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Original Research

−174 G/C polymorphism of interleukin 6 gene is not significantly different in Turkish professional short and long distance runners

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Abstract: Interleukin-6 (IL-6) is a kind of multifunctional cytokine and involved in mediating muscle repair metabolism, and therefore athletic capacity. Muscular and circulating IL-6 levels increase in response to physical exercise. Responsible gene coding for IL-6 has a functional polymorphism in its promoter region, -174 G/C (rs1800795). We aimed to analyze the association of G allele and GG genotype in Turkish professional athletes and compare the allelic and genotypic difference between short distance and long distance runners. For this purpose, we enrolled 40 (24 short distance runners and 16 long distance runners) Turkish professional athletes to the study. Real time genotyping procedure was carried out to determine the -174 G/C polymorphism. G allele and GG genotype was more prevalent than the others in our cohort. We found no statistically significant difference between short and long distance runners in the terms of genotype (p=0.07). Our study suggests that-174 G/C polymorphism of IL-6 gene differs in athletes, G allele and GG genotype is higher than the other ones, at least in Turkish athletes, and therefore should be taken into consideration when determining genetic aspects of athletes. Further studies are necessary to confirm our results and show the effect of the given polymorphism in sports science.

Key words: Sport genetics; Endurance; Athlete; Genomics.

Introduction

Interleukin-6 (IL-6) is one of the important multifunctional cytokine involving in immune functions, acute phase response and inflammation. It is a kind of glycoprotein composed of 184 amino acids with the molecular weight of 22,000 – 30,000. Mononuclear macrophages, fibroblasts and T cell are the main sources of IL-6, all aiming to boost B cell hyperplasia differentiation and secreting antibody. Additionally recent studies showed the pivotal role of IL-6 in muscle repair and hypertrophy metabolism in response to exercise-induced damage (1). Plewa (1990) reported the importance of IL-6 in exercise biology; after an acute or longtime exercise, concentration of blood plasma IL-6 increases in an exponential fashion and restored to the basis level in 24 hours. This increase is considered to be related with exercise intensity, duration, muscle mass and one's endurance capacity. It was later shown that in muscle contraction, IL-6 gene expression and mRNA levels is up-regulated, and the produced IL-6 penetrate into blood from these contracting muscles during exercise (3).

The gene responsible for coding of the protein (*IL6*) is located at 7p21. One functional polymorphism, G>C tranversion at position -174 (rs1800795), lies on the promoter region, and is associated with the expression of *IL6*, G allele with increased expression rates and IL-6 amounts when compared with C allele (4). *IL6* −174 G/C polymorphism is associated with not only immunological diseases, but also with sports biology. A study before showed that C allele is more prevalent in non-smokers who have lower maximal work capacity (5). Yamin et al. (2008) reported the association of increased total serum creatine kinase activity, which is a marker of muscle damage and therefore affect muscle metabolism, in C allele carriers following eccentric contractions of the elbow flexor muscles.

Muscles adapt to exercise in different ways. One of the function of this adaptation is to maintain optimal conditions for repetetive conctractions. To maintain these conditions, muscles secrete differents kinds of cytokines, to repair damages, and to be ready for the next task. Despite the association of the given polymorphism in muscle damage and repair after exercise, the effect of this polymorphism on sports performance is not totally clear. As the C allele is accepted with the increased risk of muscle damage following muscle eccentric contraction, we hypothesized that C allele will be underrepresented in long and short distance runners. In addition,

we also wanted to observe if this polymorphism differs in short and long distance runners, and may have a possible advantage for the sports involved.

Materials and Methods

A total of 40 professional athletes were recruitted in the present study. All the athletes were a member of athlete team and have at least 4 training sessions per week, a total of at least 16 hours. The study is completed under the principles of the Declaration of Helsinki II (2015). All the participants signed the informed consents explaining the study protocole and the outcomes of the study results.

DNA Sample Collection

For genomic DNA isolation, 200 uL peripheral blood samples were collected in the EDTA containing tubes. High Pure PCR Template Preparation Kit (Roche, Basle, Switzerland) kit was used for isolation. Manufacturer's instructions were followed for the isolation. All the samples were isolated at the same day, and the isolated DNAs were kept -20 ^{0}C until the genotyping process were carried out.

Genotyping

Functional, located in the promoter region, were genotyped with Real- time PCR (Applied Biosystem, USA) depending on the hypothesis that *IL6* −174 G/C polymorphism influences sports performance and differs in short and long distance running athletes performing. A total of 20 µl PCR mixture contained 10-20 ng genomic DNA, and spesific primers for "G" allele, and for C allele. Commercially avaible Taqman SNP Genotyping Assay (Applied Biosystem, USA) kit was used for genotyping process.

Statistical Analysis

Statistical analyses were performed by using SPSS (version 18.0 for Windows, SPSS, Chicago, IL, USA). Relationships yielding $p < 0.05$ values were considered to be significant.

Table 1. Genotype and allele distributions of the examined athlete cohort.

IL-6 (rs1800795)	Genotype			Allele	
in study cohort	GG	GC	CC	G	C
Number (n=40)	25	14	1	64	16
Percent	%62.5	35%	%2.5	80%	20%

Results

Of the 40 athletes, 25 had the genotype of GG, 14 had GC and only 1 athlete had CC for the given polymorphism. G allele was counted as 64 and had the percentage of 80, whereas C allele was counted as 16, and had the percentage of 20. Table 1 summarizes the genotype and allele distribution of the study cohort.

24 of the athletes were short distance runners in our cohort. GG, GC and CC genotypes were found as 15, 8, 1, respectively, in short distance runners. G allele was found to be as 26, and C allele as 6 in short distance runners. Of the 16 long distance runners, 10 and 6 athletes had GG and GC genotypes, respectively. In long distance athletes, we detected no CC genotype. G allele was counted as 26, and C as 6 in long distance runners. Table 2 summarizes the genotype and allele distribution in short and long distance runners.

In our cohort, we detected no significant difference in the terms of genotype and alleles in short distance runners and long distance runners (p=0.07).

Discussion

Recent studies showed the importance of genetic parameters in athletic performance and physical fitness. To date, more than 200 gene variants were associated with athletic performance, some related with structural units, some with functional factors like mediators or enzymes. One of the candidate variants related with power- endurance phenotype is -174 GC polymorphism of IL- 6 gene.

G allele of rs1800795 polymorphism is accepted to be the ancestral allele, and associated with higher expression, and therefore higher amounts of IL-6. In our cohort, GG genotype and G allele is the dominting ones when compred to others. We recruitted 40 professional athletes to the present study, and 25 of them had the GG genotype. Of these, 15 of them was short distance runners, 10 of them long distance runners. There was only one CC genotype in short distance runners, and we could not detect CC genotype in short distance runners. We detected no statistically difference in the terms of rs1800795 genotypes between short and long distance runners. 80% of the athletes had the G allele in our cohort.

Yemin et al. (6) before reported th association between increased muscle damage and C allele in response to unaccustomed eccentric exercisein non-athletes. Like Yamin et al. (6), Ruiz et al. (2009) anayzed 100 endurance athlete (cyclists,runners), 53 power athletes (jumpers,throwers,sprinters) and reported that C

Table 2. Genotype and allele distributions of the short and long distance runner athletes.

IL-6 (rs1800795)	Genotype			Allele	
Short distance runners	GG	GC	CC	G	C
Athlete number (n=24)	15	8	1	38	10
Percentage	63%	33%	4%	79%	21%
IL-6 (rs1800795)	Genotype			Allele	
Long distance runners	GG	GC	CC	G	C
Athlete number (n=16)	10	6	0	26	6
Percentage	%62.5	%37.5	0%	81%	19%

allele is underrepresented in power athletes. Our results are in agreement with the previous ones, C allele is underrepresented in athletes when compared to G allele. In our cohort, we detected no CC genotype, which is considered to have a disadvantage against power oriented athletic performance. It was established that IL-6 has important and pivotal role in muscle repair in acute exercise. Following acute exercise, IL-6 is released to circulation from muscles (3). IL-6 shows its anti-inflammatory effect by inhibiting tumor necrosis factor alpha (TNF- *α)* or interleukin-1 (IL-1) production, thats why IL-6 has important beneficial roles in muscle repair (Petersen 2005).

Eider et al. (2013) analyzed the same polymorphism in 158 Polish power-orientated athletes and 254 volunteers. Like our results, they have reported that GG genotype and G allele was higher in power athletes when compared to sedentary ones. Eynon et al. (2011) reported the high frequencies of GG genotype and G allele in Israel (Caucasian) endurance and power national and international athletes. Our results are consistent with the previous ones, to indicate the importance of G allele/ GG genotype in athletes. Before, the same polymorphism was analyzed in 486 South African Ironman triathletes and 200 sedentary controls, but no statistically difference was reported between the endurance capacity of the athletes and the polymorphism. Like our study, Cenikli et al. (2016) compared the -174 G/C polymorphism in 92 elite Turkish athletes and 100 controls, and reported that G allele and GG genotype were more prevalent, however they found no statistically significant difference between the elite athletes and the controls.

The main limitation of our study is the sample size. As athleticisms is gaining attention and importance in Turkey, there is still not enough interest. As there are not enough teams and professional athletes, it is quite hard for sport scientist to make scientific research including this subject. Another concern is that in such studies, including different ethnic populations, results are expected to each other, especially large possible cohorts. Genetic heterogeneity of the subjects, depending on different populations, may help us to explain between-studies differences. Our other limitation is the lack of biochemical results, like circulating IL-6 levels. IL-6 amount may be affected from other factors, like environmental factors, or stress; so it would be informative for us to analyze circulating IL-6 levels.

There is an increasing evidence suggesting that athletic performance is determined, partly, by the athlete's genetic endowment. Our data, with the previous ones, suggest the importance of -175 G/C polymorphism of *IL-6* G gene in determining the athletic performanceof athletes. To have more precise results, we should have more samples to genotype, and our results should be confirmed by further studies.

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Interest conflict

All the authors declare no conflict of interest.

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