chi-squared test for trend was completed on the genotype frequencies across participants reporting zero, one, and two or more concussions, to investigate possible genotype effects on the number of reported concussions.

COMT rs4680 and 5-HTTLPR were analysed as an allele construct using the haplo.score package in R (Schaid, Rowland, Tines, Jacobson, & Poland, 2002). The allele construct analysis combined the *COMT* and 5-HTTLPR genotype data to infer frequencies for each potential *COMT* and 5-HTTLPR allele construct (Met-S_A, Met-L_A, Met-L_G, Val-S_A, Val-L_A, and Val-L_G) in the control and case groups. A Pearson's Chi-squared test was then used to compare the frequencies of allele constructs between control and case groups

A one-way analysis of covariance (ANCOVA), with age as a covariate, was used to examine the relationship between TPQ scores and genotype groups. If significant differences (P<0.05) were seen in dimension (NS, HA, and RD) scores, then analysis was completed on dimension subscales and the results included in tables. A review of the codominant *COMT* rs4680 Val>Met polymorphism found an overall association between *COMT* rs4680 and NS and HA traits when the Met allele carriers (Met/Met and Met/Val genotypes) were compared to the Val/Val individuals (Montag et al., 2012). Therefore in this study, a combined *COMT* rs4680 Met/Met and Met/Val genotype group was compared to the Val/Val genotype for the TPQ analysis. Due to the dominant effect of the 5-HTTLPR S allele (Hu et al., 2006) a

combined low and intermediate 5-HTTLPR group was compared to the high genotype group.

The collective interactions between concussion history groups (control, case all, and case diagnosed only), specific genotypes (*COMT* rs4680 or 5-HTTLPR), and personality traits (NS, HA, and RD) were further investigated using generalized structural equation modeling. Concussion history was coded in the model as the case (all), or case (diagnosed only), group compared to the control group. Separate models were computed for *COMT* rs4680 and 5-HTTLPR. The model analysis tested whether each genotype was associated with concussion history or TPQ dimension when compared to a designated reference genotype. The *COMT* rs4680 Val/Val genotype and the 5-HTTLPR low genotype group were selected as the reference genotypes for the *COMT* rs4680 and 5-HTTLPR models, respectively. *P* values less than 0.05 were accepted as statistically significant.

Results

Participant characteristics

Combining all levels of play, the control group (age: 18.8 ± 3.6 years, n=140; weight: 87.7 ± 16.1 kg, n=136) was younger and lighter than the case (all) group (age: 20.4 ± 4.5 years, n=163, *P*=0.001; weight: 91.8 ± 16.8 kg, n=158, *P*=0.035, Table II). The junior control and case (all) groups were similar in age (*P*=0.721), but the senior control group (age: 21.7 ± 3.3 years, n=66) was

Table II

The general participant characteristics of junior (high school) and senior (senior amateur club and professional) rugby players without a previous suspected or diagnosed concussion (control), with a minimum of one suspected or diagnosed concussion (case all), and additionally case (all) participants with one or more diagnosed concussions (case diagnosed only).

Level of play	Control	Case (all)	*P* value[a]	Case (diagnosed only)	*P* valu
All Levels					
n	140	163		140	
Age (years)	18.8 ± 3.6 (140)	20.4 ± 4.5 (163)	**0.001**	20.2 ± 4.2 (140)	**0.00**
Height (m)	1.82 ± 0.08 (136)	1.83 ± 0.08 (161)	0.140	1.83 ± 0.09 (138)	0.19
Weight (kg)	87.7 ± 16.1 (136)	91.8 ± 16.8 (158)	**0.035**	91.8 ± 17.5 (135)	**0.04**
BMI (kg/m^2)	26.6 ± 3.6 (133)	27.2 ± 3.7 (155)	0.151	27.3 ± 3.8 (132)	0.14
Rugby exposure (years)	10.9 ± 4.1 (140)	11.2 ± 4.5 (162)	0.586	11.0 ± 4.7 (139)	0.86
Junior					
n	74	63		53	
Age (years)	16.3 ± 1.3 (74)	16.4 ± 1.4 (63)	0.721	16.2 ± 1.4 (53)	0.87
Height (m)	1.80 ± 0.09 (71)	1.80 ± 0.08 (61)	0.698	1.80 ± 0.08 (51)	0.99
Weight (kg)	79.9 ± 15.5 (71)	81.7 ± 16.5 (59)	0.545	80.7 ± 17.3 (49)	0.80
BMI (kg/m^2)	25.0 ± 3.5 (68)	25.1 ± 4.0 (56)	0.806	25.0 ± 4.1 (46)	0.95
Rugby exposure (years)	8.8 ± 2.7 (74)	8.8 ± 3.1 (63)	0.875	8.5 ± 3.3 (53)	0.60
Senior					
n	66	100		87	
Age (years)	21.7 ± 3.3 (66)	22.9 ± 3.8 (100)	**0.038**	22.6 ± 3.5 (87)	0.09
Height (m)	1.84 ± 0.08 (65)	1.85 ± 0.08 (100)	0.452	1.85 ± 0.08 (87)	0.43

Weight (kg)	96.2 ± 12.0 (65)	97.8 ± 13.9 (99)	0.436	98.2 ± 14.3 (86)	0.36
BMI (kg/m^2)	28.3 ± 2.9 (65)	28.4 ± 2.9 (99)	0.835	28.5 ± 3.0 (86)	0.71
Rugby Exposure (years)	13.3 ± 4.2 (66)	12.6 ± 4.7 (99)	0.377	12.5 ± 4.8 (86)	0.30

Data are expressed as mean ± standard deviation with the number of participants with non-missing data (n) in parentheses. Unadjusted *P* values for [a]control versus case (all) and [b]control versus case (diagnosed only) are displayed. Statistical significant differences (*P*<0.05) between control and case groups are displayed in bold. BMI: Body Mass Index.

younger than the senior case (all) group (age: 22.9 ± 3.8 years, n=100, *P*=0.038, Table II). The control and case (all) groups within the junior and senior cohorts were similar in height (junior: *P*=0.698; senior: *P*=0.452), weight (junior: *P*=0.545; senior: *P*=0.436), body mass index (BMI) (junior: *P*=0.806; senior: *P*=0.835), and rugby exposure (junior: *P*=0.875; senior: *P*=0.377, Table II).

There were no significant *COMT* rs4680 or 5-HTTLPR genotype effects on age (*COMT* rs4680: *P*=0.514; 5-HTTLPR: *P*=0.140), height (*COMT* rs4680: *P*=0.266; 5-HTTLPR: *P*=0.882), weight (*COMT* rs4680: *P*=0.988; 5-HTTLPR: *P*=0.714), BMI (*COMT* rs4680: *P*=0.718; 5-HTTLPR: *P*=0.573), or rugby exposure (*COMT* rs4680: *P*=0.865; 5-HTTLPR: *P*=0.111).

COMT rs4680 genotype and allele frequency distributions

All levels. When all participants were combined, the *COMT* rs4680 genotype distribution was significantly different between the control and the case (all) (*P*=0.040) and case (diagnosed only) (*P*=0.041) groups. Specifically, the Val/Val genotype was over-represented in the control group (31%, n=41) compared to the case (all) group (17%, n=27, Val/Val vs. Met/Met + Met/Val: *P*=0.013, OR: 2.00, 95% CI: 1.15 – 3.57) and the case (diagnosed only) subgroup (18%, n=24, Val/Val vs. Met/Met + Met/Val: *P*=0.022, OR: 1.92, 95% CI: 1.09 – 3.45; Figure 2a). Furthermore, there was a general linear trend for the Val/Val genotype frequency to decrease across participants

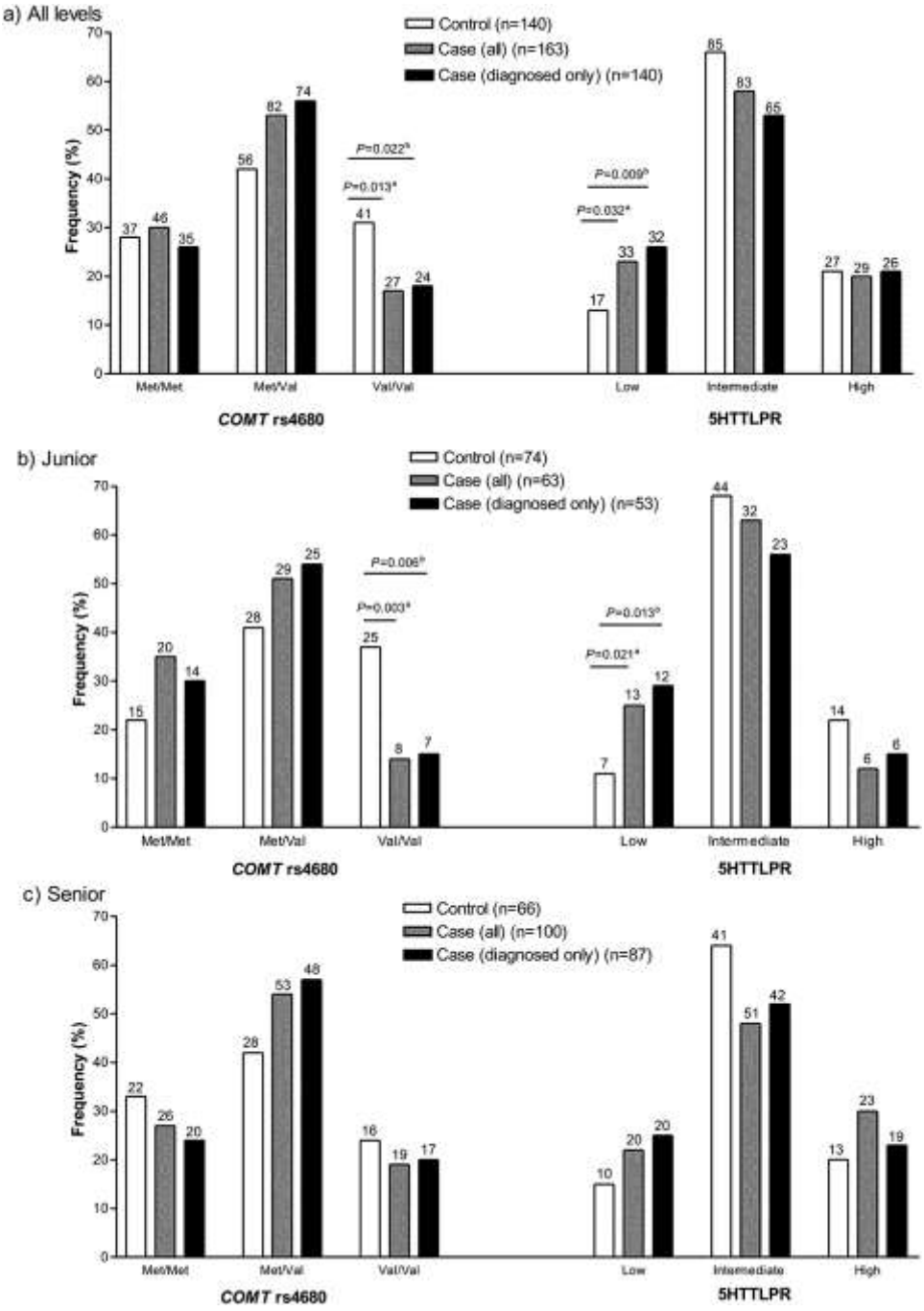

Figure 2: The *COMT* rs4680 and 5-HTTLPR, in conjunction with *SLC6A4* rs25531 ("low": S_A/S_A; "intermediate": S_A/L_A, S_A/L_G, L_A/L_G, L_G/L_G; "high": L_A/L_A), genotype frequency distributions between rugby players without a previous suspected or diagnosed concussion (control), with a minimum of one suspected or diagnosed concussion (case all), and case (all) participants with one or more diagnosed concussions (case diagnosed only) when **a)** all participants (junior and senior) were combined, **b)** in the junior cohort (high school players), and **c)** in the senior cohort (senior amateur and professional players). Significant (P<0.05) age-adjusted P values and the number of non-missing participants in each group are displayed for [a]control vs. case (all) group and [b]control vs. case (diagnosed only) subgroup.

reporting zero (31%, n=41), one (20%, n=14), or two or more concussions (15%, n=13, *P*=0.007).

Junior. When only the junior participants were analysed, the *COMT* rs4680 genotype distribution was significantly different between junior control and junior case (all) (*P*=0.012) and case (diagnosed only) (*P*=0.024) groups, with the Val/Val genotype similarly over-represented in the junior control group (37%, n=25) compared to the junior case (all) group (14%, n=8, Val/Val vs. Met/Met + Met/Val: *P*=0.003, OR: 3.57, 95% CI: 1.45 – 9.09) and junior case (diagnosed only) subgroup (15%, n=7, Val/Val vs. Met/Met + Met/Val: *P*=0.006, OR: 3.45, 95% CI: 1.35 – 9.09; Figure 2b). Furthermore, the Val allele was over-represented in the junior control (57%, n=78) group compared to the junior case (all) group (39%, n=45, *P*=0.007) and junior case (diagnosed only) subgroup (42%, n=39, *P*=0.037). There was also a general linear trend for the Val/Val genotype frequency to decrease across junior participants reporting zero (37%, n=25), one (15%, n=5) and two or more concussions (13%, n=3, *P*=0.007).

Senior. No significant differences in the *COMT* rs4680 genotype frequency distributions were noted between the senior control and the senior case groups (case all: *P*=0.333; case diagnosed only: *P*=0.210; Figure 2c). The *COMT* rs4680 polymorphism was in HWE for all control and case groups (*P*>0.05).

5-HTTLPR genotype and allele frequency distributions

All levels. The 5-HTTLPR genotype frequency distributions were not significantly different between the control and case (all) group (*P*=0.101), but there was a difference between the control and the case (diagnosed only) subgroup (*P*=0.028). Further analysis revealed that the 5-HTTLPR low group was under-represented in the control group (13%, n=17) compared to both the case (all) group (22%, n=32, low vs. intermediate + high: *P*=0.032, OR: 2.02, 95% CI: 1.05 – 3.90) and case (diagnosed only) subgroup (25%, n=31, low vs. intermediate + high: *P*=0.009, OR: 2.39, 95% CI: 1.22 – 4.65; Figure 2a). There were no significant general linear trends in the 5-HTTLPR frequency distributions across those participants reporting zero, one, or two or more concussions (*P*=0.075).

Junior. Similarly in the junior cohort, there were differences in the 5-HTTLPR genotype frequencies between the junior control and the junior case (all) (*P*=0.046) and junior case (diagnosed only) (*P*=0.042) groups. The 5-HTTLPR low group was under-represented in the junior control (11%, n=7) compared the junior case (all) (26%, n=13, low vs. intermediate + high: *P*=0.021, OR: 3.36, 95% CI: 1.16 – 9.72) and junior case (diagnosed only) subgroup (29%, n=12, low vs. intermediate + high: *P*=0.012, OR: 3.86, 95% CI: 1.30 – 11.52; Figure 2b). However, there were no significant linear trends in the frequency 5-HTTLPR groups across those reporting zero, one, or two or more concussions (*P*=0.103).

Senior. There were no significant differences in the 5-HTTLPR genotype frequencies between senior control and senior case groups (case all: *P*=0.564; case diagnosed only: *P*=0.313; Figure 2c). The 5-HTTLPR polymorphism was in HWE for all control and case groups (*P*>0.05).

Allele construct distribution

All levels. Inferred allele constructs were created from *COMT* rs4680 and 5-HTTLPR genotype data. Of the possible constructs only four (Met-S_A, Met-L_A, Val-S_A, and Val-L_A) were expressed at a frequency above 4% in all analyses. When all levels of play were combined, the Met-S_A allele construct was significantly under-represented in the control group (19%) compared to the case (all) group (32%, additive model: *P*=0.001, hap.score=3.19) and case (diagnosed only) subgroup (33%, additive model: *P*=0.003, hap.score=2.95).

Junior. The Met-S_A allele construct was also significantly under-represented in the junior control group (19%) compared to the case (all) group (34%, additive model: *P*=0.003, hap.score=3.26) and case (diagnosed only) subgroup (33%, additive model: *P*=0.005, hap.score=2.84). Furthermore, the Val-L_a allele construct was over-represented in junior control (31%, n=23) compared to the junior case (all) group (17%, n=10, additive model: *P*=0.005, hap.score=-2.78) and junior case (diagnosed only) subgroup (16%, n=8, additive model: *P*=0.015, hap.score=-2.43).

Senior. There were no differences in the frequency distribution of allele constructs in the senior cohort (*P*>0.05*)*.

COMT rs4680, 5-HTTLPR, and TPQ

All levels. There were no significant differences in the three TPQ dimension scores between *COMT* rs4680 (Met/Met + Met/Val vs. Val/Val, NS: *P*=0.865; HA: *P*=0.354; RD: *P*=0.279) and 5-HTTLPR (low + intermediate vs. high, NS: *P*=0.727; HA: *P*=0.795; RD: *P*=0.250; Table III) genotypes in all levels of play combined.

Junior. There were no significant differences in NS (*P*=0.179), HA (*P*=0.059) or RD (*P*=0.101; Table III) between the junior Met/Met and Met/Val genotype group and the junior Val/Val genotype. Although, within the HA subscales, the junior Val/Val group (HA1: 4.7 ± 2.4, n=20) had statistically higher HA1 ("anticipatory worry") scores than the junior Met/Met and Met/Val group (HA1: 3.4 ± 2.0, n=64, Met/Met + Met/Val vs. Val/Val: *P*=0.023; Table III).

The junior 5-HTTLPR low and intermediate groups (HA: 10.9 ± 5.4, n=63) had lower total HA compared to the 5-HTTLPR high group (HA: 15.3 ± 5.7, n=12, low + intermediate vs. high: *P*=0.009). Of the HA subscales, the junior 5-HTTLPR low and intermediate groups (HA1: 3.4 ± 2.1, n=66; HA2: 2.4 ± 1.7, n=66) had lower HA1 ("anticipatory worry") and HA2 ("fear of uncertainty") compared to the high 5-HTTLPR group (HA1: 4.8 ± 2.5 n=12, low +

Table III

The mean novelty seeking (NS), harm avoidance (HA), and reward dependence (RD) dimension and subscale scores, as measured by the Tridimensional Personality Questionnaire (TPQ), for South African White participants in the combined *COMT* rs4680 Met/Met and Met/Val or the Val/Val genotype groups and in the 5-HTTLPR, in conjunction with *SLC6A4* rs25531, low (S_A/S_A) and intermediate (S_A/L_A, S_A/L_G, L_A/L_G, L_G/L_G) or High (L_A/L_A) genotype group.

	***COMT* rs4680 genotype**			**5-HTTLPR genotype**		
	Met/Met and Met/Val	Val/Val	*P* value[a]	Low and intermediate	High	*P* value[b]
All levels						
n	182	52		176	43	
NS	15.9 ± 4.8 (167)	15.8 ± 5.6 (49)	0.865	16.0 ± 5.1 (162)	15.6 ± 4.8 (42)	0.727
HA	10.4 ± 5.7 (169)	11.4 ± 6.0 (50)	0.354	10.7 ± 5.5 (165)	10.7 ± 6.7 (43)	0.795
RD	18.6 ± 4.3 (170)	19.3 ± 3.9 (49)	0.279	18.6 ± 4.2 (166)	19.6 ± 4.1 (39)	0.250
Junior						
n	66	20		68	12	
NS	15.8 ± 4.4 (58)	14.3 ± 4.0 (20)	0.179	15.9 ± 4.4 (62)	13.4 ± 3.0 (11)	0.083
NS1	4.5 ± 1.4 (65)	3.9 ± 1.6 (20)	0.079	4.5 ± 1.5 (67)	3.6 ± 1.3 (12)	0.058
NS2	3.6 ± 1.9 (62)	2.8 ± 1.9 (20)	0.065	3.7 ± 1.8 (65)	2.4 ± 1.7 (11)	**0.036**
NS3	2.3 ± 1.3 (63)	2.7 ± 1.6 (20)	0.291	2.3 ± 1.4 (67)	3.0 ± 1.2 (11)	0.103
NS4	5.2 ± 2.0 (64)	5.0 ± 1.6 (20)	0.606	5.3 ± 1.9 (66)	4.5 ± 1.8 (12)	0.154
HA	11.1 ± 5.5 (59)	13.8 ± 5.4 (20)	0.059	10.9 ± 5.4 (63)	15.3 ± 5.7 (12)	**0.009**
HA1	3.4 ± 2.0 (64)	4.7 ± 2.4 (20)	**0.023**	3.4 ± 2.1 (66)	4.8 ± 2.5 (12)	**0.041**
HA2	2.5 ± 1.7 (62)	3.1 ± 1.8 (20)	0.193	2.4 ± 1.7 (66)	4.2 ± 1.3 (12)	**<0.001**
HA3	2.5 ± 2.2 (65)	3.0 ± 2.1 (20)	0.409	2.4 ± 2.1 (67)	3.5 ± 2.3 (12)	0.053

HA4	2.9 ± 2.4 (64)	3.1 ± 2.1 (20)	0.794	2.9 ± 2.3 (66)	2.8 ± 2.4 (12)	0.994
RD	17.4 ± 4.3 (59)	19.3 ± 4.2 (20)	0.101	17.6 ± 4.4 (61)	18.9 ± 3.7 (11)	0.296
Senior						
n	116	32		109	32	
NS	16.0 ± 5.1 (109)	16.9 ± 6.3 (29)	0.482	16.1 ± 5.5 (100)	16.4 ± 5.1 (31)	0.618
HA	10.0 ± 5.8 (110)	9.8 ± 5.9 (30)	0.787	10.6 ± 5.6 (102)	8.9 ± 6.2 (31)	0.186
RD	19.3 ± 4.2 (111)	19.3 ± 3.8 (29)	0.957	19.2 ± 4.0 (105)	19.8 ± 4.3 (28)	0.485

Data are expressed as mean ± standard deviation with the number of participants with non-missing data (n) in parentheses. Age-adjusted *P* values are displayed for [a]Val/Val vs. Met/Met + Met/Val and [b]low (S_AS_A) + intermediate (S_AL_A, S_AL_G, L_AL_G, L_GL_G) vs. high (L_AL_A) genotype groups. Statistically significant differences ($P<0.05$) are displayed in bold. NS1: exploratory excitability; NS2: impulsiveness; NS3: extravagance; NS4: disorderliness; HA1: anticipatory worry; HA2: fear of uncertainty; HA3: shyness/shyness with strangers; HA4: fatigability and asthenia.

intermediate vs. high: *P*=0.041; HA2: 4.2 ± 1.3, n=12, low + intermediate vs. high: *P*<0.001; Table III).

Senior. There were no differences in TPQ dimensions between the *COMT* rs4680 (NS: *P*=0.482; HA: *P*=0.787; RD: *P*=0.101) and 5-HTTLPR (NS: *P*=0.618; HA: *P*=0.186; RD: *P*=0.485; Table III) genotype groups in the senior cohort.

TPQ and concussion history

All levels. TPQ scores were similar between the control and case groups when junior and senior levels of play were combined (case all: NS: *P*=0.598; HA: *P*=0.348; RD: *P*=0.951; case diagnosed only: NS: *P*=0.763; HA: *P*=0.481; RD: *P*=0.485; Table IV).

Junior. The junior control group (HA: 12.9 ± 5.8, n=46) had higher HA than the junior case (all) (HA: 9.7 ± 5.0, n=39, *P*=0.007) and junior case (diagnosed only) groups (HA: 9.5 ± 4.8, n=31, *P*=0.004; Table IV). Specifically, the HA subscales HA1 (“anticipatory worry”) and HA2 (“fear of uncertainty”) were higher in the junior control group (HA1: 4.1 ± 2.2, n=49; HA2: 3.0 ± 1.9, n=48) than junior case (all) (HA1: 3.0 ± 1.9, n=41, *P*=0.016; HA2: 2.1 ± 1.5, n=40, *P*=0.013) and junior case (diagnosed only) groups (HA1: 2.9 ± 1.7, n=33, *P*=0.008; HA2: 1.9 ± 1.4, n=32, *P*=0.006; Table IV). There were no differences between junior control and case groups in NS or RD (case all: NS:

Table IV

The Tridimensional Personality Questionnaire dimension scores, novelty seeking (NS), harm avoidance (HA), reward dependence (RD), of junior (high school) and senior (senior amateur club and professional players) rugby players without a previous suspected or diagnosed concussion (control), with a minimum of one suspected or diagnosed concussion (case all), and additionally case (all) participants with one or more diagnosed concussions (case diagnosed only).

Data are expressed as mean ± standard deviation with the number of participants with non-

Level of play	Control	Case (all)	*P* value[a]	Case (diagnosed only)	*P* value[b]
All Levels					
n	140	163		140	
NS	16.1 ± 5.2 (100)	15.6 ± 4.9 (122)	0.598	15.8 ± 4.9 (103)	0.763
HA	10.3 ± 5.8 (102)	10.7 ± 5.8 (125)	0.348	10.6 ± 5.7 (105)	0.481
RD	18.7 ± 4.0 (99)	18.8 ± 4.4 (127)	0.951	19.2 ± 4.2 (109)	0.485
Junior					
n	74	63		53	
NS	15.4 ± 4.7 (46)	15.3 ± 3.9 (37)	0.901	15.2 ± 3.9 (30)	0.825
HA	12.9 ± 5.8 (46)	9.7 ± 5.0 (39)	**0.007**	9.5 ± 4.8 (31)	**0.004**
HA1	4.1 ± 2.2 (49)	3.0 ± 1.9 (41)	**0.016**	2.9 ± 1.7 (33)	**0.008**
HA2	3.0 ± 1.9 (48)	2.1 ± 1.5 (40)	**0.013**	1.9 ± 1.4 (32)	**0.006**
HA3	2.9 ± 2.2 (49)	2.3 ± 2.0 (42)	0.106	2.4 ± 2.1 (34)	0.149
HA4	3.1 ± 2.3 (49)	2.5 ± 2.3 (41)	0.244	2.5 ± 2.3 (33)	0.228
RD	17.7 ± 3.9 (44)	18.2 ± 4.9 (40)	0.770	18.7 ± 4.9 (33)	0.423
Senior					
n	66	100		87	
NS	16.6 ± 5.6 (54)	15.7 ± 5.3 (85)	0.481	16.0 ± 7.3 (73)	0.651
HA	8.1 ± 4.9 (56)	11.2 ± 6.0 (86)	**<0.001**	11.1 ± 5.9 (74)	**0.002**
HA1	2.1 ± 1.7 (57)	3.1 ± 2.1 (89)	**0.003**	3.1 ± 2.1 (76)	**0.004**
HA2	2.0 ± 1.9 (58)	2.5 ± 1.7 (91)	0.103	2.4 ± 1.6 (78)	0.263
HA3	1.8 ± 1.7 (59)	2.6 ± 2.1 (89)	**0.018**	2.5 ± 2.1 (77)	**0.038**
HA4	2.1 ± 2.1 (58)	3.0 ± 2.4 (90)	**0.004**	3.1 ± 2.3 (77)	**0.004**
RD	19.5 ± 4.0 (55)	19.1 ± 4.2 (87)	0.654	19.5 ± 4.0 (76)	0.955

missing data (n) in parentheses. Age-adjusted *P* values for [a]control versus case (all) and [b]control versus case (diagnosed only) are displayed. Statistical significant differences (*P*<0.05) between control and case groups are displayed in bold. HA1: anticipatory worry; HA2: fear of uncertainty; HA3: shyness/shyness with strangers; HA4: fatigability and asthenia.

P=0.901; RD: *P*=0.770; case diagnosed only: NS: *P*=0.825; RD: *P*=0.423; Table IV).

Senior. Conversely, the senior control group (HA: 8.1 ± 4.9, n=56) had lower HA compared to senior case (all) (HA: 11.2 ± 6.0, n=86, *P*<0.001) and senior case (diagnosed only) (HA: 11.1 ± 5.9, n=74, *P*=0.002) groups, with HA subscales HA1 ("anticipatory worry"), HA3 ("shyness/shyness with strangers"), and HA4 ("fatigability and asthenia") also being significantly lower in the senior control group (HA1: 2.1 ± 1.7, n=57; HA3: 1.8 ± 1.7, n=59; HA4: 2.1 ± 2.1, n=58) than the senior case (all) (HA1: 3.1 ± 2.1, n=89, *P*=0.003; HA3: 2.6 ± 2.1, n=89, *P*=0.018; HA4: 3.0 ± 2.4, n=90, *P*=0.004) and senior case (diagnosed only) groups (HA1: 3.1 ± 2.1, n=76, *P*=004; HA3: 2.5 ± 2.1, n=77, *P*=0.038; HA4: 3.1 ± 2.3, n=77, *P*=0.004, Table IV). NS and RD were similar between senior control and case groups (case all: NS: *P*=0.481; RD: *P*=0.654; case diagnosed only: NS: *P*=0.651; RD: *P*=0.955; Table IV).

Modeling the collective relationship between COMT rs4680 genotype, concussion history, and TPQ scores

Generalized structural equation modeling was used to evaluate the collective relationship between specific genotypes (*COMT* rs4680 or 5-HTTLPR), concussion history (control versus case all; control versus case diagnosed only), and personality traits (NS, HA, and RD). Due to the previous significant associations observed in this study regarding HA, the relationship between

concussion history, genotype, and HA personality scores will be reported, while all modeling results are displayed in Supplementary tables SI and SII.

All levels. When all participants were evaluated, there was a significant association between *COMT* rs4680 genotypes and concussion history, while no other associations were noted (Figure 3, Supplementary table SI). Specifically, the *COMT* rs4680 Met/Val genotype was associated with concussion history, compared to the Val/Val genotype, when comparing the control group to the case (all) (Met/Val vs. Val/Val: *P*=0.049, coefficient: 0.72, SE: 0.36) and case (diagnosed only) groups (Met/Val vs. Val/Val: *P*=0.047, coefficient: 0.75, SE: 0.38; Figure 3).

Junior. When only the juniors were examined, HA was associated with concussion history, but no other significant findings were noted (Figure 3, Supplementary table SI). HA differed between the junior control and the case group (all) (control vs. case all: *P*=0.027, coefficient: -0.11, SE: 0.05) and case (diagnosed only) subgroup (control vs. case diagnosed only: *P*=0.026, coefficient: -0.12, SE: 0.06; Figure 3).

Senior. Significant associations were found between the HA and concussion history, however, no other associations were recorded in the senior cohort (Figure 3, Supplementary table SI). Specifically, HA was significantly different between the senior control and the senior case (all) group (control vs. case all: *P*=0.005, coefficient: 0.10, SE: 0.04) and case (diagnosed only) subgroup

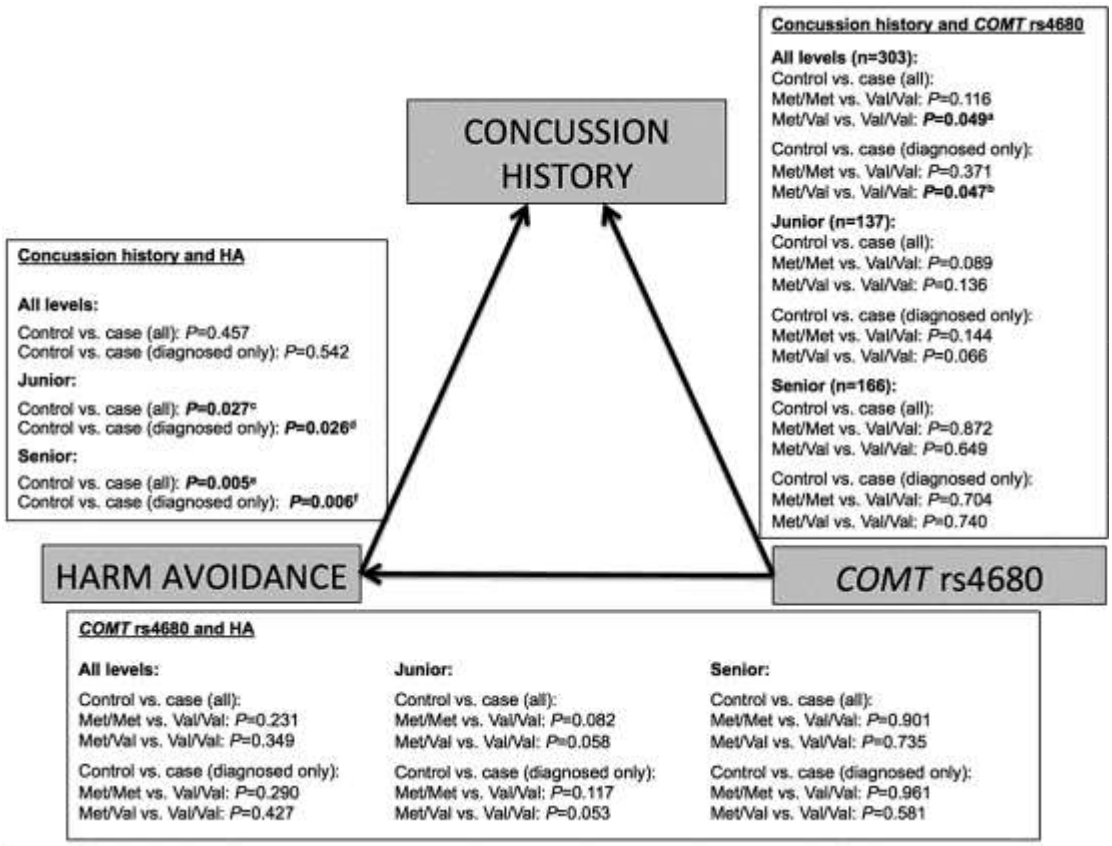

Figure 3: The results of the generalized structural equation model analysis that assessed the collective relationship between COMT rs4680 Val>Met genotype, concussion history, and harm avoidance (HA) personality trait scores in South African rugby players without a previous diagnosed or suspected concussion (control), with a diagnosed or suspected concussion (case all), and additionally case (all) participants with a diagnosed concussion (case diagnosed only). Separate models were constructed for the control versus case (all) group and the control group versus the case (diagnosed only) subgroup in each level of play (junior: high school rugby players; senior: senior community-level amateur and senior professional rugby players; all levels: junior and senior rugby players). The COMT rs4680 Met/Val genotype was associated with concussion history, compared to the Val/Val genotype in all levels of play, when [a]the control group was compared to the case (all) group (Met/Val vs. Val/Val: P=0.049, coefficient: 0.72, SE: 0.36) and [b]when the control group was compared to the case (diagnosed only) subgroup (Met/Val vs. Val/Val: P=0.047, coefficient: 0.75, SE: 0.38). HA differed between the [c]junior control and case (all) group (P=0.027, coefficient: -0.11, SE: 0.05), [d]the junior control and case (diagnosed only) subgroup (P=0.026, coefficient: -0.12, SE: 0.06), [e]the senior control and case (all) group (P=0.005, coefficient: 0.10, SE: 0.04), and [f]the senior control and case (diagnosed only) subgroup (P=0.006, coefficient: 0.11, SE: 0.04). SE: standard error

(control vs. case diagnosed only: *P*=0.006, coefficient: 0.11, SE: 0.04; Figure 3).

Modeling the collective relationship between 5-HTTLPR genotype, concussion history, and TPQ scores

All levels. When all levels of play were combined, there were significant associations between 5-HTTLPR genotype groups and concussion history, whilst no associations were noted (Figure 4, Supplementary table SII). The 5-HTTLPR intermediate (low vs. intermediate: *P*=0.011, coefficient: -1.06, SE: 0.42) and high (low vs. high: *P*=0.042, coefficient: -1.01, SE: 0.50) groups were associated with concussion history, compared to the low genotype group, but only when the control group was compared to the case (diagnosed only) subgroup (Figure 4).

Junior. In the junior cohort, HA differed between concussion history groups and the 5-HTTLPR genotype was associated with HA, but no significant associations were noted between 5-HTTLPR genotype and concussion history (Figure 4, Supplementary table SII). Specifically, the junior 5-HTTLPR high group was associated with HA compared to the 5-HTTLPR low group (control vs. case all, low vs. high: *P*=0.043, coefficient: 4.20, SE: 2.08; control vs. case diagnosed only, low vs. high: *P*=0.028, coefficient: 4.62, SE: 2.10; Figure 4). HA was significantly different between the junior control and case (diagnosed only) subgroup (*P*=0.049, coefficient: -0.12, SE: 0.06; Figure 4).

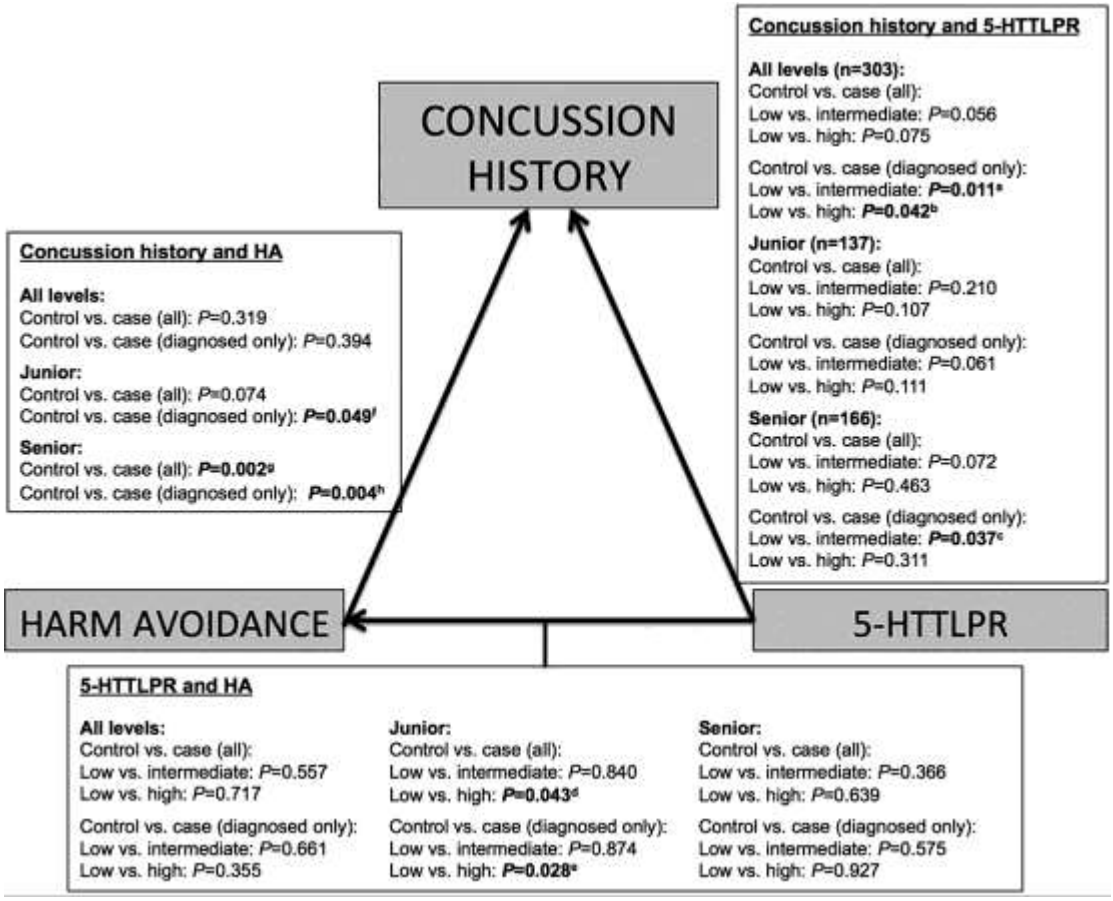

Figure 4: The summarized results of the generalized structural equation model analysis that assessed the collective relationship between serotonin-transporter-linked polymorphic region (5-HTTLPR), in conjunction with SLC6A4 rs25531, genotype groups ("low": S_A/S_A; "intermediate": S_A/L_A, S_A/L_G, L_A/L_G, L_G/L_G; "high": L_A/L_A), concussion history, and harm avoidance (HA) personality trait scores in South African rugby players without a previous diagnosed or suspected concussion (control), with either a diagnosed or suspected concussion (case all), and case (all) participants with diagnosed concussions (case diagnosed only). Separate models were constructed for the control versus case (all) group and the control group versus the case (diagnosed only) subgroup in each level of play (junior: high school rugby players; senior: senior amateur and senior professional rugby players; all levels: junior and senior rugby players). When the control group was compared to the case (diagnosed only) subgroup in all levels of play, the 5-HTTLPR [a]intermediate (low vs. intermediate: P=0.011, coefficient: -1.06, SE: 0.42) and [b]high (low vs. high: P=0.042, coefficient: -1.01, SE: 0.50) groups were associated with concussion history. [c]The 5-HTTLPR intermediate group was associated with concussion history when the control group was compared to the case (diagnosed only) subgroup in the senior cohort (low vs. intermediate: P=0.037, coefficient: -1.19, SE: 0.57). The junior 5-HTTLPR high group was associated with HA, compared to the 5-HTTLPR low group, when the junior control group was compared to the junior [d]case (all) (low vs. high: P=0.043, coefficient: 4.20, SE: 2.08) and [e]case (diagnosed only) subgroup (low vs.

high: P=0.028, coefficient: 4.62, SE: 2.10). HA was significantly different between [f]the junior control and case (diagnosed only) subgroup (P=0.049, coefficient: -0.12, SE: 0.06), the senior control and [g]case (all) group (P=0.004, coefficient: 0.12, SE: 0.04), and [h]the senior control and case (diagnosed only) subgroup (P=0.002, coefficient: 0.12, SE: 0.04). SE: standard error.

Senior. In the senior cohort, 5-HTTLPR and HA were both associated with concussion history, but there were no significant associations between 5-HTTLPR genotypes and HA (Figure 4, Supplementary table SII). The 5-HTTLPR intermediate group was associated with concussion history only when the control group was compared to the case (diagnosed only) subgroup in the senior cohort (low vs. intermediate: *P*=0.037, coefficient: -1.19, SE: 0.57; Figure 4). HA was significantly different between the senior control and the case (all) (*P*=0.004, coefficient: 0.12, SE: 0.04) and case (diagnosed only) subgroup (*P*=0.002, coefficient: 0.12, SE: 0.04; Figure 4).

Discussion

The main finding of this study was that the *COMT* rs4680 Val>Met and the 5-HTTLPR genotype frequencies significantly differed, both independently and as an inferred allele construct, between concussion history groups. Specifically, the *COMT* rs4680 Met allele carriers (Met/Met and Met/Val genotypes) and the low expressing 5-HTTLPR genotype group (S_A/S_A genotype) were twice as likely to have a history of concussion compared to other genotypes. In addition, the Met-S_A *COMT-SLC6A4* inferred allele construct was over-represented in those with a concussion history. Similar genetic associations were noted in the junior, but not senior cohorts.

When junior and senior participants were combined there were no associations between personality traits and concussion history groups. However, when splitting the participants by level of play, the junior case groups displayed significantly lower HA scores than the junior control group. There is emerging evidence linking increased risk-taking behaviour and lower injury risk appraisal to heightened injury susceptibility in sport. A recent study of over 1200 college students found that athletes with a previous concussion had higher risk-taking impulsivity and sensation seeking trait scores (Beidler, 2016). Specifically in rugby, players with high impulsivity measures also had increased rates of concussion (Hollis et al., 2009). In the present cohort, junior players with a concussion history had significantly lower HA scores, indicative of decreased risk appraisal. Lower levels of risk appraisal have been shown to result in increased risk-taking and impulsive behavior (Morrongiello & Matheis, 2007; Siegel et al., 1994). It is therefore reasonable to propose that increased risk-taking behaviour, due to low risk appraisal or elevated impulsivity, may lead to heightened concussion risk in the junior rugby participants.

The senior case groups exhibited higher HA scores compared to the senior control group, in contrast to the findings in the junior cohort. Furthermore, the associations between genotypes and concussion history were largely absent in this cohort. Personality traits strongly influence the choice of recreational activities in adulthood (Gomà-i-Freixanet, Martha, & Muro, 2012). Therefore at the senior level, where rugby participation is more of a personal preference, individuals with high risk appraisal may represent a

natural, self selective exclusion from the cohort, reducing the chance of associations. Furthermore, the senior cohort included professional players and it may be argued at the elite level personality traits may have a very low influence on the style of play or subsequent injury risk. However, the sample size of this study is a limitation particularly when the cohorts were stratified by age. Further investigation is thus required to understand the conflicting findings between the levels of play.

In this study, the *COMT* rs4680 Met/Met and Met/Val participants were 2 and 3.6 times more likely to report a previous concussion when all levels of play were combined and in the junior cohort, respectively. In addition, there was a general linear trend for the frequency of the Met allele carriers to increase across those reporting zero, one, or two or more concussions. In the model analysis, which collectively assessed the relationships between *COMT* genotype, concussion history, and personality scores, the Met/Val genotype was associated with concussion, compared to the Val/Val genotype, but only when all levels of play were combined.

Elevated dopamine signaling is proposed to increase impulsive (Dalley & Roiser, 2012; Pine et al., 2010) and risk-taking (Riba et al., 2008) traits and therefore the Met allele, with decreased dopamine clearance, should theoretically be linked to greater impulsivity and risk-taking. In support, the Met allele was previously associated with higher risk taking propensity (Amstadter et al., 2012), and elevated self-reported impulsivity scores (Soeiro-De-Souza et al., 2013) in healthy adults. Hence, the increased concussion susceptibility in *COMT* rs4680 Met carriers may possibly be due to increased

risk-taking and impulsive traits, which have previously been suggested to increase concussion susceptibility in sport (Beidler, 2016; Hollis et al., 2009).

The associations between the *COMT* rs4680 and the personality traits measured in this study were inconsistent across the levels of play, with an absence of significant findings outside of the junior cohort and in the modeling analysis. The junior Met allele carriers tended to have lower total HA and had significantly lower “anticipatory worry” (HA1) compared to the junior Val/Val genotype, suggesting that the junior Met carriers may have decreased risk appraisal, which has been linked to risk-taking behaviour (Morrongiello & Matheis, 2007; Siegel et al., 1994). Previous studies have implicated both the Val (C. Chen et al., 2011; Kim, Kim, Kim, Lee, & Kim, 2006) and the Met (Enoch, Xu, Ferro, Harris, & Goldman, 2003; Hashimoto, Noguchi, Hori, & Ohi, 2007) allele with increased HA scores. The variability in the previously reported associations is supposedly due to differences in sex, ethnicity, pre-existing diagnosis, and sample size between study populations (C. Chen et al., 2011; Montag et al., 2012). Therefore, the findings of the study need to be interpreted with caution until further research has comprehensively established, firstly, the role of *COMT* rs4680 on HA, impulsive, and risk-taking traits, and secondly, whether the *COMT* rs4680 SNP influences behaviours leading to concussion on the rugby field.

In addition to the *COMT* rs4680 polymorphism, the 5-HTTLPR genotypes also differed between concussion history groups. The 5-HTTLPR low groups were 2 and 3.4 times more likely to have a history of concussion

when in all participants were combined and in the junior cohort, respectively. In partial support, the modeling analysis found significant associations between the 5-HTTLPR intermediate and high genotype groups with diagnosed concussion history in all levels of play, but not in the junior cohort.

The 5-HTTLPR S allele has been linked to higher levels of impulsive and risk-taking behaviours in a number of studies (Aluja, Garcia, Blanch, De Lorenzo, & Fibla, 2009; Hallikainen et al., 1999; Kogan et al., 2010; Paaver et al., 2007; Paaver, Kurrikoff, Nordquist, Oreland, & Harro, 2008; Sonuga-Barke et al., 2011; Walderhaug et al., 2010). Therefore, there is evidence that the S_A/S_A homozygous participants, that comprise the low expressing 5-HTTLPR group, may exhibit higher impulsive or risk-taking behaviours. Following on, it may thus be plausible that, similar to *COMT* rs4680, the increased concussion susceptibility observed in the 5-HTTLPR low genotype group may be related to increased risk-taking and impulsive behaviours. Although it must be noted that several studies have also failed to show a relationship between 5-HTTLPR and impulsivity (Lage et al., 2011; Malloy-Diniz et al., 2011; Patkar et al., 2002).

The associations between 5-HTTLPR and HA were only evident in the junior cohort. Specifically, the combined low and intermediate 5-HTTLPR genotype groups had lower total HA, “anticipatory worry”, and “fear of uncertainty”, while the collective modeling analysis associated the junior 5-HTTLPR high (L_A/L_A genotype) group with HA compared to the 5-HTTLPR low genotype group. These associations suggest that junior carriers of the S_A

allele may have had lower risk appraisal. However, the absence of significant findings across all levels of play requires these results to be interpreted cautiously.

The association between the low 5-HTTLPR group and increased HA appears to be is in contrast with the previous evidence. Six meta-analysis studies have investigated the relationship between 5-HTTLPR and HA. Three found a significant association between the S or S_A allele and increased HA (Minelli, Bonvicini, Scassellati, Sartori, & Gennarelli, 2011; Munafò et al., 2003; Munafò, Clark, & Flint, 2005), while the remaining studies found no significant relationships (Munafo et al., 2009; Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004). The exact reasons for the contrasting findings are unknown, but previous inconsistencies between studies have been attributed to sample heterogeneity, small sample sizes, population stratification effects, the incorporation of the *SLC6A4* rs25531 SNP, and comorbid psychiatric or medical diagnosis (Balestri et al., 2014). Therefore, similar to *COMT* rs4680, the findings regarding 5-HTTLPR needs to be interpreted with caution until further research has more thoroughly established whether, or how, the 5-HTTLPR might affect personality traits, rugby-related behaviours, and concussion susceptibility in rugby players.

Although this study highlighted novel genetic associations with concussion, there were several limitations. The main limitation was that the concussion history was self-reported. Several external factors, including concussion knowledge, attitudes, and age (Register-Mihalik et al., 2013), may

influence concussion reporting. Future studies should implement a large-scale prospective design with medical doctor confirmed diagnosis. In addition, the study did not directly measure rugby-specific behaviors and therefore investigation is still required to identify whether, or how, the *COMT* rs4680 and 5-HTTLPR genotypes affect playing style on the rugby field, and to what extent risky or dangerous behavior contributes to concussion in rugby.

Overall, the study findings suggest that concussion risk in rugby may be modulated by personality traits and personality-associated genetic polymorphisms. Specifically, we hypothesize that junior rugby players with lower HA scores may more easily partake risky or dangerous play, while individuals with high HA may be more cautious, thereby reducing their exposure to possible concussion causing impacts. Education interventions highlighting the risks of reckless or dangerous play may therefore be beneficial to combat concussion, especially in youth rugby. Furthermore, rule changes aimed to limit aggressive and reckless play in ice hockey were found to reduce injury rates by 3 to 12 fold (Cusimano, Nastis, & Zuccaro, 2012), and hence may also be beneficial in rugby to discourage risky play and thereby possibly reduce concussions. On an individual level, coaches and medical staff should aim to assess whether personality traits, or dangerous on-field behaviours, are contributing to the heightened susceptibility of concussion observed in rugby players that sustain frequent concussions.

In conclusion, this study provided provisional evidence for two novel genetic risk factors for concussion and further implicated impulsive and risk-

taking behaviours in concussion susceptibility. Further research is required to clarify the effect of *COMT* rs4680 and 5-HTTLPR on rugby specific behaviours and their relation to concussion risk.

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Supplementary tables

Table SI:

The results of the generalized structural equation model analysis that assessed the collective relationship between concussion history, novelty seeking (NS), harm avoidance (HA), and reward dependence (RD) personality scores, and *COMT* rs4680 Val>Met genotype in junior (high school) and senior (senior community-level amateur and senior professional) rugby participants without a previous suspected or diagnosed concussion (control) compared to those with a minimum of one suspected or diagnosed concussion (case all) or case (all) participants with one or more diagnosed concussions (case diagnosed only). Separate models were constructed for the control versus case (all) group (control vs. case all) and the control group compared to the case (diagnosed only) subgroup (control vs. case diagnosed only) in each level of play (all levels, junior, and senior).

		Control vs. case (all)				Control vs. case (diagnosed only)			
		Coefficient	**SE**	**95% CI**	***P* value**	**Coefficient**	**SE**	**95% CI**	***P* value**
All levels									
NS									
COMT rs4680	Met/Val	-0.03	0.86	-1.71 to 1.66	0.975	0.24	0.88	-1.48 to 1.96	0.784
	Met/Met	0.25	0.96	-1.63 to 2.14	0.792	0.13	1.01	-1.85 to 2.11	0.896
HA									
COMT rs4680	Met/Val	-0.92	0.99	-2.86 to 1.01	0.349	-0.80	1.01	-2.77 to 1.17	0.427
	Met/Met	-1.30	1.09	-3.44 to 0.83	0.231	-1.21	1.14	-3.44 to 1.03	0.290
RD									
COMT rs4680	Met/Val	-0.60	0.73	-2.02 to 0.83	0.414	-0.57	0.73	-2.00 to 0.85	0.430

	Met/Met	-0.79	0.81	-3.44 to 0.83	0.329	-0.50	0.83	-2.12 to 1.12	0.543
Concussion history									
NS		-0.02	0.03	-0.08 to 0.04	0.461	-0.02	0.03	-0.08 to 0.04	0.592
HA		0.02	0.03	-0.03 to 0.07	0.457	0.02	0.03	-0.04 to 0.07	0.542
RD		0.02	0.04	-0.05 to 0.09	0.575	0.04	0.04	-0.03 to 0.12	0.253
COMT rs4680	Met/Val	0.72	0.36	0.00 to 1.43	**0.049**	0.75	0.38	0.01 to 1.48	**0.047**
	Met/Met	0.63	0.40	-0.16 to 1.42	0.116	0.38	0.42	-0.45 to 1.21	0.371
Junior									
NS									
COMT rs4680	Met/Val	1.40	1.18	-0.92 to 3.72	0.237	1.60	1.21	-0.78 to 3.97	0.188
	Met/Met	1.57	1.32	-1.01 to 4.15	0.234	1.29	1.40	-1.46 to 4.04	0.359
HA									
COMT rs4680	Met/Val	-2.85	1.50	-5.80 to 0.09	0.058	-3.02	1.56	-6.08 to 0.04	0.053
	Met/Met	-2.89	1.66	-6.14 to 0.36	0.082	-2.79	1.78	-6.27 to 0.69	0.117
RD									
COMT rs4680	Met/Val	-2.27	1.18	-4.50 to 0.03	0.053	-2.37	1.20	-4.72 to -0.03	**0.047**
	Met/Met	-0.95	1.30	-3.49 to 1.59	0.462	-1.16	1.36	-3.83 to 1.51	0.395
Concussion history									
NS		-0.04	0.06	-0.15 to 0.08	0.543	-0.05	0.06	-0.17 to 0.08	0.482
HA		-0.11	0.05	-0.22 to -0.01	**0.027**	-0.12	0.06	-0.23 to -0.01	**0.026**
RD		0.04	0.06	-0.08 to 0.16	0.522	0.09	0.07	-0.05 to 0.23	0.189
COMT rs4680	Met/Val	1.05	0.70	-0.33 to 2.43	0.136	1.45	0.79	-0.10 to 2.99	0.066
	Met/Met	1.22	0.72	-0.19 to 2.63	0.089	1.18	0.81	-0.40 to 2.77	0.144
Senior									
NS									

COMT rs4680	Met/Val	-1.03	1.17	-3.33 to 1.26	0.379	-0.71	1.19	-3.04 to 1.62	0.550
	Met/Met	-0.68	1.31	-3.25 to 1.89	0.605	-0.71	1.36	-3.38 to 1.97	0.605
HA									
COMT rs4680	Met/Val	0.43	1.27	-2.05 to 2.91	0.735	0.70	1.27	-1.79 to 3.19	0.581
	Met/Met	-0.18	1.40	-2.92 to 2.57	0.901	-0.07	1.44	-2.89 to 2.75	0.961
RD									
COMT rs4680	Met/Val	0.27	0.90	-1.49 to 2.04	0.760	0.39	0.89	-1.34 to 2.13	0.656
	Met/Met	-0.69	1.00	-2.65 to 1.26	0.486	-0.12	1.01	-2.09 to 1.86	0.909
Concussion history									
NS		-0.01	0.04	-0.08 to 0.06	0.719	0.00	0.04	-0.08 to 0.07	0.910
HA		0.10	0.04	0.03 to 0.18	**0.005**	0.11	0.04	0.03 to 0.18	**0.006**
RD		0.01	0.05	-0.09 to 0.10	0.867	0.03	0.05	-0.07 to 0.13	0.591
COMT rs4680	Met/Val	0.22	0.49	-0.73 to 1.17	0.649	0.16	0.49	-0.80 to 1.13	0.740
	Met/Met	0.09	0.54	-0.97 to 1.14	0.872	-0.21	0.56	-1.31 to 0.88	0.704

The *COMT* rs4680 Val/Val genotype was used as the reference genotype for the analysis. Statistically significant differences ($P<0.05$) are displayed in bold. SE: Standard error of the mean; 95% CI: 95% confidence interval.

Table SII:

The summarized results of the generalized structural equation model analysis that assessed the collective relationship between concussion history, novelty seeking (NS), harm avoidance (HA), and reward dependence (RD) personality scores, and 5-HTTLPR, in conjunction with *SLC6A4* rs25531, genotype ("low": S_A/S_A; "intermediate": S_A/L_A, S_A/L_G, L_A/L_G, L_G/L_G; "high": L_A/L_A) in in junior (high school) and senior (senior community-level amateur and senior professional) rugby participants without a previous suspected or diagnosed concussion (control) compared to those with a minimum of one suspected or diagnosed concussion (case all) or case participants with one or more diagnosed concussions (case diagnosed only). Separate models were constructed for the control versus case (all) group and the control group compared to the case (diagnosed only) subgroup, in each level of play (all levels, junior, and senior).

		Control vs. case (all)				Control vs. case (diagnosed only)			
		Coefficient	**SE**	**95% CI**	***P* value**	**Coefficient**	**SE**	**95% CI**	***P* value**
All levels									
NS									
5-HTTLPR	Intermediate	-0.81	0.95	-2.68 to 1.05	0.392	-0.72	0.97	-2.61 to 1.17	0.456
	High	-1.07	1.14	-3.30 to 1.17	0.350	-0.97	1.16	-3.25 to 1.31	0.403
HA									
5-HTTLPR	Intermediate	0.62	1.06	-1.46 to 2.71	0.557	0.56	1.08	-1.55 to 2.67	0.601
	High	0.46	1.28	-2.05 to 2.97	0.717	1.20	1.29	-1.34 to 3.74	0.355
RD									
5-HTTLPR	Intermediate	0.17	0.76	-1.33 to 1.66	0.827	0.24	0.77	-1.27 to 1.75	0.753
	High	1.06	0.94	-0.79 to 2.91	0.260	1.11	0.95	-0.75 to 2.98	0.241

Concussion history									
NS		-0.01	0.03	-0.07 to 0.05	0.769	0.00	0.03	-0.07 to 0.06	0.895
HA		0.03	0.03	-0.03 to 0.08	0.319	0.02	0.03	-0.03 to 0.08	0.394
RD		0.01	0.04	-0.06 to 0.09	0.714	0.03	0.04	-0.04 to 0.11	0.383
5-HTTLPR	Intermediate	-0.79	0.41	-1.59 to 0.02	0.056	-1.06	0.42	-1.88 to -0.24	**0.011**
	High	-0.87	0.49	-1.84 to 0.09	0.075	-1.01	0.50	-1.99 to -0.04	**0.042**
Junior									
NS									
5-HTTLPR	Intermediate	-0.03	1.31	-2.60 to 2.55	0.985	0.01	1.35	-2.64 to 2.65	0.997
	High	-2.56	1.72	-5.94 to 0.82	0.138	-2.56	1.74	-5.97 to 0.85	0.141
HA									
5-HTTLPR	Intermediate	-0.32	1.59	-3.43 to 2.79	0.840	0.26	1.65	-2.98 to 3.50	0.874
	High	4.20	2.08	0.13 to 8.27	**0.043**	4.62	2.10	0.51 to 8.73	**0.028**
RD									
5-HTTLPR	Intermediate	0.16	1.24	-2.28 to 2.60	0.896	0.18	1.29	-2.36 to 2.72	0.890
	High	1.47	1.67	-1.81 to 4.75	0.379	1.24	1.69	-2.07 to 4.56	0.462
Concussion history									
NS		-0.02	0.06	-0.13 to 0.10	0.790	-0.01	0.07	-0.14 to 0.12	0.865
HA		-0.10	0.05	-0.20 to 0.01	0.074	-0.12	0.06	-0.24 to 0.00	**0.049**
RD		0.01	0.06	-0.11 to 0.13	0.901	0.05	0.07	-0.09 to 0.18	0.502
5-HTTLPR	Intermediate	-0.83	0.67	-2.14 to 0.47	0.210	-1.31	0.70	-2.68 to 0.06	0.061
	High	-1.64	1.02	-3.63 to 0.35	0.107	-1.65	1.04	-3.69 to 0.381	0.111
Senior									

NS									
5-HTTLPR	Intermediate	-1.26	1.28	-3.76 to 1.24	0.322	-1.13	1.29	-3.65 to 1.39	0.380
	High	-0.69	1.48	-3.58 to 2.21	0.641	-0.47	1.50	-3.42 to 2.48	0.754
HA									
5-HTTLPR	Intermediate	1.24	1.35	-1.41 to 3.89	0.360	0.76	1.35	-1.89 to 3.41	0.575
	High	-0.74	1.57	-3.82 to 2.34	0.639	-0.14	1.59	-3.25 to 2.96	0.927
RD									
5-HTTLPR	Intermediate	0.05	0.94	-1.79 to 1.89	0.957	0.19	0.94	-1.65 to 2.02	0.840
	High	0.61	1.12	-1.59 to 2.82	0.586	0.87	1.13	-1.34 to 3.07	0.440
Concussion history									
NS		-0.01	0.04	-0.08 to 0.06	0.817	0.00	0.04	-0.08 to 0.07	0.932
HA		0.12	0.04	0.04 to 0.20	**0.002**	0.12	0.04	0.04 to 0.20	**0.004**
RD		0.02	0.05	-0.08 to 0.12	0.713	0.03	0.05	-0.08 to 0.14	0.599
5-HTTLPR	Intermediate	-1.03	0.57	-2.14 to 0.09	0.072	-1.19	0.57	-2.31 to -0.07	**0.037**
	High	-0.48	0.65	-1.75 to 0.80	0.463	-0.66	0.65	-1.94 to 0.62	0.311

The 5-HTTLPR low genotype group was used as the reference genotype for the analysis. Statistically significant differences (P<0.05) are displayed in bold. SE: Standard error of the mean; 95% CI: 95% confidence interval.