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Original Research

A pilot study for determination of anxiety related *SLC6A4* promoter "S" and "L" alleles in healthy Turkish athletes

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Abstract: We aimed to analyze the allelic distribution of solute carrier family-6 member-4 promoter region in Turkish athletes. Recent studies showed the association of lesser expressing "S" allele with anxiety. Genotype percentages for LL, LS and SS genotypes were found as 46, 35 and 19, respectively. 38% of the males had LL, %38 had LS and 24% had SS genotypes. Percentages of LL, LS and SS genotypes were 54, 31 and 15 in females, respectively. 15 (58%) male and 18 (69%) females had L, 11 (42%) male and 8 (31%) females had S alleles. Variations in the association of the SLC6A4 alleles with neuropsychiatric disorders according to different nationalities have been reported. This is the first report showing that LL genotype and L allele in Turkish athletes was more frequent than SS genotype and S allele.

Key words: Anxiety; Polymorphism; Serotonin transporter; Sports; Sport psychology.

Introduction

Psychological components, especially anxiety, are among the factors that determine sport perfomance. In the publications regarding anxiety disorders, the prevalence has been reported as high as %20. Consequently, the rate of the individuals who have anxiety symptoms is much higher when added the individuals who do not meet criteria of the diagnosis (1). Sport events constitute environments in which anxiety is quintessentially hightened. Accordingly, relationship between anxiety and sport performance has become one of the main areas of research (2).

There are several chemicals in the body which are related with anxiety, such as CRF, cortisol, serotonin, norepinephrine, etc. As a monoamin neurotransmiter, serotonin (5-hydroxytryptamine; 5-HT), plays an important role in sleep, mood, learning, appetite etc., (3). Serotonin's role in psychiatric symptoms such as depression, anxiety, obsession, compulsions, addiction has also been shown (4). Physiologically, serotonin is an inhibitory neurotransmitter most of the time (5), and lack of serotonin causes an increase in impulsive behaviour (6,7).

Serotonin, after being released into synaptic cleft, is taken by the presynaptic neuron (reuptake) by a transmembranal carrier protein, referred to as serotonin transporter (SERT) or (5-HTT). After the reuptake, serotonin is stored in the presynaptic vesicles, and ready to be released again when needed. The number of 5-HTT is an indicator of the activity of serotonergic system (8).

5-HTT is produced by solute carrier family 6 member 4 (SLC6A4) gene, located on 17th chromosome (9). A polimorphism seen in the promotor region (serotonin transporter gene linked polymorphic region; 5-HTTLPR) which controls the transcription, affects the production of 5-HTT, hence impacts serotonergic system. The polymorphism comprises a 44-bp insertion (L allele) or deletion (S allele) and has been shown to be linked to various neuropsychiatric disorders, making the individual susceptible against stressful life events (10).

Current study aims investigating the distribution of S and L alleles of SLC6A4 gene in athletes. Since LL carriers are more resistant against stress (11), professional players are expected to have LL genotype in greater possibility due to their necessity to cope with stressful conditions in their life such as sport events and trainings, strict rules in their private lives, and sense of competition that dominates their life. Those individuals who have LL genotype have more possibility to be successful, be selected, and be kept in the team.

Materials and Methods

26 marathon runner athletes, aged between 18-20, all with Turkish nationality were enrolled for the study. All the athletes were not diagnosed with any physciatric disorder. Of the 26 athletes, 13 were male and 13 were female. These athletes have 4 training sessions per week, a total of 15 hours. The athletes were qualified for the national team at least once through their sports life. Üsküdar University Ethics Committee approved the study protocol and the study protocol is in accordance with the principles of the Declaration of Helsinki II. Athletes

signed the informed consents prior to enrollment to the study.

DNA Sample Collection

200 uL peripheral blood samples were collected in the tubes containing EDTA. Isolation procedure was carried out by using High Pure PCR Template Preparation Kit (Roche,Basle, Switzerland), by following manufacturer's instructions.

Genotyping

Functional deletion/ insertion polymorphism located in the promoter region of SLC6A4 was amplified by polymerase chain reaction (PCR) by using specific primers (primers can be sent upon request). 50 µlPCR mixture contained 100 ng genomic DNA, 5 µl 10X Taqbuffer (1X final concentration), 1,5 mM MgCl2, 0,5 mMdNTP, 10 pmol of each primer and 2 U Taq DNA polymerase. PCR reaction were completed after 35 cycles and included pre-denaturation at 95˚C for 5 min, denaturation at 95˚C for 45 sec, annealing at 64˚C for 45 sec, extension at 72˚C for 45 sec, and a final extention at 72˚C for 10 min. "L " allele amplicon gave rise to 528 bp, whereas "S" allele amplicon to 484 bp in 3% agarose gel electrophoresis when visiulized under UV light.

Results

All of the athletes were genotyped for the SLC6A4 promoter polymorphism (Table 1) by two different investigators to avoid false- negative results. In males, genotype numbers and percentages were 5 (38%), 5 (38%) and 3 (24%) for the LL, LS and SS, respectively. In our study cohort, male genoype frequencies were 19%, 19% and 1,5% for the LL, LS and SS genotypes, respectively. In females, genotype numbers and percentages were 7 (54%), 4 (31%) and 2 (15%) for the LL, LS and SS genotypes, respectively. Genotype frequencies for LL, LS and SS genotpyes were 27%, 15,5% and 7,5%, respectively, for the female athletes. When we consider our study cohort, 46% had LL, 35% had LS and 19% had SS genotypes.

Allelic count were assessed by direct counting of the genotypes. L allele was 15 (58%) and 18 (69%) in males and females, respectively, whereas S allele was 11 (42%) in males and 8 (31%) in females. In our total cohort, L allele was found as 33 (61%) and S was found as 19 (39%).

Discussion

There is a high emotional pressure on players in sports events and training sessions. Since anxiety affects athletic and tactical performance, endurance to stress of a player is considered important as well as staying away from stress. There is evidence regarding the relatedness of psychopathology and sports performance (1). Players and athletes should reduce and manage their anxiety and aggression for better performance. A study by Sysoeva et al. (2009) on professional synchronized swimmers showed that the swimmers have lower scores on assault, irritability, negativism and verbal hostility compared to age-matched control group.

Genetic factors play crucial roles not only in determining skills and endurance, but also have effects on psychology of players. One of the important systems affecting psychology is serotonergic system, which regulates mood and controls stress. Decreased brain serotonin levels, causing emotional problems and impulsivity, have negative effects on sports performance. There are also studies which have shown that running increases serotonin tissue levels (13-16).

One of the genes that control serotonergic system is *SLC6A4,* it produces serotonin transporter protein (5-HTT). After being released, serotonin is taken back (reuptake) into the presynaptic neuron by 5-HTT found in presynaptic membrane. Therefore the density of 5-HTT is an important factor determining the amount of serotonin in synapses. 44 bp insertion/deletion polymorphism within the promotor region of *SLC6A4* impacts the transcription rate, the presynaptic 5-HTT density (17), therefore serotonergic system and mood. "L" allele is associated with high production of the transporter protein, whereas "S" allele with low production. Recent studies showed the association of "S" allele with anxiety (18,19). SS genotype was found to be associated with agression in children (20). Sanhueza et al. (2016) has found significant associations for the same polymorphism with athletic performance.

In this study cohort, *SLC6A4* promoter polymorphism of 26 male and female athletes has been analyzed. According to results, 12 of the players had LL (L allele being greater expressing). 5 of the players had SS genotype (S allele being lesser expressing). 9 of the players had LS, which is regarded as intermediate genotype. 33 players had at least one L allele while 19 players had at least one S allele.

The studies investigating the relationship between *SLC6A4* promoter polymorphism and sport performance are limited. Saunders et al. (2006) analyzed *SLC6A4* polymorphism in male triathletes, and found that LL and LS genotypes were much higher than SS genotype. Trushkin et al. (2011) studied on male endurance athletes and found that LL genotype was higher in athletes when compared to non-athletes. Sysoeva et al. (2009) examined female swimmers, and showed the higher percentage of LL genotypes when compared to non-sport group. Ulucan et al. (2014) found that LL and LS genotypes were higher than SS genotype in young Turkish basketball players. Our findings were in concordance with those previous studies. In our cohort, L

Table 1. Genotpyes, allelic numbers and percentages of the analyzed male and female athletes.

Subjects	Numbers	Genotype (percentage in same gender)			Alleles	
		LL	LS	SS	L	S
Male	13	5 (38)	5 (38)	3 (24)	15 (58)	11 (42)
Female	13	7 (54)	4 (31)	2 (15)	18 (69)	8 (31)
Total	26	12	9	5	33	19

allele number (n=33) was higher than the S allele number (n=19).

It is difficult to associate the *SLC6A4* promoter polymorphism with the metabolism of the athletes. However, endurance against stress is a well-known positive factor in sports (2). LL carriers have been shown to be more resistant against stress (11). Professional players are more expected to have LL genotype because they have to cope with stress in their life such as sport events and trainings, strict rules in their private lives, and due to the sense of competition that dominates their life. Those athletes who carry SS genotype have a negative genetic factor in terms of stress, whereas those who have LL genotype are more possibly successful, hence, are selected, and stay in the national team. Because S-allele carriers showed exaggerated amygdala reactivity in stressful conditions, and can be controlled less compared with the LL-allele group (24), while LL group is protected (11).

Determination of a genetic influence on qualified athletes requires large study cohorts. Our study group was consisted of 26 healthy athletes. In the present study, we analyzed the distribution of L and S allelles of *SLC6A4* in Turkish national athletes, and this is the first reporting the relationship between Turkish athletes and L and S allelles of *SLC6A4*. Despite the limitation, our results suggested the role of the *SLC6A4* promoter polymorphism in success in sports performance. Identification of SS genotype in athletes can be considered as an occasion to investigate anxious states of the athletes, their predispositions to anxiety symptoms, and may help to implement early prevention programs. Further studies with high numbers of athletes and on various sports types are needed to determine the role of *SLC6A4* promoter polymorphism in sports.

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