The anxiety level of an athlete is important. The serotonin transporter protein is encoded by the SLC6A4 gene. Serotonin is one of the most important members of anxiety metabolism. Serotonin transporter protein plays a role in bringing serotonin back from the synaptic space to the presynaptic neuron. We aimed to determine the genotype and allele distributions of the SLC6A4 promoter L/S polymorphism in football players and compare it with the control group. For this purpose, 20 football players participated in our study. Genotyping was performed by PCR after DNAs were isolated from buccal epithelial cells. When we examined the genotype distributions, the LL, LS and SS were found as 4 (20%), 8 (40%) and 8 (40%), respectively. In the control group, the same genotype number and percentages were determined as 10 (23%), 25 (57%) and 9 (20%), respectively. In terms of genotype distribution, no statistically significant significance was found between the two groups (p = 0.2493). When allelic distributions were examined in the athlete group, the L allele was counted as 16 (40%) and the S allele as 24 (60%). In the control group, the L allele 45 (51%) and the S allele were counted as 43 (49%). When the two groups were compared statistically in terms of allelic distribution, no statistically significant difference was found (p = 0.2423). As a result, LS and SS genotype were co-dominant and at the same time higher than LL genotype. At the same time, when we compared the S allele with the L allele, it was found that the S allele was more in percentage. Studies have found that the S allele is associated with anxiety. Sports psychologist can support athletes with S allele who are prone to anxiety problems. This is the first study to report that S allele is more common than L allele in Turkish football players.

Keywords: sports, genetics, polymorphism, SLC6A4, football
1. Introduction

Athletic performance is the combination of an individual's innate genetic abilities as well as abilities acquired under the influence of environmental factors (Ulucan et al., 2014). Sports genetic was accepted as a new branch of science that studies the functioning and regulation of genes that affect athletic performance (Ulucan, 2016). Studies in the field of sports genetics also include analysis of genetic factors that determine psychological mood, such as serotonergic and dopaminergic systems, which affect sports activity. Psychological characteristics of athletes negatively affect their athletic performance (Yiğit et al., 2020). Psychological parameters such as stress, anxiety, and aggression are effective in determining the performance of athletes (Eken et al., 2018). Serotonin is one of the endogenous molecules affecting human psychology. The serotonin neurotransmitter has been shown to play a role in symptoms and syndromes such as depression, anxiety, stress, and addiction (Ateş et al., 2017).

Serotonin transporter protein (S-HTT or SERT) is one of the most important members of serotonin metabolism and is encoded by the SLC6A4 gene. Serotonin is stored in vesicles within the neurons after synthesized. Serotonin is released into the synaptic space between two neurons to stimulate receptors on other neurons when the cell is stimulated. Excess serotonin released between neurons is taken back to the neuron where it was released. The serotonin transporter protein synthesized by the SLC6A4 gene on chromosome 17 performs this process (Ulucan et al., 2014). There are 14 repeat short (S) and 16 repeat long (L) alleles in the promoter region of the SLC6A4 gene, depending on the deletion / insertion of a 44 bp repeat sequence (Heils et al., 1996). The L allele increases the transcriptional efficiency of the SLC6A4 gene and accordingly increases the rate of serotonin. The S allele has the opposite effect (Collier et al., 1996). Studies conducted to date are associated with the "S" allele from some personality traits associated with anxiety, hostility and depression (Lesch and Merschdorf, 2000). Previous studies have shown that the distribution of SLC6A4 polymorphism among athletes (synchronized swimming, football, and hockey) is different from non-athletes (Sysoeva et al., 2009). In another study, it was reported that students who were aggressive and used drugs had more SS genotypes compared to students who did not use drugs (Gerra et al., 2005). On the other hand, individuals with LL genotype and L allele have been reported to be more resistant to stress and trauma (Caspi et al., 2006).

In this study, we aimed to examine the distribution of "S" and "L" alleles of the promoter region of the SLC6A4 gene in professional Turkish football players and compared the results with sedentary individuals.

2. Materials and Methods

The protocol used in the present study was approved by the Uskudar University Ethics Committee and was performed in accordance with the principles of the Declaration of Helsinki II. All participants signed consent forms containing all the information such as the study protocol, results and evaluation of the results.

2.1. Study group

20 professional football players were participated in our study. As a control group, 44 individuals who did not exercise regularly participated in our study. The study protocol was prepared in accordance with the Helsinki Declaration-2 (2015) guidelines and was approved by the Uskudar University Non-Interventional Ethics Committee. Before the study, all participants signed consent forms containing all the information such as the study protocol, results and evaluation of the results.

2.2. Genotyping

DNA isolations from buccal epithelial cells of the athletes participating in our study were performed with the commercially obtained PureLink DNA isolation kit (Invitrogen, Van Allen Way, Carlsbad, CA, USA) and the polymerase chain reaction (PCR) technique was used for genotyping. Briefly, a total volume of 50 μL of 100 ng DNA, 0.5 mM dNTP, 10 pmol primers and 2U Taq polymerase was performed (Fermantas, Vilinus, Lithuanian). PCR conditions 95 °C 3 min pre-denaturation, 35 cycles in total, 95 °C 30 s, 53 °C 45 s, 72 °C 1 min and after the last cycle at 72 °C 10 min final elongation It was applied in the form. The amplicons obtained were imaged under Ultraviolet (UV) light in 2.5% agarose gel electrophoresis with ethidium bromide.

2.3. Statistical analysis

Genotype distribution and allele frequencies between groups of athletes and controls were then compared by χ² testing using the SPSS (version 18.0 for Windows, SPSS, Chicago, IL, USA) program in the statistical analysis of the obtained results. P <0.05 value was accepted as statistically significant.

3. Results

In the SLC6A4 analysis, it was determined that 4 (20%) of 20 players had LL, 8 (40%) of them LS and 8 (40%) of them had SS genotype. When allele distributions were examined, it was observed that the percentage of L allele was 40% and the S allele was 60%. In the control group (n = 44), 10 individuals had LL, 25 individuals LS and 9 individuals had SS genotype. L allele was counted as 45 (51%) and S allele as 43 (49%). When the two groups were compared statistically, in terms of genotype distribution, no statistically significant significance was found between the two groups (p = 0.2493) and allelic distribution, no statistically significant difference was found (p = 0.2423). The genotype and allele number distributions of the athletes are summarized in Table 1.

Table 1. Genotype and Allelic Distribution of players for the SLC6A4 Gene Region

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>P Value</th>
<th>Allelic Frequency</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL</td>
<td>16</td>
<td>0.2493</td>
<td>40%</td>
<td>0.05</td>
</tr>
<tr>
<td>LS</td>
<td>24</td>
<td>0.2423</td>
<td>40%</td>
<td>0.05</td>
</tr>
<tr>
<td>SS</td>
<td>8</td>
<td>0.2493</td>
<td>40%</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Significance was assessed at least at the p <0.05 level. Comparison with the control group was made using the x² test.
In our study, LS and SS genotype were co-dominant and at the same time higher than LL genotype. At the same time, when we compared the S allele with the L allele, it was found that the S allele was more in percentage. Our findings differ from those of previous studies. The L allele is high expression allele and is associated with resistance to stress and trauma. The S allele is associated with anxiety. This study is the first study investigating the relationship between the SLC6A4 gene and anxiety in Turkish athletes and finding a higher S allele. Thus, our work will contribute to the genetic information pool and support other studies in this field. Our study can help to take early precautions for anxiety disorders encountered in athletes.

Patient informed consent: Informed consent was obtained.
Ethics committee approval: The protocol used in the present study was approved by the Üsküdar University Ethics Committee and was performed in accordance with the principles of the Declaration of Helsinki II. All participants signed consent forms containing all the information such as the study protocol, results and evaluation of the results.
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Tolga Polat (10%): data collection, statistics
Çisem Şılar (10%): laboratory assistance
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Sezgin Kapıcı (10%): laboratory assistance
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Başak Eken (5%): data collection
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