

Preliminary Findings of the Distribution of Alpha-2-Adrenergic Receptor (*ADRA2A*) rs1800544 Polymorphisms in Kickboxing Players

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ABSTRACT

Objective: Adrenergic receptors play an important role in providing neurotransmitter release from the sympathetic nervous system and adrenergic neurons. The rs1800544 polymorphism in 3'-untranslated region of Alpha-2-Adrenergic Receptor (*ADRA2A*) is if caused by C>G tranversion at position -1291. In this study, we objective to analyze the rs1800544 distribution in kickboxing players.

Methods: A total of 12 male players and 101 sedentary individuals (as a control group) participated in the study. Following DNA isolation, rs1800544 polymorphism of *ADRA2A* gene was determined by real-time polymerase chain reaction (Rt-PCR). Statistical analysis was carried out by chi-square test.

Results: CC, CG and GG genotypes and percentages for rs1800544 polymorphism were analyzed as 5 (41,7%), 4 (33,3%) and 3 (25%), respectively. C allele was counted as 14 (58,3%) and G allele as 10 (41,7%). In the control group CC, CG and GG were found as 34 (33,6%), 53 (52,5%), 14(13,9%), respectively. C allele was counted as 121 (60%) and G allele as 81 (40%). When we compare athletes and controls in the terms of genotypes, we detected no statistically significant difference ($p>0,3940$). For the allele comparison, we detected no significant difference between groups ($p>0,0871$).

Conclusion: Determination of gene combinations in athletes is of great importance for determination of the effect of genetic factors in athletic performance. In our cohort, CC genotype was more frequently found, and C allele was also higher in players. We think that the effect of *ADR2A2* gene on athletic performance might be determined more clearly by the fact that this study is an example of similar studies involving higher numbers of subjects.

Keywords: *ADRA2A*, Polymorphism, Sports, Kickboxing players

1. INTRODUCTION

Sport is a physical and cognitive contest that pursues rules for a specific purpose such as war or hunting preparations to determine the best, which comes from past to present activity. In both individual or team sports, athletic performance is limited by player's sporting ability or capacity that is influenced by several factors including physical, physiological, environmental and mental (1,2). All factors are directly or indirectly related with genetic/hereditary background even for diet type or exercise characteristics (3,4). This is why, the intrigue of association between sports and genes or continues to fascinate both athletes and researchers, despite current findings.

Kickboxing has historically been developed from karate, Thai Boxing and western boxing, and is a fighting sport that is generally used for standing and self-defense based on kicking

and punching. Worldwide interest in kickboxing is increasing due to its beneficial effects such as personal protection, increasing muscle strength and keeping the body in shape (5). However, heavy blows to the head, neck and abdomen of the athletes during the competitions cause serious injuries and negatively affect the athlete's health in the short and long term.

Adrenergic receptors (adreno receptors) are members of the G protein-coupled receptor family class. It is a catecholamine linked to norepinephrine (noradrenaline) and epinephrine (6). It plays a major role in the release activity of the adrenaline hormone and the neurotransmitter of noradrenaline. There are 3 adrenergic receptor variants; $\alpha 1$, $\alpha 2$ and β (7). These receptors play an important role in the release activity of neurotransmitters from sympathetic

nerves and adrenergic neurons in our central nervous system. Studies in mice revealed that both the $\alpha 2A$ and $\alpha 2C$ subforms of the receptors are required for presynaptic control of transmitter release from sympathetic nerves in the heart and central noradrenergic neurons. In the study, the $\alpha 2A$ subtype was found to inhibit transmitter release at high stimulation frequencies, while the $\alpha 2C$ subtype modulates neurotransmission at lower nerve activity levels (8).

Studies have shown that methylphenidate improves attention by acting on alpha2-adrenergic receptors found in dopamine-containing neurons. The functional rs1800544 polymorphism, which is formed by C>G transversion at a position -1291, is located in the promoter region of *ADRA2A* gene. Another functional rs553668 polymorphism is located in the 3'-UTR region of the gene and occurs as a result of A>G transition. These are the two main polymorphisms investigated in Attention-Deficit/Hyperactivity Disorder (ADHD) patients. In pharmacogenetic studies conducted to date, the role of rs1800544 in the *ADRA2A* gene promoter region was investigated and the relationship of the G allele with MPH was evaluated (9). Also, attention deficit symptoms are associated with rs1800544. The *ADRA2A* gene is a small gene with a genomic size of <4000 bp. The α -2A-adrenergic receptor (*ADRA2A*) also plays a role in the regulation of sympathetic nervous system activity.

The aim of our study is to evaluate the distribution of *ADRA2A* (rs1800544) polymorphism in kickboxing players. To date, there are no studies on Turkish kickboxing players in the terms of *ADRA2A* polymorphism yet. Therefore, this report will be the first to determine the allelic distribution of rs1800544 polymorphism of kickboxing players.

2. METHODS

2.1. The Participants

A total of 12 male professional kickboxing players and 101 sedentary controls, all with Turkish ancestry, were recruited for the study. Our study protocol were prepared in accordance with the Helsinki Declaration-2 (2015) guidelines and approved by Uskudar University Non-Interventional Ethics Committee. (Protocol number 61351342/2019-550). The volunteers participating in the study were given detailed information about the analyzes and outputs before the study and their consent forms were obtained from them.

2.2. *ADRA2A* Genotyping

DNA Isolation

Oral epithelium cells were collected by DNA collection sticks from the volunteers who participated in the study, and DNA isolation was completed by using a PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, CA, USA). Briefly, 20 μ L proteinase K was vortexed by adding 10 μ L of RNAase

to 200 μ L of DNA isolation. After 2 min at room temperature, 200 μ L of binding buffer was added and homogenized with stirring. After incubation for 10 minutes in a 55 $^{\circ}$ C water bath, 200 μ L of ethanol was added and vortexed for 5 seconds. It was taken to the filtered tube and centrifuged at 10000g for 1 minute. The supernatant was discarded and 500 μ L of washing buffer was added to the pellet and centrifuged at 10000 g for 1.15 seconds. 80 μ L of elution buffer was added and incubated and centrifuged at maximum speed for 1 minute. An average of 20ng of DNA was isolated from each sample and the isolated DNAs were evaluated according to the OD260/280 spectrophotometric ratio. The DNA samples obtained were stored at - 20 $^{\circ}$ C until the analysis of the relevant gene regions was completed.

Genotyping of *ADRA2A* rs1800544

Genotyping of *ADRA2A* rs1800544 was performed from the isolated DNA by using 7500 Fast Real-Time PCR System (Applied Biosystems). TaqMan Genotyping Assays (Applied Biosystems Foster City, CA, USA) genotyping kit was used for allelic determination. C and G alleles were determined using VIC and FAM primers, respectively (Table 1). Genotyping was completed using 5 μ L master mix, 3.75 μ L H₂O, 0.25 μ L assay and 1 μ L (10ng) DNA.

Table 1. VIC/FAM labelled times primer use to *ADRA2A* rs1800544 polymorphism

qPZR	Sequence (5'3')
VIC/FAM	CCGTTGCGTTCTGCTCCGTCGGCCC[C/G] GAGCTGCATGGCCAACTCCCAGCAG

2.3. Statistics

All data were analyzed by using SPSS 20, 0 for windows (SPSS Inc., Chicago, IL, USA). Statistical analysis was conducted by using the chi-square test. Values less than P <0.05 were considered significant.

3. RESULTS

12 kickboxing and 101 control group participated in the study. Genotypes and alleles for the *ADRA2A* gene region are summarized in the Table 2. CC, CG and GG genotypes and percentages for rs1800544 polymorphism were analyzed as 5 (41,7%), 4 (33,3%) and 3 (25%); respectively. C allele was counted as 14 (58,3%) and G allele as 10 (41,7%). CC, CG and GG genotypes and percentages for rs1800544 polymorphism in controls were as 34 (33,6%), 53 (52,5%) and 14 (13,9%); respectively. For allelic distributions, C allele was counted as 121 (60%) and G allele as 81 (40%) (Table 2). When we compare athletes and controls in the terms of genotypes, we detected no statistically significant difference ($p > 0,3940$). For the allele comparison, we detected no significant difference between groups ($p > 0,0871$).

Table 2. Genotype and allele distributions of ADRA2A rs1800544 polymorphism of kickboxing players.

	ADRA2A Genotype			P value	Allele Frequency		P Value
	CC	CG	GG		C	G	
Players (12)	5	4	3	0,3940	14	10	0,0871
Percentage	41,7%	33,3%	25%		58,3%	41,7%	
Control (101)	34	53	14	0,0871	121	81	0,0871
Percentage	33,6%	52,5%	13,9%		60%	40%	

*Significance was assessed at least at the $p < 0,05$ level. Comparison with the control group was made using the χ^2 test.

4. DISCUSSION

Kickboxing is a sport in which two competitors compete to get the most score points with the technique and speed in accordance with the rules. While punches and kicks are strictly controlled, points are awarded by hitting the hands or feet in accordance with the rules, with proper techniques on the permitted areas.

In the formation and development of athletic performance, muscle metabolism is expected to be optimal. The effect of genetic variations on metabolism on and cellular basis of activities, such as exercise, is important a lot through article crucial, therefore optimal to more favorable forms are expected to be included in the genotypes of athletes (10).

Recent studies have shown that genetic parameters are important in athletic performance and physical health. In studies to date, more than 200 gene variants have been associated with athletic performance, some with structural and some with functional factors such as enzymes (11). Recent studies that aimed to determine the athletic performance in the terms of environmental (epigenetic) and genetic factors had not been fully identified the association of genetic and epigenetic factors and warrants further research in sports genetics (12).

CC genotype was more dominant compared with other genotypes and C allele in the terms of ADRA2A rs1800544 polymorphism in our cohort. According to the best of our knowledge, this report is the first which investigate reflects the genotype distribution of rs1800544 polymorphism in Turkish kickboxing players.

Studies have reported that individuals with the C allele of ADRA2A rs1800544 polymorphism are higher than the G allele. It is stated that adrenergic receptors play a role in the regulation of adipose tissue lipolysis, which is one of the most important steps in meeting the energy needs of athletes during endurance sports training (13).

Wolfarth *et al.* (14) examined the ADRA2A and ADRB2 gene polymorphisms in the study comparing 148 Caucasian elite endurance athletes and 149 the control group. Professional endurance athletes and a control group were compared. A significantly higher frequency of the 6.7-kb allele was observed in athletes, suggesting that this genetic variation in the ADRA2A gene may play a role in sustaining the endurance

training necessary for enhanced maximal aerobic power. It is stated that genetic variation in the ADRA2A gene or in a locus in the immediate vicinity can be effective in sports that require endurance to achieve high levels of maximum aerobic power.

5. CONCLUSION

Currently, genetic factors are gaining importance importance in determining athletic performance. As athletic performance involves multifactorial elements, it is thought that analyzing gene groups instead of single gene analyzes may provide precise results in the studies. Determination of gene combinations in Turkish kickboxing is of increasing importance whilst carrying out these studies. Therefore, instead of comparing the alleles between athletes' sedentary individuals, we aimed to determine their distributions on successful athletes.

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