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Research article

Association of Anxiety-Related Polymorphisms with Sports Performance in Chilean Long Distance Triathletes: A Pilot Study

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Abstract
Different factors affecting athletic performance are well established: intensity and type of training, anthropometric characteristics as well as an important psychological component. However, the contribution of the genetic background has been less investigated. The aim of the present study was to investigate the influence of polymorphisms within genes associated with stress and anxiety (*5HTT*, *CRH2R*, *ACE*, *NK1R*, *5HT1AR* and *CRF-BP*) on the physical capability and sports performance in triathletes. One hundred and ninety two (192) unrelated Chilean triathletes who participated in the 2014 70.3 Pucón city triathlon were divided into opposite subgroups of sports performance according to their time results. We identified significant associations for five polymorphisms (*5HTT* 5-HTTLPR, *ACE* I/D, *NK1R* rs6715729, *5HT1AR* -1019C>G and *CRF-BP* CRF-BPs11) with athletic performance. Our results indicate that these polymorphisms are associated with differential sports performance in Chilean triathletes, establishing an initial background for better understanding the relationship between physical performance, genetics and anxiety disorders.

Key words: Sports performance, polymorphisms, genetics, anxiety disorders.

Introduction

The effects of anxiety on athletic performance have been the main target of study in sports psychology recently. Each anxiety disorder has different symptoms, but they cluster around an irrational and excessive fear or dread (Issler et al., 2014). In triathletes, changing situations during training and competition together with the presence of anxiety disorders might cause the maladaptive fatigue syndrome (overtraining syndrome) characterized by: anger, hostility, anxiety, confusion, depression, sadness, lack of energy and apathy, finally resulting in poor performance and/or abandonment of training and competition (Patel et al., 2010). The mental health and athletic performance current model suggests a relationship between psychopathology and athletic performance (Patel et al., 2010). Studies have shown that between 70% to 85% of successful and unsuccessful athletes can be identified using general psychological measures of personality structure and mood, a level above chance, but insufficient for the purpose of athletes selection (Del Coso et al., 2014). Other studies show a deleterious effect of stress and anxiety on athletic performance in various sports (Raglin, 2001).

The biological basis of anxiety disorders focuses on a dysfunctional hypothalamus-pituitary-adrenal (HPA) axis, leading to increased activity and exaggerated response mediated by the neuroendocrine system of cortisol and catecholamines (Drabant et al., 2012). An important modulator is the serotonergic system, controlling HPA axis function on at least two levels: on one hand, activating the corticotropin-releasing factor (CRF) and secondly, by regulating cortisol and CRF activity at the synaptic level (Drabant et al., 2012). In this context, the serotonin transporter (SERT or 5-HTT) regulates serotonin (5-HT) concentrations at the synaptic level (Lee et al., 2004), and more than twelve different traits of human behavior and other systemic diseases have been linked to SERT variations (SLC6A4) (Sysoeva et al., 2009). SLC6A4 repression and the function of important variations in the transcriptional control region (serotonin transporter gene linked polymorphic region; 5-HTTLPR) have been linked to multiple psychopathological conditions, including anxiety disorder (Gonda et al., 2008).

The 5-HTTLPR polymorphism corresponds to a genetic variant in which an insertion-deletion of a 44 base pairs (bp) fragment occurs within *SLC6A4*, where the shorter variant (deletion, short/short or s/s) results in reduced transcriptional activity and increased vulnerability to affective disorders (Trushkin et al., 2011).

Within the serotonergic system, a crucial study target corresponds to the 5-HT1A receptor, which plays an important role in the self-regulatory function of the central serotonergic system (Noro et al., 2010). Studies evaluating the C(-1019)G polymorphism within this gene show an association with suicide risk, without being associated with depression (Lemonde et al., 2003). Dysfunctions associated with this receptor in knockout 5-HT1A -/- mice show increased anxious features and stress sensitivity (Lesch, 2001). Animal and cell culture studies demonstrate that increased activity of the HPA axis is associated with decreased expression of the postsynaptic 5-HT1A receptor (Lanfumey et al., 2008).

Another important regulation mechanism of the HPA axis occurs during CRF release and its binding to type 1 and type 2 specific receptors, modulating HPA axis activity (Van Den Eede et al., 2005). Current evidence shows that CRF release regulation is mediated by CRF binding protein (CRF-BP), producing an additional feedback on the HPA axis (Issler et al., 2014). Different studies showed increased expression of CRF-BP in the amygdale, anterior pituitary and portal circulation follo-

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wing increased CRF release (Van Den Eede et al., 2005). Furthermore, CRF-BP knockout mice showed an anxious behavior together with increased CRF concentrations and elevated ACTH and cortisol levels (Van Den Eede et al., 2005).

Corticotropin-releasing factor 2 receptor (CRF2R) is suggested to play a fundamental role in the recovery from stress to calm (Bale et al., 2002). Reports show that CRF2R receptors are required for proper 5-HT1A receptors function in the raphe nuclei, and that they are key for successful stress recovery (Issler et al., 2014).

In addition to its neurotransmitter/modulator in pain perception, substance P (SP) is involved in mood regulation, as demonstrated by its neurokinin-1 receptor (NK1R) antagonists to have antidepressant effects in humans (Noro et al., 2010). In rodents, treatment with NK1R antagonists showed increased 5-HT liberation from the dorsal raphe nucleus (DRN), suggesting local interactions between SP and serotonin in 5-HT1A receptors desensitization, representing a new element in the complex neural circuits proposed for mood regulation (Koller et al., 2006).

Multiple studies have associated angiotensin converting enzyme (ACE) with sports performance, and recently, ACE has been proposed as an important cortisol secretion and HPA axis regulator (Ancelin et al., 2013). The I/I genotype of the ACE rs1799752 polymorphism (I/D) has been associated with reduced plasma levels and tissue activity of ACE, while the D/D genotype was associated with higher plasma concentration and increased cardiac activity of the enzyme together with improved performance in sprint sports (Saber-Ayad et al., 2014). The I allele has been also associated with increased endurance in elite long distance runners, rowers and trail runners (Cam et al., 2005). Moreover, the presence of the D allele increases the ejection fraction and systolic pulmonary artery pressure (Saber-Ayad et al., 2014) together with an increase in CRH and ACTH levels of the HPA axis (Ancelin et al., 2013). In addition, higher ACE plasma levels are associated with lower performance in cycling and jogging in a group of athletes competing in the South Africa Ironman (Domingo et al., 2013).

Based on the background aforementioned, we aimed to explore a possible relationship between the presence of anxiety-related polymorphisms and their association with athletic performance in a group of Chilean long-distance triathletes.

Methods

Participants

One hundred and ninety two triathlon male competitors (1.9 km swimming, 90 km bike and 21 km of jogging) were evaluated. Clinical assessment consisted in physical measures of body composition by bioelectrical impedance, and a psychiatric interview using the MINI international neuropsychiatric interview, version 5.0, which allows categorizing various Axis I DSM-IV-TR disorders. Anthropometric characteristics are resumed in Table 1.

Every triathlete had experience on the Half Ironman as the entire group ran the same triathlon at least one time. All athletes fulfilled six mesocycles consisting of 3 microcycles each. Different categories were set to establish their performance outcomes. Following the participation of the athletes in the Half Ironman 70.3 competition (Pucón city), overall performance stratification was completed dividing the 192 participants into two opposite performance subgroups, designated as superior performance group (SP, n = 92) and inferior performance group (IP, n = 100), according to times registered in their respective categories. The study was conducted according to the Declaration of Helsinki. All participants accepted to participate by signing an informed consent previously approved by the Research Ethics Committee of Universidad de La Frontera (Protocol number CEC 112/2013).

Table 1. Anthropometric characteristics of performance subgroups. Data are means (±SD).

	SP (n = 92)	IP (n = 100)
Age (years)	31.07 (8.40)	27.88 (3.96)
Weight (kg)	73.35 (7.26)	74.30 (6.04)
Height (m)	1.74 (6.12)	1.76 (5.51)
BMI (kg·m^{-2})	24.27 (1.52)	23.89 (1.60)

SP: superior performance (triathlon´s top performance participants); IP: inferior performance (triathlon´s inferior performance participants); BMI: body mass index.

Blood samples

Venous blood samples were obtained for leukocyte DNA extraction and subsequent polymorphisms genotyping by PCR, PCR-RFLP and qRT-PCR. Genomic DNA was extracted using a protocol previously described by Salazar et al. (Salazar et al., 1998). Afterwards, DNA was quantified by spectrophotometry and diluted to 100 ng/100µl.

Genotyping

Genotyping of *ACE* rs1799752 (I/D) and serotonin transporter *5HTT* (5-HTTLPR) polymorphisms was completed by conventional PCR, observing for the I/D polymorphism a fragment of 190 bp in the presence of the D allele, and a fragment of 490 bp in the presence of the I allele. For the 5-HTTLPR polymorphism, a fragment of 528 bp was observed for the insertion L/L homozygote genotype, two fragments of 484 bp and 528 bp for the heterozygote S/L, and one 484 bp fragment for deletion genotype S/S.

The presence of *CRF-BP* rs1875999 polymorphism was detected by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) using *Taq*I restriction endonuclease (Fermentas, Lithuania). Wild-type genotype (C/C) was identified by the presence of a 503 bp fragment; the heterozygous genotype (C/T) for fragments of 503, 325 and 178 bp and the homozygous genotype (T/T) by two fragments of 325 and 178 bp.

For the detection of polymorphisms *CRF2R* rs2267717, *5HT1AR* -1019C>G and *NK1R* rs6715729, we used the C__15872907_10, C__11904666_10, C__25473413_10 TaqMan® SNP Genotyping Assays (Life Technologies, CA, USA), respectively. PCR assays contained 12.5 µL of Universal Master Mix (2X) (Life Technologies CA, USA), 1.25 µL of TaqMan SNP Genotyping Assay (20X) and 1 µL of DNA (20 ng) diluted in

nuclease-free water. The thermal cycling protocol performed was initiated with a cycle for 10 min. at 95°C and followed by 50 cycles at 92°C for 15 sec., and 60°C for 1 min. using standard conditions for real-time system (Life Technologies). A list of the primers and assays used are provided in Table 2. Genotype calling was performed using the StepOne software v. 2.2 (Life Technologies). No template controls (NTC) were included per triplicate in each genotyping experiment plate. Genotyping was randomly repeated on 20% of the samples for quality control purposes, without finding differences.

Statistical analysis

All statistical analyses were performed using SPSS software version 20.0 for Mac OSX. Chi-square test (x^2) was used to analyze differences in allelic frequencies and to verify Hardy-Weinberg equilibrium. Gaussian distribution was assessed by D'Agostino and Pearson normality test. Following ANOVA, we performed the Tukey post test to compare all pairs of columns. The OR was calculated assuming a model of genetic dominance and using the minor allele frequency as a risk factor for IP. Two-tailed p values <0.05 were considered as statistically significant.

Results

Genotypes distribution and relative frequency of alleles was consistent with Hardy-Weinberg equilibrium for all polymorphisms evaluated (Table 3). From the 192 participants undergoing the MINI neuropsychiatric interview, 85 individuals met the criteria to be categorized into anxiety disorder, and 107 were excluded from this category. When comparing the presence of this disorder in IP (n = 57) and SP (n = 28) individuals, we observed significant differences, which indicated that the presence of anxiety disorder correlates with deficient athletic performance in IP triathletes (p = 0.011). The results from the neuropsychiatric interview according to genotypes are shown in Table 4.

In addition, significant differences were observed when evaluating genotype distributions. Considering a codominance genetic inheritance model, the presence of the genotypes affected athletic performance, being more frequent in the IP subgroup for five polymorphisms. The same results were observed when analyzing allelic frequencies, showing significant differences between both groups and identifying more frequently the presence of the minor allele in the IP subgroup (Table 5).

Discussion

One of the main areas of research in sports psychology is the study of the relationship between anxiety and athletic performance. Even when the model of the inverted U determines that in the extremes, i.e., low and high anxiety levels condition poor performance, there are several sports that contradict this theory (Woodman and Hardy, 2003). Among them, Lopez-Perez and Labrador (1992) show that high anxiety levels improve athletic performance in a group of basketball players. Certainly, these results contradict those observed in our study, in which IP athletes present anxiety more frequently.

Anxiety can affect multiple sports aspects, and is often associated with a lack of continuity, loss of pleasure for competing and poor sports performance (Raglin, 2001). We show that anxiety disorder associates with inferior sports performance in Chilean triathletes, which is consistent with previous results where anxiety displays a negative impact on penalty definitions, climbing, golf and tennis (Raglin, 2001).

None of the athletes participating from this study declared their belonging to a particular ethnicity, neither orally nor written. Nonetheless, is important to point out that a rich admixture dominates the phenotype of Chileans (Lagos et al., 2015), determining a strong Amerindian background, which could affect one genetic variant that often represents a marker of successful sports performance, the angiotensin-converting enzyme (ACE)

Table 2. Sequence of the primers used for genotyping.

Gene / Polymorphism	Primers sequences
ACE / rs1799752	5'-CTG GAG AGC CAC TCC CAT CCT TTC T- 3' 5'-GAC GTG GCC ATC ACA TTC GTC AGA T- 3'
5HTT / 5HTTLPR	5`-GGC GTT GCC GCT CTG AAT GC-3` 5`-GAG GGA CTG AGC TGG ACA ACC AC-3`
CRF-BP / CRF-BPs11	5´-AGC CCA ACA TCA TGG TGC CAA C-3´ 5´-ACC AGT CAG TAT TCC CAG CCT TGA-3´
CRF2R / rs2267717	5'-CCA CTT CTG GCC AAA CCA CTT CCA TA-3' 5'-GCT AAT CCA CTT CCT TTC GGC CTA CA-3'
5HT1AR / -1019C>G	5'-GAG AAC GGA GGT AGC TTT TTA AAA AC-3' 5'-GGA AGA CAC ACT CGG TCT TCT TCC AT-3'
NK1R / rs6715729	5'-TAC TGG CGA AGA CAG CGG CGA TGG GA-3' 5'-GAA GAA GTT GTG GAA CTT GCA GTA GA-3'

Table 3. Genotypic distribution and relative allelic frequencies for the studied polymorphisms.

Gene / Polymorphism	Genotypes % (n)			Alleles		H-W
ACE / rs1799752	I/I: 39.1 (75)	I/D: 44.8 (86)	D/D: 16.1 (31)	I: 0.62	D: 0.38	χ^2 = 0.6; 1 df, p = 0.75
5HTT / 5HTTLPR	L/L: 23.4 (45)	L/S: 43.2 (83)	S/S: 33.4 (64)	L: 0.45	S: 0.55	χ^2 = 3.1; 1 df, p = 0.21
CRF-BP / CRF-BPs11	C/C: 35.9 (69)	C/T: 43.8 (84)	T/T: 20.3 (39)	C: 0.58	T: 0.42	χ^2 = 2.0; 1 df, p = 0.36
CRF2R / rs2267717	G/G: 55.2 (106)	G/A: 35.9 (69)	A/A: 8.9 (17)	G: 0.73	A: 0.27	χ^2 = 1.4; 1 df, p = 0.50
5HT1AR / -1019C>G	C/C: 30.2 (58)	C/G: 48.4 (93)	G/G: 21.4 (41)	C: 0.54	G: 0.46	χ^2 = 0.1; 1 df, p =0.94
NK1R / rs6715729	A/A: 33.3 (64)	A/G: 45.3 (87)	G/G: 21.4 (41)	A: 0.56	G: 0.44	χ^2 = 1.2; 1 df, p = 0.53

H-W= Hardy-Weinberg Equilibrium (Number in parenthesis indicates number of individuals from the total population studied).

Table 4. Genotypes according to results of the neuropsychiatric interview.

Gene / Polymorphism	Genotype	Anxiety disorder (-)		Anxiety disorder (+)	
		IP	SP	IP	SP
ACE / rs1799752	**I/I**	12	29	13	21
	I/D	18	26	33	9
	D/D	9	2	15	5
		χ^2=9.93 p=0.007		**χ^2=14.63 p=0.001**	
5HTT / 5HTTLPR	**L/L**	7	16	7	15
	L/S	20	26	26	11
	S/S	12	15	28	9
		χ^2=1.31 p=0.520		**χ^2=12.63 p=0.002**	
CRF-BP / CRF-BPs11	**C/C**	10	23	28	41
	C/T	24	27	46	38
	T/T	5	7	26	13
		χ^2=2.34 p=0.311		**χ^2=5.35 p=0.069**	
CRF2R / rs2267717	**A/A**	4	4	3	6
	A/G	14	25	21	9
	G/G	21	28	37	20
		χ^2=0.75 p=0.686		**χ^2=4.13 p=0.127**	
5HT1AR / -1019C>G	**C/C**	4	27	14	13
	C/G	23	25	29	16
	G/G	12	5	18	6
		χ^2=17.26 p=0.0001		**χ^2=2.97 p=0.227**	
NK1R / rs6715729	**A/A**	5	26	19	14
	A/G	21	17	32	17
	G/G	13	14	10	4
		χ^2=11.72 p=0.003		**χ^2=0.95 p=0.622**	

insertion/deletion (I/D) polymorphism. Despite the abundant evidence showing significant associations for the I allele with enhanced sports outcomes, results can be controversial, as there seems to be a differential role for each allele of the ACE I/D polymorphism; while the I allele has been associated with endurance sports, a higher D allele frequency has been observed in power-orientated sports (Nazarov et al., 2001; Puthucheary et al., 2011). The association between IP athletes and the ACE D/D genotype is similar to previous findings obtained in 2009 in Greek athletes (Papadimitriou et al., 2009), in 2011 in Lithuanian athletes (Gineviciene et al., 2011), and other studies associating the D/D genotype with poor sports performance (Holdys, 2011). In 2006, Hruskovicova et al. (2006) observed differences in genotype and allele distributions between marathon runners compared with sedentary controls. In Koreans, no differences were observed between elite athletes and unrelated nonathletes (Oh, 2007). The ACE I/D polymorphism is one of the main reported factors impairing sports performance in athletes (Woods et al., 2000) mainly due to its effect on cardiovascular system homeostasis (Izzicupo et al., 2013). The D/D genotype has been also associated with effort intolerance in humans (Mota et al., 2013). Besides, previous reports indicate that genotype I/I enables a better energetic adaptation and vascular resistance to endurance exercises (Holdys, 2011), situation that coincides with our findings, which is also consistent with a comparative study between elite and amateur athletes, identifying the same deleterious effects of the D allele and D/D genotype on athletic performance (Cam et al., 2005). However, inconsistencies between studies evaluating this polymorphism can exist, and may rely on several factors: study design, sports evaluated, inclusion/exclusion criteria, and age of the participants, just to mention a few. Nonetheless, the most important issue that may confront results corresponds to the population's dissimilar genetic background, which is key when considering that we are precisely determining the genetic contribution across, in our case, highly admixed individuals, an issue that can underlie different results.

On the other hand, we found a higher frequency for the 5-HTTLPR S/S genotype and S allele in IP athletes, which is concordant with results described by Trushkin and colleagues (2011) in a group of athletes undergoing maximum stress testing, where individuals carrying the S/S genotype had lower tolerance to fatigue than L/L carriers. A common finding is that sports have a similar effect than serotonin reuptake inhibitors (SRI) antidepressants. However, Rethorst and colleagues (2010) showed that the presence of the S/S genotype and S allele reduced the positive impact of sports in depression patients. Nevertheless, SRI consumption did not improve athletic performance in another group of athletes under study (Parise et al., 2001). In addition, our results disagree with those reported in long distance South African triathletes, finding no association with this polymorphism, however, it should be noted that in the South African cohort, performance was evaluated in two different years, where diverse variables could be influencing the final outcome. For instance, the study by de Milander included Caucasian population only, where the S/S genotype is known to have a lower frequency than South Americans (Ospina-Duque et al., 2000). De Milander reported a 20% frequency for the S/S genotype, while our results showed a 33.4% frequency. Moreover, de Milander and colleagues performed their study in Ironman competitors (3.9 km swimming, 180 km biking, 42 km jogging), whilst our work included half-ironman participants (1.9 km swimming, 90 km biking and 21 km jogging). Temperatures

Table 5. Genotypic distribution and relative allele frequency for the studied polymorphisms in SP/IP subgroups.

Gene / Variant	Genotype Frequency (%)			H-W	Allele Frequency (%)	
ACE I/D	**I/I**	**I/D**	**D/D**		**I**	**D**
Total (192)	39.1 (75)	44.8 (86)	16.1 (31)		0.62 (119)	0.38 (73)
SP (92)	54.3 (50)	38.0 (35)	7.6 (7)	$\chi^{2=}$0.06; p=0.799	0.73 (135)	0.27 (49)
IP (100)	25.0 (25)	51.0 (51)	24.0 (24)	$\chi^{2=}$0.04; p=0.841	0.51 (101)	0.49 (99)
		χ^2 = 20.34 ; 2 df; p = 0.006			χ^2 = 21.16; 1 df; p = 0.0001	
		OR: 2.701 (C.I. 95% = 1.759 – 4.146) P = 0.0001				
5HTT / 5HTTLPR	**S/S**	**S/L**	**L/L**		**S**	**L**
Total (192)	23.4 (45)	43.2 (83)	33.4 (64)		0.45 (86)	0.55 (106)
SP (92)	33.7 (31)	40.2 (37)	26.1 (24)	$\chi^{2=}$3.36; p=0.066	0.54 (99)	0.46 (85)
IP (100)	14.0 (14)	46.0 (46)	40.0 (40)	$\chi^{2=}$0.02; p=0.894	0.37 (74)	0.63 (126)
		χ^2 = 11.08; 2 df; p = 0.004			χ^2 = 10.93; 1 df; p = 0.001	
		OR: 1.983 (C.I. 95% = 1.318 –2.982) P = 0.001				
CRF-BP / CRF-BPs11	**C/C**	**C/T**	**T/T**		**C**	**T**
Total (192)	35.9 (69)	43.8 (84)	20.3 (39)		0.58 (111)	0.42 (81)
SP (92)	44.6 (41)	41.3 (38)	14.1 (13)	$\chi^{2=}$0.70; p=0.390	0.65 (120)	0.35 (64)
IP (100)	28.0 (28)	46.0 (46)	26.0 (26)	$\chi^{2=}$0.60; p=0.4258	0.51 (102)	0.49 (98)
		χ^2 = 7.22; 2 df; p = 0.027			χ^2 = 7.94; 1 df; p = 0.005	
		OR: 1.801 (C.I. 95% = 1.194 – 2.717) P = 0.005				
CRF2R / rs2267717	**C/C**	**C/G**	**G/G**		**C**	**G**
Total (192)	55.2 (106)	35.9 (69)	8.9 (17)		0.73 (140)	0.27 (52)
SP (92)	10.9 (10)	37.0 (34)	52.1 (48)	$\chi^{2=}$1.09; p=0.296	0.20 (37)	0.80 (153)
IP (100)	7.0 (7)	35.0 (35)	58.0 (58)	$\chi^{2=}$0.29; p=0.589	0.17 (32)	0.83 (162)
		χ^2 = 1.16; 2 df; p = 0.561			χ^2 = 1.15; 1 df; p = 0.2841	
		OR: 1.280 (C.I. 95% = 0.814 – 2.012) P = 0.447				
5HT1AR / -1019C>G	**C/C**	**C/G**	**G/G**		**C**	**G**
Total (192)	30.2 (58)	48.4 (93)	21.4 (41)		0.54 (104)	0.46 (88)
SP (92)	43.5 (40)	44.6 (41)	12.0 (11)	$\chi^{2=}$0.01; p=0.921	0.66 (121)	0.34 (63)
IP (100)	18.0 (18)	52.0 (52)	30.0 (30)	$\chi^{2=}$0.30; p=0.581	0.44 (88)	0.56 (112)
		χ^2 = 18.15 ; 2 df; p = 0.0001			χ^2 = 18.3; 1 df; p = 0.0001	
		OR: 2.444 (C.I. 95% = 1.617-3.694) P = 0.0001				
NK1R / rs6715729	**A/A**	**A/G**	**G/G**		**A**	**G**
Total (192)	33.3 (64)	45.3 (87)	21.4 (41)		0.56 (108)	0.44 (84)
SP (92)	43.5 (40)	37.0 (34)	19.6 (18)	$\chi^{2=}$4.29; p=0.038	0.62 (114)	0.38 (70)
IP (100)	24.0 (24)	53.0 (53)	23.0 (23)	$\chi^{2=}$0.36; p=0.547	0.51 (101)	0.49 (99)
		χ^2 = 8.44 ; 2 df; p = 0.015			χ^2 = 6.02; 1 df; p = 0.014	
		OR: 1.596 (C.I. 95% = 1.063 – 2.397) P = 0.024				

H-W= Hardy-Weinberg Equilibrium; df = Degree of Freedom; C.I. = Confidence Interval.

were also different, having extremes of 9-26 °C in Pucón, and 17-23.9 °C in South Africa. Finally, de Milander divided their participants into fast, middle and low triathletes, a different measure from the lower and upper performance shown in our work (de Milander et al., 2009).

For the *5HT1AR* -1019C>G polymorphism, our

results show that the G/G genotype is more frequent in the IP group (p = 0.0001), which is similar to the presence of the G allele (p = 0.0001). However, there are no previous reports linking athletic performance and the presence of this polymorphism, but there could be a connection if the -1019C>G polymorphism can be related to the presence of anxiety disorders. Reports show that 5-HT1A receptors are distributed in high density in the limbic system, and are involved in the regulation of emotional states, being found pre and post-synaptically (Huang et al., 2004). Recent studies in animal models indicate that knockout mice for this protein show and increased overall response to anxiety states (Zetzsche et al., 2008). In human, studies revealed an association between -1019C>G polymorphism and the presence of panic disorder and agoraphobia, two determinants of anxiety disorders (Bosia et al., 2011; Rothe et al., 2004).

For the *NK1R* rs6715729 polymorphism, our results show an association between G/G genotype and G allele with the IP group, which could possibly be due to changes in relation to stress homeostasis. Human and animal studies suggest that the P substance mediates the response to stress, where the biological responses of the P substance are mainly transduced through NK1R, widely expressed in pathways controlling stress, and tissues such as intestine, joints, tendons and skin (Seneviratne et al., 2009); antagonists for this receptor are very effective in treating depression and anxiety (Stein et al., 2006). This polymorphism is also associated with alcohol dependence and abuse (Seneviratne et al., 2009). Nonetheless, we associated the presence of the G/G genotype with agoraphobia (p = 0.029) and generalized anxiety disorder (p = 0.024), major determinants of anxiety disorder. Finally, although reporting significant findings, some issues may be limiting the extent of the results and need to be further considered. For instance, the lack of a control group, therefore, we cannot conclusively rule out other unaccounted factors that may be influencing the differential performance observed as well. Secondly, the small sample size, which brings the necessity to replicate this study encompassing a greater number of participants to reproduce the associations identified.

Conclusion

Multiple physiological, pathophysiological and psychological aspects influence athletic performance. Clarifying how these factors affect the organism in endurance sports is critical to achieve a better understanding in long-distance competitions outcomes. Here, we show that genetic variants within stress- and anxiety-related genes affect athletic performance in long-distance Chilean triathletes; contributing evidence that includes a novel factor to consider is sports physiology, which is an important advance in the comprehension of the HPA axis functionality. Further studies are necessary to disclose the role of additional components involved affecting sports performance.

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Key points

- Genetic factors influencing sports performance in the Chilean population are unknown.
- Differential outcomes from athletes who completed a triathlon competition were associated with five polymorphisms (*5HTT* 5-HTTLPR, *ACE* I/D, *NK1R* rs6715729, *5HT1AR* -1019C>G and *CRF-BP* CRF-BPs11).
- We show that genetic variants within stress- and anxiety-related genes affect athletic performance.

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