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VEGFR2 Gene Polymorphism and Aerobic Performance in Turkish Elite Endurance Athletes

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ABSTRACT The present study analyzes the effect of *VEGFR2* gene rs2305948 polymorphism on the aerobic performance of Turkish elite endurance athletes. This study included 170 participants (110 sedentary controls and 60 elite endurance athletes). Maximal oxygen uptake capacity was used to define the aerobic performance of participants (both athletes and sedentary controls). Peripheral blood was used for genomic DNA isolation. Genotyping of the *VEGFR2* gene polymorphism was performed by PCR–RFLP methods. For statistical analysis SPSS software programme and ki-kare analysis were used. According to the results of the study, no statistically significant importance was detected between the elite athletes and control groups with respect to *VEGFR2* genotype distributions ($p>0.05$). To the researchers' best knowledge, in elite endurance athletes, this is the first study in Turkey that examined *VEGF2* gene rs2305948 polymorphism. The researchers' results suggest that on aerobic performance of Turkish elite endurance athletes *VEGFR2* gene has no effect. To confirm these results further studies are needed.

INTRODUCTION

Factors that affect athletic performance are important research topics in sports sciences and sports medicine. Top sports performance is the result of a combination of many factors such as genetics, nutrition, motivation, equipment. Epigenetics is also important factor in recent years. Genetic effects on sporty performance and ability have been studied for many years. High sporty performance; It is the result of a combination of favorable environmental conditions such as training, nutrition and genetic potential (Ahmetov and Fedotovskaya 2012). After obtaining important information about the human genome, the effects of individual differences in the genome on the phenotype attracted attention. In addition to the fact that individual differences and variations in the genome are effective in susceptibility to diseases, studies have also been shown to be effective in the field of sports. Especially factors such as vascular differences, differences in muscle structure/strength and the effect of maximum oxygen capacity in endurance sport are noteworthy. For this reason, especially due to genetic predisposition, sports preferences have become popular in recent years (Ahmetov and Fedotovskaya 2012). As a result of all these studies, strength or endurance in the sport performed as a result of the effects of physical activity levels and long-term exercise (> 12 months) on the mechanism of genes. It has been stated that it has very important effects in terms of increasing the aging and resistance against aging and diseases. Especially in competitions between elite athletes, seconds or even the importance of the differences (except doping / gene doping) arising from the variables in the individual's genetic background has been revealed.

Several autosomal and mitochondrial genes that affect performance in sporting activities in human have been identified. Regarding relationship with performance, the most investigated genes are *ACE, ACTN3, PPARGC1A, PPAR-α, HIF1A, NOS3, Myostatin, VEGFR2 and VEGFA* (Ahmetov and Fedotovskaya 2012). Aerobic training in endurance athletes enables the adaptation of skeletal muscles to training. Exercise induced angiogenesis enhances aerobic capacity by expanding the existing capillary surface area for oxygen diffusion (Kraus et al. 2003; Kim et al. 2019). The increase in maximum oxygen consumption (MaxVO2) by aerobic training leads to an increase in maximum blood flow in

the capillary and proliferation of muscle capillaries in the active tissues (Ahmetov et al. 2008).

Vascular Endothelial Growth Factor (VEGF) is a signal protein. It is a type of vascular growth factor and is produced by cells that stimulate vasculogenesis and angiogenesis. VEGF takes a role in new vascularization during embryonic period and in post-injury vessel repairing.

VEGF also plays an active role in peripheral circulation (Bruno et al. 2018). VEGF, together with hypoxia inducible factors (HIF-1 alpha), stimulates angiogenesis when oxygenation of tissues is reduced, thereby meeting the increased demand for oxygen (Kim et al. 2019; Mason et al. 2007). The effect of VEGF is mediated by receptors, such as *VEGFR1, VEGFR2* and *VEGFR3* (Bagyura et al. 2017; Salles et al. 2016; Ahmetov et al. 2009).

Kinase insert domain-containing receptor (KDR), also known as *VEGFR2* or fetal liver Kinase-1, is expressed in a wide variety of cells, such as endothelial progenitor cells (EPC), endothelial cells and stem cells (Wang et al. 2007). Many studies have shown that VEGF activation produces angiogenic response; It is also suggested that muscle proliferation and VEGFR2 protein expression are highly associated with VEGFR2 mRNA expression in acute systemic exercise (Conway et al. 2001; Milkiewicz et al. 2003; Gavin et al. 2004; Gavin et al. 2007).

Objective

Aerobic exercise workouts are a powerful angiogenic stimulant. Skeletal muscle capillarization increases by 30 percent with 1-3 months of training (Kim et al. 2019; Mason et al. 2007). VEGF is important in angiogenesis. Genetic factors cause individual differences; therefore, in athletic performance, genetic variations and polymorphisms are important. In this regard, the researchers investigated the effect of *VEGFR2* gene rs2305948 polymorphism on aerobic performance.

MATERIAL AND METHODS

Subjects

This study included 170 individuals (110 sedentary controls and 60 elite endurance athletes). Elite endurance athletes mean age was 21.38 ± 2.83 years, and healthy sedentary individuals mean age was 25.92 ± 4.88 years. Selected athletes had participated in national or international track and field championships. From all the participant informed consent was taken. The ethical committee of Ondokuz Mayis University approved the study. Peripheral blood (2ml) was obtained from participants for DNA isolation. DNA isolation was performed by GeneJet (Lithuanian) kit. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis were used for genotyping of *VEGFR2* gene polymorphisms. *VEGFR2* gene polymorphism applied according to the method of O'Brien et al. (2014) and Shahsavari et al. (2015) (O'Brien et al. 2014; Shahsavari et al. 2015). Amplification of PCR was performed by using primers F5'-TTTCCAAGACCATAGCTTACCAT-32 and primer R5'- CAGCATCAGCATAAGAAACTTGTA-3' (O'Brien et al. 2014). The PCR reaction mixture was prepared using 1x Taq pol 25 μL of master mix (Solis Biodyne) (master mix containing $MgCl_2$ 1.5 μM 1x reaction); 5 μL of Firepol Master Mix (Ready-to-Load), 14.5 μL of H2O (MiliQ water) and 2 μL of sample DNA (total volume of 25 μL) were added. PCR conditions included the initial denaturation step at 95°C for 5 min for 1 cycle, 30 cycles of denaturation at 95°C for 60 s, annealing step at 59°C for 60 s, elongation step at 72°C for 60 s and a final extension step at 72°C for 10 min. *Rsa*I enzyme was used for digestion (of GT↓AC). Electrophoresis was performed using 2 percent agarose gel. GeneRuler Low Range DNA Ladder (Thermo fisher scientific) was used as marker. For statistical analysis SPSS software programme and ki-kare analysis were used.

RESULTS

Table 1 presents the physical characteristics of the athlete and control groups. The mean age of the athletes was 21.38 ± 2.83 years (range of age: 18–29), and the control group's mean age was 25.92 ± 4.88 years (range of age: 18–35). The mean body mass index (BMI) of the athletes was 23.91 ± 2.82 kg/m^2 (20-32) and that of the controls was 25.42 ± 3.03 (18-33) (Table 1). Demographic variables and baseline characteristics are shown in Table 2. There were 57 males (95%)

Table 1: Physical characteristics of athletes and control groups

Study group	*n*	*Median ±SD*	*Minimum-Maximum*
Age (year)			
Athletes	60	21.38±2.83	18-29
Controls	110	25.92±4.88	18-35
Height (cm)			
Athletes	60	173.65±5.50	163-188
Controls	110	171.45±6.159	158-189
Weight (kg)			
Athletes	60	72.27±10.04	56-105
Controls	110	74.83±10.82	44-115
BMI (kg/m²)			
Athletes	60	23.91±2.82	20-32
Controls	110	25.42±3.03	18-33
Total	170		

and 3 females (5%) in the athlete group as well as 100 males (90.9%) and 10 females (9.1%) in the control group. Smoking status and alcohol consumption were low in both athlete and control groups. The mean number of sport years of the athletes was 9.23 ± 2.46 (Table 2). The mean maximal oxygen consumption of the athletes ($MaxVO_2$) was 42.14 ± 7.6 ml/(kg min) and that of the controls was 34.33 ± 5.43 ml/(kg min) (Table 3). Other athletic performance values, such as time, speed and levels of participants, are given in Table 3. The results of agarose gel electrophoresis are presented in Figure 1. Heterozygous genotypes have two bands. The distribution of the *VEGFR2* genotype frequencies of athletes and controls are presented in Table 4. The results of the study indicate that there were no statistically significant differences between the groups (p>0.05).

Table 2: Demographic variables and baseline characteristics of athletes and controls

	Athletes n (%)	*Controls n (%)*
Gender		
Male	57 (95)	100 (90.9)
Female	3 (5)	10 (9.1)
Smoking		
Yes	11 (18.3)	30 (27.3)
No	49 (81.7)	80 (72.7)
Alcohol		
Yes	5 (8.3)	13 (11.8)
No	55 (91.7)	97 (88.2)
Sport History (Year)	9.23±2.46	0
Total	60 (100)	110 (100)

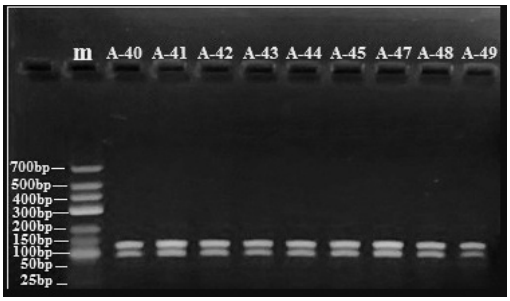

Fig. 1. Results of agarose gel electrophoresis. m: marker (GeneRuler Low Range DNA Ladder)

Table 4: Distribution the genotype frequencies of *VEGFR2* gene

Genotype	*Athletes (n=60) (%)*	*Controls (n=110) (%)*	*P value*
G T	60 (31.1)	110 (49.1)	
GG	0 (0.00)	0 (0.00)	>0.05
T T	0 (0.00)	0 (0.00)	

DISCUSSION

It is known that specific polymorphic regions are related to some physical characteristics of athletes; therefore, genetic variations/ polymor-

Table 3: Sport performance of athletes and controls

Performance values	*Study group*	*n*	*Mean±SD*	*Minimum-Maximum*
Time (s)	Athletes	60	222.4± 77.2	125-590
	Controls	110	149.3± 46.34	19.5-300
Speed (m/s)	Athletes	60	11.36± 1.34	9.49-17.5
	Controls	110	10.03± 1.01	8.4912.5
Level	Athletes	60	3.95± 1.28	2-10
	Controls	110	2.76± 0.83	1-5
VO_2Max (ml/kg/m)	Athletes	60	42.14± 7.6	31.7-78.1
	Controls	110	34.33± 5.43	21.6-49.1

phisms are importanat in sport selection (Ahmetov et al. 2008). Systemic aerobic exercises enhance endurance and the numerous adaptive responses associated with it. Examples include the increase in capillaries around the muscle fiber and the improvement of gas and heat exchange.

Maximum oxygen efficiency increases after training; therefore, blood flow and muscle capillaries increase in active tissues (Ahmetov et al. 2008). In this perspective, genetic factors cause individual differences. Protein concentration is also influenced by the polymorphisms of *VEGF* and *KDR*. These polymorphisms may also alter the process of angiogenesis (Bruno et al. 2018; Bagyura et al. 2017; Wang et al. 2007; Watson et al. 2000). The signaling pathway of angiogenesis is importana especially in remodeling. After cyclic sterchinhg of tendon fibroblast increased levels of aniogenic cytokines were reported in different studies (Kim et al. 2019; Rahim et al. 2016). In a study conducted with Turkish endurance athletes, it was demonstrated for the first time that there is a strong relationship between PPAR-alpha and PPARGC1A genes and endurance performance (Tural et al. 2014). Polymorphisms in *VEGF* gene affect maxVO2. AAG or MaxVO2 levels of individuals with CGC haplotype should be given to individuals in AGG and / or CGG haplotype before and after aerobic training program. It was found to be higher. It was also found that this allele was associated with sportive performance in high-end Russian endurance athletes.VEGFR2 is required in VEGF's angiogenic response to aerobic training. As a result of rsl1870377T / A polymorphism occurring in exon 11 in the gene, glutamine replaces the amino acid of histidine. VEGFR2 472Gln allele is high in endurance athletes ($p = 0.00006$). MaxVO2 levels are also high in those carrying this allele. In addition, the rate of type 1 fiber in the vastus lateralis muscle has been shown to be high in those carrying the 472Gln allele (Kim et al. 2019; Rahim et al. 2016).

In the present study, the effect of *VEGFR2* gene rs2305948 polymorphism was investigated, and it was found that there was no statistically significant importance for *VEGFR2* genotype distributions ($p>0.05$). The researchers emphasize that *VEGFR2* gene rs2305948 polymorphism has no effect. Recently, Rahim and colleagues studied Kinase insert Domain receptor (*KDR*) (rs 1870377, rs(2071559) polymorphism *VEGFA* (rs699947, rs1570360, rs2010963) polymorphisms for the risk of Achilles tendinopathy, and they implicated that the *VEGFA* A-G-G inferred haplotype was related with Achilles tendinopathy. Their new findings link *VEGFA* gene with Achilles tendinopathy risk (Rahim et al. 2016; Salles et al. 2016). Their results revealed that *KDR 1192 GA* and *GA + AA* genotypes showed an association with lower risk of tendinopathy (Salles et al. 2016). Brodal et al. (1977) indicated in their study that individual differences determine the genetic predisposition compared with the performance of physical exercises of different intensities and durations (Brodal et al. 1977; Richardson et al. 2000). VEGF receptors mediate the effect of VEGF (*VEGFR1*, *VEGFR2*, and *VEGFR3*) (Liu et al. 2002). The *VEGFR2*C allele is associated with aerobic physical exercise (Prior et al. 2006). Consequently, Ahmetov et al. (2008) suggested that VEGFR2 C allele carriers may have advantages for aerobic performance capacity (Ahmetov et al. 2008). In another study by Ahmetov et al. (2009), *VEGFR2* gene G-634C polymorphism showed an association with physical performance. This findings emphasize that it may have a key role in sports selection.

CONCLUSION

Genetic infrastructure in sports has a great impact on strength, endurance, muscle mass, muscle type and ratio of muscle fibers. Lung capacity is especially necessary for endurance sports. When the structure of skeletal muscles is examined, it is known that it consists of two different types of fibrils, which can contract quickly and slowly. What fibril in our body genetically predominant type. While fibrils, which can contract fast and produce excessive strength, get tired quickly, those that can contract slowly allow us to sustain a certain effort for a long time. This it is known that the number of mitochondria that provide the energy production of the cell is also related to the genetic structure while playing an active role in the selection of athletes of the type required by endurance and speed sports. Whether athletic ability is inherent or later acquired is always a matter of debate, but whether these abilities and the resulting performance have a limit, has become a subject that has become more important and emphasized today. Genetic fac-

tors cause individual differences; therefore, in athletic performance, genetic variations and polymorphisms are important. In this regard, we investigated the effect of *VEGFR2* gene rs2305948 polymorphism on aerobic performance. The present study infers that *VEGFR2* gene has no effect on aerobic performance of Turkish elite athletes.

RECOMMENDATIONS

In light of the advances in molecular biology and genetics, along with technological advances, genetic factors, especially in diagnosis and risk profiling, candidate gene identification and mapping, as a result of the response to pharmacogenetics and physiological genomics and environmental stimuli, gene and gene groups affecting performance in humans are determined in more detail every day. In recent studies, it has been the subject of research how the increase in sportive performance is affected depending on the genetic infrastructure of individuals. To what extent this effect is successful in the athlete. It is tried to be determined that it affects. In this context, candidate gene studies come to the fore. It should be remembered that the genetic infrastructure of the athlete determines the potential to be superior only in sports. The information received by an athlete from different disciplines contributes positively to his performance. To the researchers' knowledge, this is the first study in Turkey to examine *VEGFR2* gene rs2305948 polymorphism. Further studies with a larger study group are needed to confirm these results.

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