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The Determination of *IL-6* rs1800795 Polymorphism Distribution in Turkish National Cross-Country Skiing Athletes Sub-groups Created Referring to the 1km CCSTAs

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ABSTRACT

Objective: Interleukin-6 (IL-6) is an effective and functional protein with a cytokine structure. IL-6 produces a pro/anti-inflammatory response in the immune system and muscle tissue hypertrophy and repair. We aimed to investigate the *IL-6* rs1800795 polymorphism in athletes who were members of the Turkish national cross-country skiing team in order to determine the advantageous genotype for endurance performance.

Methods: A total of 34 athletes within three groups–general (group-1), female (group-2), and male (group-3)–were enrolled in the study. Each group was also divided into two sub-groups as faster and slower referring to their 1-kilometer cross-country skiing time averages (1km CCSTAs). Blood samples were used for DNA isolation, and genotyping was completed by real-time PCR. Chi-square Fisher's and descriptive tests were used for statistical analysis.

Results: The groups' 1km CCSTAs were 188.7 ± 22.4, 212.3 ± 17.8, and 177.4 ± 14.1 seconds, respectively. We detected the genotypes as follows: 17 GG (50%) and 17 GC (50%) in Group 1, 5 GG (45%) and 6 GC (55%) in Group 2, and 12 GG (52%) and 11 GC (48%) in Group 3. We detected no CC genotype in the groups.

Conclusion: Although the GC genotype ratio was higher in the faster athletes of each group compared to the slower sub-groups, we detected a statistically significant difference only in Group 3 ($p<0.0001$). Additionally, the C-allele frequency was higher in the faster sub-groups. However, the significant allele gene distribution was obtained only in Group 3 ($p=0.0002$). According to our results, we can speculate that the GC genotype is more advantageous than the GG genotype in cross-country skiing athletes.

Keywords: *IL-6* Genotypes, Cross-country skiing, Endurance, Athletic performance, Sports genetics

1. INTRODUCTION

The first goal of sports genetics research is to identify the genes that affect athletic performance which is a concept with multifactorial components determined by both polygenetic and epigenetic mechanisms. Today, we know that there are quite a few genes associated with athletic performance (1). We can categorize these genes into groups such as those that affect endurance, muscle strength, energy metabolism, muscle repair, ligament-tendon strength, and heart-lung capacity. The second goal of sports genetics research is to analyze the polymorphism distribution of the determined genes in successful athletes. The third goal of sports genetics research is to use the data obtained to direct individuals in childhood to sports branches in which they may be predisposed according to their genotype.

Cross-country skiing is a winter sport based on completing a heat in the shortest time possible on snow with very heavy protective clothing using skis and poles. It is an endurance sport that requires long-term muscle activity depending on the type of competition (2). There are many national and international competitions for this sport branch. The experimental group in our research included athletes who are specialists in this field.

The Interleukin-6 (*IL-6*) gene is located on chromosome 7 (7p15.3). IL6 is a cytokine protein consisting of 184 amino acids. IL-6 effects the immune system, muscle tissue repair, and muscle hypertrophy. It is accepted that it creates a pro-inflammatory effect as a cytokine as well as an anti-inflammatory effect as a myokine (3).

IL-6 rs1800795 polymorphism attracts a great deal of attention in sports genetics studies (4, 5, 6, 7, 8, 9). According to *IL-6* rs1800795 polymorphism, the G allele is associated

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with more IL-6 plasma levels and is considered to be more advantageous in strength-oriented sports while the C allele, which causes a low IL-6 plasma level, is considered to be more advantageous in endurance sports.

It is the purpose of our study to investigate the relationship between the 1km CCSTA of the athletes of the Turkish cross-country skiing team and *IL-6* rs1800795 polymorphism. We aim to determine the advantageous allele and genotype for cross country skiing, an endurance-focused sport. We can hypothesize that the GC genotype is more advantageous than the GG genotype in cross-country skiing athletes. We believe that our study will be a meaningful part of big data to better understand the paradoxical identity of *IL-6* and contribute scientifically to the spread of cross-country skiing sport in our country and the world.

2. METHODS

2.1. Sample Selection

A total of 34 athletes participated in our study. We formed three experiment groups among the athletes: general (Group 1), female (Group 2), and male (Group 3). There were 34, 23, and 11 athletes in Group 1, Group 2, and Group 3 respectively. There was no sedentary group in our study. We divided each group into faster and slower sub-groups referring to their 1km CCSTAs. There were 19 athletes in the faster sub-group and 15 athletes in the slower sub-group of Group 1, and 7 athletes in the faster sub-group and 4 athletes in the slower sub-group of Group 2, and 12 athletes in the faster sub-group and 11 athletes in the slower sub-group of Group 3. Our study was carried out with a protocol under the Declaration of Helsinki. Ethics committee approval was given by the Uskudar University ethics committee (Approval Number: B.08.6.YOK.2.US.0.05.0.06/2018/553).

2.2. Genotyping

DNA isolation for the blood samples taken from all athletes was carried out with a PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, USA) following the user protocol. Generation of gene amplicons from isolated DNA and genotyping of *IL-6* rs1800795 was realized by the real time-PCR method. For this purpose, the PCR device Roch Light Cycler Nano, Taqman's genotyping kit (Applied Biosystems, Foster City, CA, USA), and VIC/FAM probes were used.

2.3. Statistical Analysis

The data obtained were analyzed using the statistics program for the social sciences (SPSS, version 25.0, IBM, USA). While descriptive statistics of demographic data was performed with a descriptive test, predictive statistics of the data related to genotypes and alleles gene distribution between the sub-groups was performed with the chi-square Fisher's test. Significance was accepted as less than 0.05 ($p<0.05$).

3. RESULTS

A total of 34 national cross country skiing athletes, 23 (68%) male and 11 (32%) female, participated in our study. According to the *IL-6* rs1800795 polymorphism, we formed three experimental groups.

Table 1 shows the phenotypic features of the groups and the number of athletes in the sub-groups. We summarized the age, height, and weight averages, 1km CCSTAs, and the sub-groups athlete numbers for each group. The respective average ages were 18.0 ± 1.4, 17.2 ± 0.8, and 18.4 ± 1.5, for Group 1, Group 2, and Group 3. The height (cm) averages were 168.7 ± 6.5, 163.5 ± 6, and 171.1 ± 5.4, respectively; and the weight (kg) averages were 59.6 ± 6.2, 71.1 ± 5.4, and 62 ± 5.4, respectively. The 1km CCSTAs were 188.7 ± 22.4 s, 212.3 ± 17.8 s, and 177.4 ± 14.1 s, respectively for the groups. There were 19 athletes in the faster sub-group and 15 athletes in the slower sub-group of Group 1, there were 7 athletes in the faster sub-group and 4 athletes in the slower sub-group of Group 2, and there were 12 athletes in the faster sub-group and 11 athletes in the slower sub-group of Group 3. The faster and slower sub-groups were formed referring to the 1km CCSTA of each group.

Table 1. *Groups' phenotypic features and sub-groups athletes*

Phenotypic features	Group 1 General Athletes (n=34)	Group 2 Female Athletes (n=11)	Group 3 Male Athletes (n=23)
Average age	18 ± 1.4	17.2 ± 0.8	18.4 ± 1.5
Average height (cm)	168.7 ± 6.5	163.5 ± 6	171.1 ± 5.4
Average weight (kg)	59.6 ± 6.2	54.5 ± 4.5	62 ± 5.4
1km CCSTA* (s)**	188.7 ± 22.4	212.3 ± 17.8	177.4 ± 14.1
Faster sub-group	19 athletes	7 athletes	12 athletes
Slower sub-group	15 athletes	4 athletes	11 athletes

**1km CCSTA: 1-kilometer cross-country skiing time average, **s: second*

The *IL-6* rs1800795 genotype polymorphism distribution of the three groups is summarized in Table 2. There were 17 GG (50%) and 17 GC (50%) genotypes in Group 1, there were 5 GG (45%) and 6 GC (55%) in Group 2, and there were 12 GG (52%) and 11 GC (48%) genotypes in Group 3.

Table 2. *IL-6 rs1800795 genotype polymorphism distribution of groups*

Genotype	Group 1 (n=34)	Group 2 (n=11)	Group 3 (n=23)
GG	17 (50%)	5 (45%)	12 (52%)
GC	17 (50%)	6 (55%)	11 (48%)

Table 3 shows the *IL-6* rs1800795 allele gene polymorphism distribution of the three groups we formed. The distribution of the G and C alleles of the *IL-6* rs1800795 polymorphism in the groups were counted as 51 G (75%) and 17 C (25%) in Group 1; 16 G (73%) and 6 C (27%) in Group 2, and 35 G (76%) and 11 C (24%) in Group 3. The distributions were not normally distributed. The common allele was found to be G allele, as expected.

Table 3. *IL-6 rs1800795 allele polymorphism distribution of groups*

Allele	Group 1 (n=34)	Group 2 (n=11)	Group 3 (n=23)
G	51 (75%)	16 (73%)	35 (76%)
C	17 (25%)	6 (27%)	11 (24%)

Table 4 shows the genotypes and their average 1km CCSTAs time. Individuals with the GC genotype completed the 1km cross-country skiing heat 7.16 ± 22.3 s earlier in Group 1, 3.6 ± 10.3 s earlier in Group 2, and 10 ± 7.3 s earlier in Group 3 than the group averages. In all groups, it was observed that the individuals with the GC genotype completed the 1km cross-country skiing heat in less time.

Tablo 4. *The genotypes differences of 1km CCSTAs*

GROUPS	IL-6 rs 1800795 polymorphism	The differences of genotypes' 1km CCSTAs* from groups' 1km CCSTAs* (s)**
Group 1 (n=34)	GG (n= 17)	7.16 ± 20.9 (slower than average)
	GC (n= 17)	-7.16 ± 22.3 (faster than average)
Group 2 (n=11)	GG (n=5)	4.2 ± 24.9 (slower than average)
	GC (n=6)	-3.6 ± 10.3 (faster than average)
Group 3 (n=23)	GG (n=12)	9.8 ± 11.5 (slower than average)
	GC (n=11)	-10 ± 7.3 (faster than average)

**1km CCSTA: 1-kilometer cross-country skiing time average, **s: second*

Table 5. *Significance value of IL-6 rs1800795 polymorphism genotype distribution between the sub-groups*

GROUPS	SUB-GROUPS	GG	GC	CC	Significance value
Group 1 (n=34)	Faster (n=19)	8 (42%)	11 (58%)	0 (0%)	p= 0.490
	Slower (n=15)	9 (60%)	6 (40%)	0 (0%)	
Group 2 (n=11)	Faster (n=7)	2 (29%)	5 (71%)	0 (0%)	p= 0.240
	Slower (n=4)	3 (75%)	1 (25%)	0 (0%)	
Group 3 (n=23)	Faster (n=12)	1 (8%)	11 (92%)	0 (0%)	p < 0.0001
	Slower (n=11)	11 (100%)	0 (0%)	0 (0%)	

The genotype distribution values between the faster and slower sub-groups formed referring to their 1km CCSTAs are listed in Table-5. In Group 1, the faster sub-group had 8 GG (42%) and 11 GC (58%) genotypes, and the slower sub-group had 9 GG (60%) and 6 GC (40%) genotypes (p=0.49). In Group 2, the faster sub-group had 2 GG (29%) and 5 GC (71%) genotypes, and the slower sub-group had 3 GG (75%) and 1 GC (25%) genotypes (p=0.24). In Group 3, the faster sub-group had 1 GG (8%) and 11 GC (92%) genotypes, and the slower sub-group had only 11 GG (100%) genotypes. Only the allele genes distribution of Group 3 was statistically significant (p<0.0001).

The ratio of GC genotypes in all the faster sub-groups was higher than in the slower sub-groups. However, only the genotype distribution of Group 3 was statistically significant (p<0.0001). In Group 3, it was observed that the ratio of GC genotypes was higher in the faster sub-group than the slower sub-group. The ratios of GC genotypes of Group 3 were 0% in the slower sub-group and 92% in the faster sub-group. These ratios had a significant distribution (p<0.0001).

Table 6 shows the significance values of the allele gene distribution between the faster and slower sub-groups formed referring to their 1km CCSTAs. In Group 1, the faster sub-group had 27 G (71%) and 11 C (29%) allele genes, and the slower sub-group had 24 G (80%) and 6 C (20%) allele genes (p = 0.57). In Group 2, the faster sub-group had 9 G (64%) and 5 C (36%) alleles genes, and the slower sub-group had 7 G (88%) and 1C (12%) alleles genes (p=0.35). In Group 3, the faster sub-group had 13 G (54%) and 11C (46%) alleles genes, and the slower sub-group had 22 G (100%) and 0 C (0%) alleles genes (p=0.0002).

Table 6. *Significance value of IL-6 rs1800795 polymorphism allele distribution between sub-groups*

GROUPS	SUB-GROUPS	G	C	Significance value
Group 1 (n=34)	Faster (n=19)	27 (71%)	11 (29%)	p= 0.57
	Slower (n=15)	24 (80%)	6 (20%)	
Group 2 (n=11)	Faster (n=7)	9 (64%)	5 (36%)	p= 0.35
	Slower (n=4)	7 (88%)	1 (12%)	
Group 3 (n=23)	Faster (n=12)	13 (54%)	11(46%)	p= 0.0002
	Slower (n=11)	22 (100%)	0 (0%)	

The G allele gene's rate was over 50% in all groups, and the frequency of C alleles in all the faster sub-groups was higher than in the slower sub-groups. However, only the allele gene distribution of Group 3 was statistically significant (p=0.0002). The ratios of C alleles in Group 3 were 0% in the slower sub-group and 46% in the faster sub-group. These ratios had a significant distribution (p=0.0002). In our cohort, there was only the GC as genotypes with C, but there was no CC genotype.

4. DISCUSSION

IL-6 is an effective cytokine in the immune system, in muscle tissue repair, and in muscle hypertrophy. In recent studies, it has been reported that the IL-6 molecule produces a pro-inflammatory response in monocytes or macrophages and an anti-inflammatory response in the muscle (10). The IL-6 molecules can rise to 100 times the normal level in blood and muscle tissue during exercise, depending on the amount of physical activity (11).

Factors that increase *IL-6* gene expression in skeletal muscles include the increase in muscle contraction activity, the increase in the number of calcium ions in the cytoplasm, the increase in the amount of calcineurin, some metabolic changes as a result of sports activity (decrease in glucose and glycogen content, increase in oxidative stress, increase in temperature, increase in catecholamines); and it can be counted as an increase in some hormones (12).

Some studies have reported that vigorous and intense exercise reduces the number of pro-inflammatory receptors on the surface of monocytes and some other cells (13). Based on information in the literature, we thought that the reason for the decrease in the number of these receptors is to reduce the destructive effect of the excessively increased number of cytokines. Therefore, we hypothesized that the GC genotype may be more advantageous than GG for endurance-type athletes.

Our study is one of the first to investigate the relationship among the 1km CCSTA of the Turkish cross-country skiing athletes with *IL-6* rs1800795 polymorphism distribution. In our study, we formed three experimental groups among the athletes on the Turkish national cross-country skiing team as Group 1, Group 2, and Group 3. We also divided each group into two sub-groups that were relatively faster and slower referring to their 1km cross-country skiing time averages (1km CCSTAs). In addition, the genotype distribution between these sub-groups according to the *IL-6* rs1800795 polymorphism was investigated.

There are some studies that attempt to associate the rs1800795 SNP polymorphism of the *IL-6* with athletic performance in terms of strength or endurance. In the literature, significant relationships have been established between the G allele of the *IL-6* rs1800795 polymorphism and increased power performance (4, 6). In a meta-analysis of this topic, nine polymorphisms were defined for strong athlete status, and, according to the *IL-6* rs1800795 polymorphism, the G allele and thus the GG genotype was found to be associated with the power phenotype. (14).

However, there are also studies in the literature reporting that there is a significant relationship between the G allele of the *IL-6* rs1800795 polymorphism and the endurance phenotype (8). However, there are also studies reporting a significant relationship between the C allele and the endurance phenotype (9).

When we look at the difference in genotypes and 1km CCSTAs, it was observed that the athletes with GC genotype completed the 1km cross-country skiing faster than the group averages in all three groups.

The GC genotype ratio was higher in the faster sub-groups of each group compared to the slower sub-groups. However, a significant genotype distribution was detected only in Group 3 ($p<0.0001$). The ratios of the GC genotypes of Group 3 were 0% in the slower sub-group and 92% in the faster sub-group. We can say that the GC genotype is more advantageous than GG for endurance performance, and there was no CC genotype in our cohort.

The C allele frequency was higher in the faster sub-groups of each group compared to the slower sub-groups. However, a significant allele gene distribution was detected only in Group 3 ($p=0.0002$). The ratios of C alleles of Group 3 were 0% in the slower sub-group and 46% in the faster sub-group. In our cohort, there was only the GC as genotypes with C, and there was no CC genotype.

According to our results, we can speculate that the GC genotype is more advantageous than GG in sports branches that require endurance. We believe that the GC genotype, which may be less affected by the destructive effect of excessively increased cytokine amount, may be more advantageous in sports branches that require endurance compared to the GG genotype. However, there was no CC genotype in our cohort, which makes it difficult to make a hypothesis about CC. The main limitation of our study was the low number of subjects. The reason for this was to eliminate the environmental differences of the athletes. The athletes who enrolled in our study had the best average times and represented the national team. They have similar training programs and very similar nutritional habits; that was the reason we just wanted to have a single team to analyze the effect of the *IL-6* rs1800795 polymorphism. The second limitation of our study, which we are planning to complete in further studies, was the lack of serum IL-6 amounts. In spite of these limitations, we believe that this study will guide researchers to further studies.

5. CONCLUSION

High performance occurs in athletes as a result of the interaction of appropriate genetic structure and environmental factors such as proper nutrition and training. Therefore, genetic and environmental factors that can affect athletic performance should be analyzed separately. We can suggest that the GC genotype is more advantageous than GG for endurance performance; however, since a limited number of athletes were included in our study, we think that this gene polymorphism should be re-studied in experimental groups with larger numbers of participants, and its results should be evaluated with meta-analyzes. Our results should also be confirmed by further studies.

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