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ANALYSIS OF SOLUTE CARRIER FAMILY 6 MEMBER 4 GENE PROMOTER POLYMORPHISM IN YOUNG TURKISH BASKETBALL PLAYERS.

GENÇ TÜRK BASKETBOL OYUNCULARINDA SLC6A4 PROMOTOR POLİMORFİZMİNİN ANALİZİ

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Abstract

The serotonin transporter (5-hydroxytryptamine transporter, 5- HTT) gene (SLC6A4) is considered to be one of the most important candidate genes for genetic involvement in psychiatric conditions like anxiety, self- confidence and motivation. In the present study, we aimed to analyze the distribution of SLC6A4 promoter long and short (L and S, respectively) polymorphism in young Turkish basketball players. We enrolled 24 players in the study, 17 were females and 7 were males. 12%, 35% and 53% of the females had SS, LS and LL genotype, respectively; whereas 28,5% of the males had SS and the same percentage of them had LS, and 43% had LL genotype. When we examined the allelic counts, L allele was recorded as 71% in females and 57% in males; S allele was 29% in females and 43% in males. Our results were in agreement with the previous ones, indicating the presence of L allele in individuals dealing with sport. We suggest that SLC6A4 promoter analysis is important for genetic counseling for the individuals who are prone to be successful in sports.
Keywords: Serotonin transporter, sports, genetics, polymorphism

Özet

Serotonin taşıyıcı (5- hidroksitriptamin taşıyıcı, 5-HTT) geni (SLC6A4), anksiyete, kendinden eminlik ve motivasyon gibi psikolojik durumlara genetik yatkınlıklara neden olabilecek en önemli genlerden biri olarak nitelendirilmektedir. Bu çalışmada, genç Türk basketbol oyuncularında SLC6A4 geninin promotor bölgesi uzun ve kısa (sırasıyla L ve S) polimorfizminin dağılımını analiz etmeyi amaçladık. Çalışma da 17' si kız, 7' si erkek 24 kişi dahil olmuştur. 17 kız basketbolcunun sırasıyla %12, %35 ve %53' ü SS, LS ve LL genotipindedir, erkek basketbolcuların ise %28.5' inin genotipleri SS ve LS, %43' ünün de LL olarak bulunmuştur. Allel sayıları incelendiğinde L alleli kızlarda %71, erkeklerde %57; S alleli de kızlar da %29 ve erkeklerde %43 olarak bulunmuştur. Sonuçlarımız, sporla uğraşan bireylerde L allelinin bulunmasını destekleyen önceki çalışmaları desteklemektedir. Sonuçlarımıza dayanarak başarılı sporcu olma potansiyali olan bireylere genetik danışma verilmesinde SLC6A4 promotor bölgesinin genotiplenmesini önermekteyiz.
Anahtar Kelimeler: Serotonin taşıyıcı, spor, genetik, polimorfizm.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter synthesized from amino acid, tryptophan. Approximately, up to 90% of serotonin is located in enterochromaffin cells in the gut, regulating the intestinal movements; the rest is synthesized in serotonergic cells of central nervous system (CNS) (Berger et al., 2009). In CNS, 5-HT system, including serotonin, its receptors and downstream molecules, has various functions, like memory, learning, in cognitive and neuroendocrine functions and it is considered to be a contributor of the feelings happiness and self- confidence (Young, 2007). 5-HT system also involves in stress- related mood disorders in humans (Loughridge et al., 2013).

Serotonin is released to the synaptic cleft by serotonergic neurons, activates its receptors on the postsynaptic neurons, and the excess serotonin is taken back to the presynaptic neuron by carrier proteins and recycled in serotonin metabolism. This uptake process is maintained by serotonin transporters (5-HTT). The gene that encodes this carrier protein is solute carrier family 6 (neurotransmitter transporter), member 4 gene (SLC6A4) and is located at 17q11.1–q12 (Nakamura et al., 2000). Promoter region of SLC6A4 contains a functional

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polymorphism (5-HTTLPR), 14 or 16 repeats of a sequence leading to short (S) or long (L) alleles, respectively (Heils et al., 1996). Short allele and SS genotype is associated with decreased transcription of the gene, leading to the decreased number of carrier proteins, and increase in extracellular 5-HT. Decreased transcription rate and increased extracellular 5-HT may trigger the self-inhibition of 5-HT1A receptors. Therefore, S allele is considered to be responsible for some of the anxiety-related personality traits, hostility and depression (Lesch et al., 1996; Lesch and Merschdorf, 2000). On the other hand, L allele is the high- expression allele, and associated with resistance to stress and trauma (Caspi et al., 2003). Effect of this allele in psychological development of English national-level swimmers was reported before (Golby and Sheard, 2006).

Recent studies reported the association of 5-HT system and exercise. Rethorst et al. (2010) showed the antidepressant effects of exercise across 5-HTTLPR genotypes in 18–23 aged individuals and suggested the effect of this polymorphism in the treatment for depression. Prolonged exercises have influences in the development of fatigue due to the increase in extracellular serotonin concentrations (Meeusen et al., 2006). Works from the 1980s expressed the possible effects of exercise on serotonin metabolism in rats and the effect of exercise on positive mood psychology (Chaoulof, 1997). Animal models showed the increase of 5-HT after exercise (Jacobs, 1994). Chronic wheel running decreased 5-HTT mRNA levels in rat dorsal and medial raphe nuclei (Greenwood et al., 2005). Newsholme et al. (1987) were the first to relate 'central fatigue hypothesis' to the CNS 5-HT levels, other studies followed that to support the hypothesis (Trushkin et al, 2011). However, forced swimming, which is accepted as a model for depressive- like behavior in rodents, decreased the 5-HT concentrations in rats hypothalamus and amygdala (Shishkina et al., 2008). But the molecular studies including human subjects are not adequate to explain the exact mechanism in humans.

To date, reports trying to explain the effect of SLC6A4 5-HTTLPR polymorphism in sports are limited. The aim of the study is to examine the allelic distribution of SLC6A4 in young male and female basketball players and evaluate the possible effects of allelic differences on their sport motivation.

2.Methods

2.1. Subjects

A total of 24 basketball players, composed of 7 male and 17 females, aged between 15 and 18, were enrolled in the study. All the participants were the members of high school team and also playing for club teams, as well. They had at least 4 training sessions in different days of the week. All the players enrolled in the study showed their willingness to be a successful player, and doing their training for at least 3 years. The players who showed their unwillingness to be a basketball player and the one who had a decision to give up playing basketball were excluded from the study.

The study protocol was approved by the Üsküdar University Ethics Committee and the study procedure was in accordance with the principles of the Declaration of Helsinki II. Written informed consents were provided prior to enrollment from one of the parents who were in charge.

2.3. DNA sample collection: DNA was isolated from buccal cells by using Quick Extract DNA Extraction solution (Epicenter, Madison, WI, USA). The procedure for isolation was conducted according to the manufacturers' instructions.

2.4. SLC6A4 Genotyping

Functional polymorphism located in the promoter region of SLC6A4 is genotyped by using the primers 5'- GGCGTTGCCGCTCTGAATGC-3' and 5'-GAGGGACTGAGCTGGACAA CCAC-3'. PCR reactions were carried out in a 50 µl mixture containing 100ng genomic DNA, 5 µl 10X Taq buffer (1X final concentration), 1,5 mM MgCl2, 0,5 mM dNTP, 10 pmol of each primer and 2 U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). The reaction was completed after 35 cycles and included pre-denaturation at 94°C for 7 min, denaturation at 94°C for 30 sec, annealing at 64°C for 30 sec, extension at 72°C for 30 sec and a final extension at 72°C for 10 min. 'L' allele gave rise to 528 bp after electrophoresis in 2,5% agarose gel visualized with ethidium bromide (0.2 g/mL) under ultraviolet light, whereas 'S' allele had 484 bp (Figure 1).

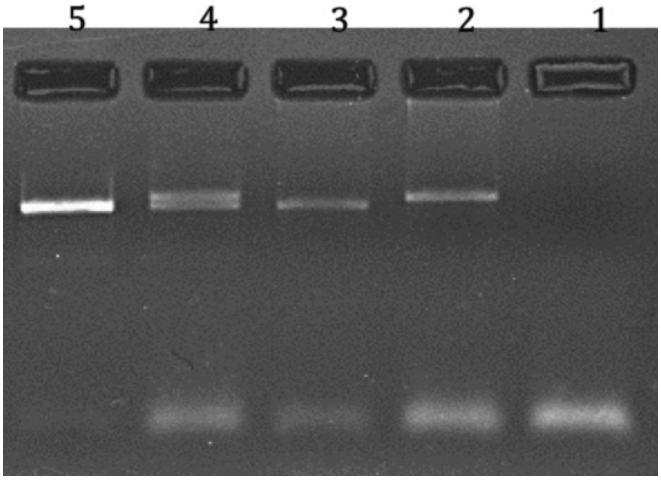

Figure 1. Agarose gel electrophoresis and the genotype results. Lanes; 1: SS genotype (484 bp); 2: LS genotype (484 and 528 bp); 3: SS genotype; 4: LL genotype (528 bp); 5: negative control.

3. Results

A total of 24 young basketball players were genotyped for the SLC6A4 promoter polymorphism in the present study (Table 1). Males constituted 29% of the study cohort and the females the rest. 2 of the males had SS genotype, again the same number of the males had LS, and 3 had LL genotype. In females 2, 6 and 9 had the genotypes SS, LL and LL, respectively (Table 1). Direct allelic count showed that 'L' allele dominated the 'S' allele in both genders (Table 1). 'L' allele direct count was found as 32 in the study cohort and lead to a 67% of the all genotyped individuals (data not shown in Table 1). Similarly, 'L' allele had a percentage of 71% in females, when compared to males, which was 57%.

Genders	Number (Percentage to the study cohort)	Genotype (Percentage in the same gender cohort)			Alleles (Percentage in the same gender cohort)	
		SS	LS	LL	S	L
Male	7 (29)	2 (28.5)	2 (28.5)	3 (43)	6 (43)	8 (57)
Female	17 (71)	2 (12)	6 (35)	9 (53)	10 (29)	24 (71)
Total	24 (100)	4	8	12	16	32

Table 1: Genders, genotype and allelic distribution of the study cohort. Percentage of the genotype and allelic counts were determined within the same gender groups, percentage of the genders were determined within the study cohort.

4. Discussion

Genetic and environmental factors such as nutrition play crucial roles on the development of individuals who have predisposition to sports. Hard-working, discipline, self and high motivation are some of the personal traits that are found in most successful players, or athletes, regardless to the nature of the sport they involve. Sport is associated with a high emotional and psychological pressure on players and endurance of stress has gaining great importance for sportive achievements. Therefore, not only training and nutritional stability, being in the best mood conditions, or staying away from depression and anxiety feelings is also important for the players or athletes during sports activity or in daily life.

Serotonin system is one of the key important systems regulating our mood condition, happiness or stress, and the role of serotonin in this system and motor control is complex (Takahashi et al., 2000). The biological metabolism underlying the process of serotonin and willingness to exercise is still not clear, but the possible role of serotonin in exercise was evaluated in some model organism. Meeusen et al. (1996) showed a rapid increase in the hippocampus 5-HT level at the onset of a moderate running in food-deprived rats. Gomez-Merino et al. (2001) reported a delayed increase in extracellular 5-HT in ventral hippocampus and the frontal cortex parts of rat brain after an acute intensive running. In addition to these findings, the increase in tissue 5-HT after prolonged or exhaustive exercise had been shown (Blomstrand et al., 1989). Another study showed the activation of serotonergic system in pedal exercise and reported that this exercise improved the negative emotion (Ohmatsu et al., 2014).

The studies including human subjects and 5-HTTLPR polymorphism in sport predisposition are very limited. In our study cohort, we analyzed the SLC6A4 promoter 5-HTTLPR polymorphism. We examined 24 young basketball players. Analysis revealed us that 12 of the players were LL, this genotype and L allele is regarded as active allele, and 4 of the players had SS genotype, this genotype and S allele refers the under- expression allele. 8 of the players were LS, which is regarded as intermediate genotype. 20 players had at least one L allele. We also expressed the gender differences; but this polymorphism and sport activities were not associated with gender differences before. Saunders et al. (2006) examined the related polymorphism in 428 Caucasian male triathletes, and showed that LL and LS genotypes, genotypes that are regarded as L allele, were much higher than the SS genotypes. Trushkin et al. (2011) evaluated the same polymorphism in 223 male endurance athletes and found that LL genotype was higher in athletes when compared to non- athletes. Our findings were similar with the findings of previous studies. In our cohort, L allele number (n=32) was twice as the S allele number (n=16). Sysoeva et al. (2009) examined 62 synchronized Caucasian female swimmers, and showed the higher percentage of LL genotypes when compared to control non- sport group.

It is very difficult to associate the 5-HTTLPR polymorphism with the sport metabolism. This gene was associated with neuroticism and anxiety before (Lesch and Merschdorf, 2000), but not in all studies (Willis-Owen et al., 2005). Also Sysoeva et al. (2009) showed the high aggressiveness and Novelty Seeking scores for the SS compared to LL carriers in synchronized swimmers. As the L allele was associated with resistance to stress and trauma, the possible explanation for this allele to dominate in our study cohort is that individuals are more prone to be successful in aggressive and stressful sports, like basketball. It is a known fact that genetic and environmental factors have roles on being an elite and successful player, or an athlete. But the exact biological system underlying this situation is hard to explain by only with the 5-HTTLPR polymorphism.

However our results evaluated the importance of L allele in basketball players, the number of the players enrolled in the analysis is the main limitation of the present study. Detection of the impact of the genetic influence to a certain phenotype requires large study cohorts. Our study group was consisted of 24 players, and this was the number of the individuals playing in that team. Despite the limitation, our results suggested the role of the 5-HTTLPR genotype in basketball activities. This was the first study carried out in Turkish young basketball players, according to best of our knowledge. Before, we reported ACTN3 analysis in young sprinters (Ulucan et al., 2014) and ACTN3 and ACE analysis in wind- surfers (Ulucan et al., 2013; Ulucan and Göle, 2014) but this study is the first trying to associate the serotonergic system and sport in young Turkish basketball players. Further studies with the high numbers of samples and including different sports types are needed to be carried out to fulfill the role of 5-HTTLPR genotype in sports.

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