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Investigation of PPAR-Alpha gene polymorphism in female's soccer players

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Abstract

The aim of this study was to investigate the effect of PPAR-Alpha gene polymorphism on female soccer players. 24 female soccer players and 24 female sedentary volunteers aged between 18-26 years participated in the study. For DNA isolation, 2 ml of peripheral blood was collected from the study and control groups in EDTA tubes by a specialized nurse from the right or left forearm anterior venous vein. After informed consent forms were filled out, genotyping was performed at the Black Sea Advanced Technology Research and Application Center (KITAM) Laboratory. The polymorphic region of PPAR-α gene (rs4253778) was identified using PCR-RFLP method. A 266 bc segment in intron 7 of the PPAR-α gene containing the C-T substitution was amplified by PCR. PCR products were obtained by creating the amplification reaction. The obtained products were run on agarose gel and 266 bç fragments were evaluated as GG genotype; 253 bç, 216 bç and 50 bç fragments were evaluated as GC genotype and 232 bç and 21 bç fragments were evaluated as CC genotype and statistical analyzes were performed. As a result of the chi-square analysis, there was no difference between the athlete and sedentary groups (p=0.913). The chi-square analysis revealed no significant difference in the distribution of G and C alleles between athletes (G: 23, C: 8) and sedentary individuals (G: 24, C: 6) (p=0.480). In this study, PPAR-Alpha gene polymorphism data will be an important factor in identifying athletes and determining the predisposition of athletes to which sports branches. It can be used to create a database for similar studies on the distribution of PPAR-Alpha genotypes in Turkiye.

Keywords: Polymorphism, PPAR-Alpha, soccer, women

Kadın futbolcularda PPAR-Alpha geni polimorfiziminin incelenmesi

Öz

Bu çalışmanın amacı, PPAR-Alpha gen polimorfizminin kadın futbolcular üzerindeki etkisini araştırmaktır. Çalışmaya yaşları 18-26 arasında değişen 24 kadın futbolcu ve 24 kadın sedanter gönüllü katılmıştır. DNA izolasyonu için çalışma ve kontrol gruplarından uzman bir hemşire tarafından sağ veya sol ön kol ön venöz damarından EDTA tüplerine 2 ml periferik kan alınmıştır. Bilgilendirilmiş onam formları doldurulduktan sonra genotipleme, Karadeniz İleri Teknoloji Araştırma ve Uygulama Merkezi (KITAM) Laboratuvarı'nda gerçekleştirilmiştir. PPAR-α geninin (rs4253778) polimorfik bölgesi, PCR-RFLP yöntemi kullanılarak tanımlanmıştır. PPAR-α geninin intron 7 bölgesindeki C-T substitüsyonunu içeren 266 bp'lik bir segment, PCR ile amplifiye edilmiştir. Amplifikasyon reaksiyonu oluşturularak PCR ürünleri elde edilmiştir. Elde edilen ürünler agaroz jel üzerinde çalıştırılmış ve 266 bp'lik fragmanlar GG genotipi; 253 bp, 216 bp ve 50 bp fragmanlar GC genotipi; 232 bp ve 21 bp fragmanlar ise CC genotipi olarak değerlendirilmiş ve istatistiksel analizler yapılmıştır. Ki-kare analizi sonucunda sporcu ve sedanter gruplar arasında fark bulunmamıştır (p=0,913). Ki-kare analizi, sporcular (G: 23, C: 8) ve sedanter bireyler (G: 24, C: 6) arasında G ve C allellerinin dağılımında anlamlı bir fark olmadığını ortaya koymuştur (p=0,480). Bu çalışmada, PPAR-Alpha gen polimorfizmi verileri, sporcuları tanımlamada ve sporcuların hangi spor dallarına yatkın olduğunu belirlemede önemli bir faktör olacaktır. Ayrıca, Türkiye'deki PPAR-Alpha genotiplerinin dağılımına ilişkin benzer çalışmalara bir veri tabanı oluşturmak için kullanılabilir.

Anahtar Kelimeler: Futbol, kadın, PPAR-Alpha, polimorfizm

This study is derived from the master's thesis of the first author under the supervision of the second author.

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INTRODUCTION

Until today, it has been a common thought that the maximum improvement of athletes' performance is related to special training methods and developing nutrition programs. However, it is not sufficient that these innovations alone can increase these sportive performances. Nowadays, it is thought that genetic predisposition is an important factor in complicated physical performances. Although genetic predisposition is not the most prominent factor, it seems to have an important role in making an individual a high-level athlete (Altıntaş et al., 2011; Sarıakçalı et al., 2020; Sarıakçalı et al., 2022).

It is beginning to be discovered that there are many genes in the gene map that will affect the physical performance of the individual. In this case, research focuses on the changes in the levels of physical performance parameters by examining the genes (Ceylan, 2022).

Genetic studies have become an important factor in the discovery of sports talents. Therefore, genetic studies on athletes have recently started to increase. Gene screening in the early period will reveal that a child has a great potential with the creation of specific training programs for the development of a child in a special sports branch (Eroğlu & Zileli, 2015).

Small polymorphic changes in the genetics of individuals can lead to different outcomes in athletes with the influence of environmental variables. Polymorphisms based on one nucleotide are generally equivalent to (<1%) of the polymorphism in the population. These can lead to inter-individual differences in physiological function. This may lead to one athlete being superior to another athlete (Eynon et al., 2010).

The peroxisome proliferator-activated receptor gamma coactivator 1a (ppargc1a) and peroxisome proliferator-activated receptor-alpha (ppar-α) genes investigated in the studies are known as transcription factor and coactivator responsible genes that regulate lipid, glucose and energy balance and control body mass and circulatory inflammation (Qian et al., 2024).

One transcription coactivator, ppargc1a, is hypothesized to be involved in diversity in the mitochondrial response. It has been suggested that ppar-α and ppargc1a are expressed at high levels in tissues that degrade fatty acids such as skeletal muscle, liver and cardiac muscle and less in other tissues such as pancreas (Villegas et al., 2014).

Endurance sport is known to increase fatty acid utilization outside plasma. Endurance sport increases ppargc1a mrna level and may increase striated muscle oxidative capacity by regulating ppar-α gene expression. Ppar-α and ppargc1a genes also regulate fatty acid oxidation in striated and cardiac muscle. Changes in these genes may cause an increase or decrease in

energy metabolism and may affect the performance of endurance athletes negatively or positively (Ahmetov et al., 2006; Ahmetov et al., 2015). Therefore, it is necessary to determine how the relationship between ppar-α gene polymorphisms of female athletes in soccer, which is one of the branches requiring endurance, and ppar-α gene polymorphisms of sedentary women (Kaynar et al., 2021).

In this study, it was aimed to understand how female athletes are associated with the ppar-α gene compared to sedentary women and to determine the genetic effect of early sports orientation. In line with these results, it will be determined that if there is a hereditary predisposition, incentives for sports at an early age can form a basis. It is planned that examining this hereditary predisposition and examining the relationship between athlete sedentary will have a facilitating effect on coaches to have an idea, to identify and direct athletes.

METHOD

Research group (Population-sample)

24 female amateur soccer players and 24 female sedentary volunteers aged between 18-26 years participated in the study. For DNA isolation, 2 ml of peripheral blood was collected from the control and study groups in EDTA tubes from the right or left forearm venous vein by a specialized nurse. After the informed consent forms were filled out, genotyping was performed at the Black Sea Advanced Technology Research and Application Center (KITAM) Laboratory.

Data collection tools

The polymorphic region of the PPAR-α gene (rs4253778) was identified using the PCR-RFLP method (Eynon et al., 2010). A 266 bc segment in intron 7 of the PPAR-α gene containing the C-T substitution was amplified by PCR. F-5'-ACAATCACTCTCCTTAAATATATGGTGG3'and(R)5'AAGTAGGGACAGACAGACAG GACCAGTA -3' primers were used for this process. Amplification reaction was performed and PCR products were obtained. The products obtained were run on agarose gel and 266 bc fragments were evaluated as GG genotype; 253 bc, 216 bc and 50 bc fragments as GC genotype and 232 bc and 21 bc fragments as CC genotype and statistical analyzes were performed (Figure 1, Figure 2). Analyses were performed at the Laboratory of the Black Sea Advanced Technology Research and Application Center (KITAM) of Ondokuz Mayıs University.

PCR conditions were as follows: initial denaturation at 95°C for 30 s, 95°C for 30 s, 56°C for 301 min, 68°C for 1 min, 40 cycles at 68°C for 1 min and extension at 68°C for 5 min. After PCR, the products were run on a 1% agarose gel, stained using Ethidium Bromide and visualized on a Biorad Chemidoc MP. PCR products were then subjected to enzyme digestion

reaction. For enzyme digestion, PCR products were incubated with Thermo Scientific FastDigest TaqI enzyme at 65°C for 8 min (this is the process of keeping microorganisms at the optimum temperature at which they can grow for a certain period of time in a device called an oven or incubator).

After enzyme digestion, the samples were run on a 3% agarose gel at 80 volts for 90 min. The bands obtained were visualized and genotypes were determined.

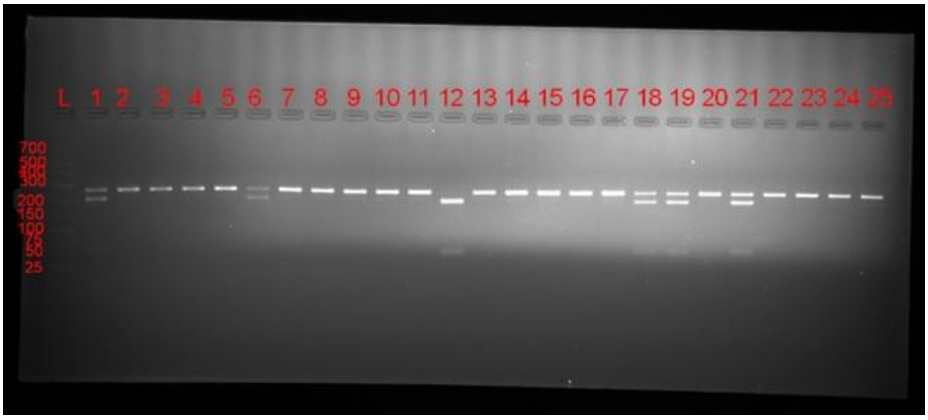


Figure 1. PPAR Alpha PCR Results of sedentary subjects

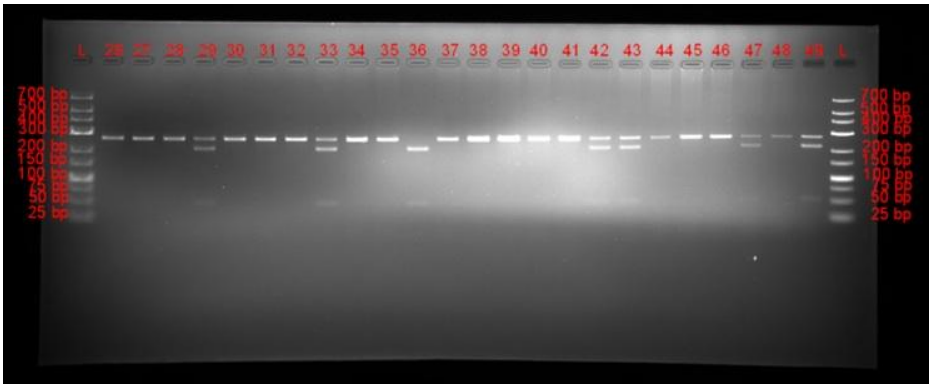


Figure 2. PPAR Alpha PCR Results of athletes and sedentaries

Data analysis

Age, body length, and body weight characteristics of athletes and sedentary people were provided as minimum, maximum, mean, and standard deviation values. To compare the genotypes of athletes and sedentary people, the chi-square test was used. This method was chosen as it is well-suited for analyzing categorical data and determining whether there is a significant association between two variables. Despite the small sample sizes in some genotype categories (e.g., CC genotype), the chi-square test remains appropriate due to the distribution of observed frequencies across groups. The significance level was set at 0.05.

FINDINGS

The findings of the study are given below.

Table 1. Participant descriptive statistics by group

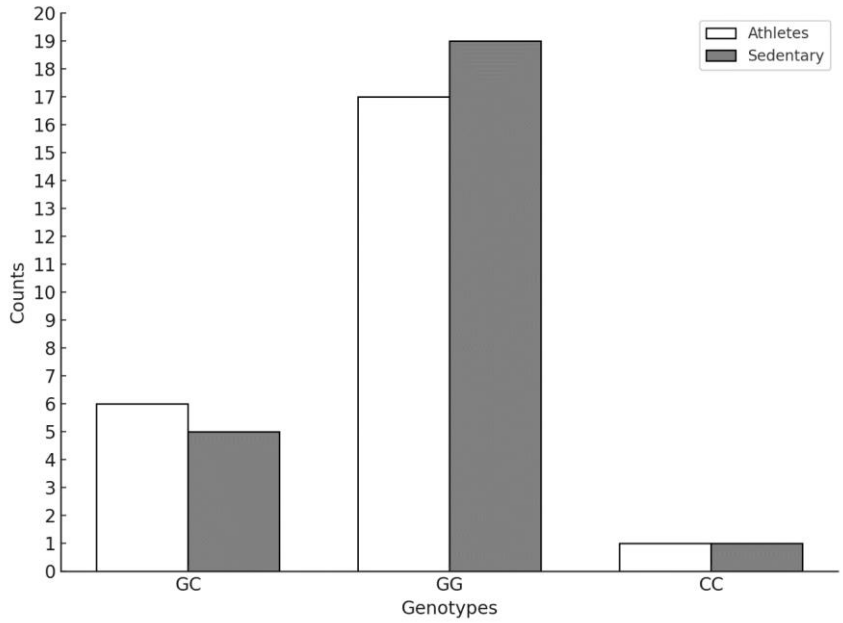
		N	Average	S.D.	Min.	Max.
Athlete	Age (year)	24	20.04	3.06	18.00	29.00
	Height (cm)	24	166.08	5.82	158.00	180.00
	Weight (kg)	24	57.08	8.46	40.00	75.00
Sedentary	Age (year)	25	21.24	2.92	18.00	29.00
	Height (cm)	25	164.88	6.33	150.00	175.00
	Weight (kg)	25	58.72	11.23	43.00	90.00

As shown in Table 1, the average age of the athletes was 20.04 years, 21.24 years for sedentary athletes, 166.08 years for athletes, 164.88 years for sedentary athletes, 57.08 years for athletes and 58.72 years for sedentary athletes.

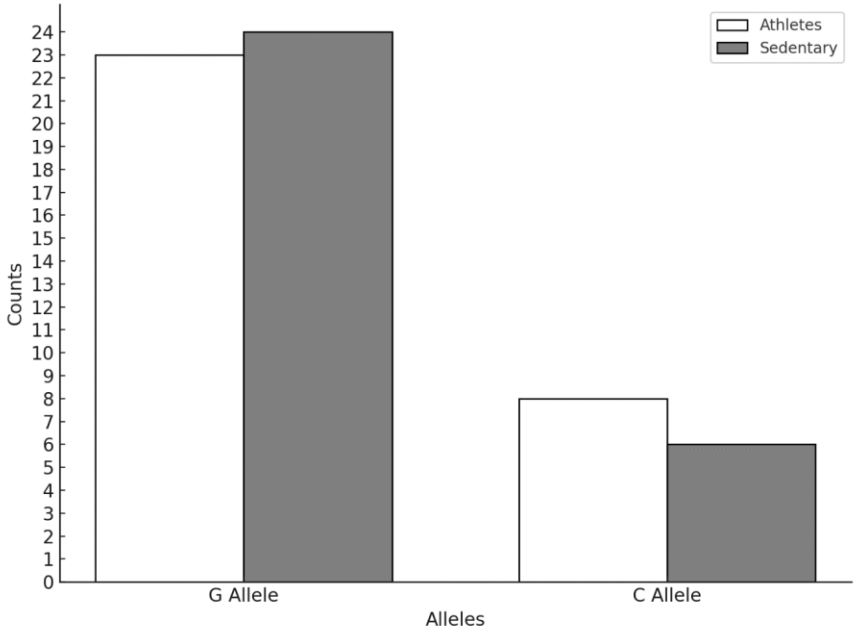
Table 2. Comparison of genotype ratios of athletes and sedentary people

			Athlete	Sedentary	Total	p
Genotype	GC	F	6	5	11	0.913
		%	54.5	45.5	100	
	GG	F	17	19	36	
		%	47.2	52.8	100	
	CC	F	1	1	2	
		%	50	50	100	
Total		F	24	25	49	
		%	49.0	51.0	100.0	

According to Table 2, there was no difference between the groups of athletes and sedentary people (p=0.913). The GC genotype was found in 6, the GG genotype in 17 and the CC genotype in 1 athlete and the GC genotype in 5, the GG genotype in 19 and the CC genotype in 1 sedentary person.



Graphic 1. Graph of genotype ratios of athletes and sedentary



Graphic 2. Graph of allele ratios of athletes and sedentary people

Table 3. Comparison of allele ratios of athletes and sedentary people

			Athlete	Sedentary	Total	p
Alel	G	F	23	24	47	0.408
		%	48.9	51.1		
	C	F	8	6	14	
		%	57.1	42.9		
Total		F	31	30	61	
		%	50.8	49.2	100.0	

As can be seen in Table 3, the distribution of G alleles was 23 in athletes and 24 in sedentary people, while the distribution of C alleles was 8 in athletes and 6 in sedentary people, with no significant difference between the values ($p>0.05$). These results indicate a comparable distribution of alleles between the groups, supporting the lack of statistical significance.

DISCUSSION AND CONCLUSION

In order to achieve sportive success, basic motoric skills must be exhibited at a high level. Endurance, which is a performance element, is influenced by many factors. One of the genes associated with endurance and strength is the gene encoding peroxisome proliferator-activated receptor alpha (PPARα). Peroxisomes are round-shaped organelles located in the cytoplasm of eukaryotic cells and contain catalase, peroxidase and oxidase enzymes. It can be found especially in cells of organs with high metabolic activity such as liver, muscle, heart and kidney. It is an organelle with an important role in the metabolism of fatty acids in the cell. The shortening of long chain fatty acids and the toxic compound resulting from denaturation in mitochondria are removed by catalase enzymes with antioxidant effect of peroxisomes. Peroxisome receptors function as transcription factors after stimulation. These receptors have 3 forms as PPAR-alpha (PPARα), PPAR beta/delta (PPARβ/δ) and PPAR-gamma (PPARγ) (Mollashahi et al., 2018). In this study, allele and genotype distributions of PPARα gene, which is associated with endurance and strength, in female soccer players were examined.

As a result of the studies, it is stated that PPAR-α gene is a transcription factor belonging to the nuclear receptor family that regulates lipid, glucose and energy homeostasis and controls body weight and vascular inflammation (Ahmetov et al., 2009; Maciejewska et al., 2011).

When the genotype ratios of athletes and sedentary groups were compared in Table 2, GC genotype was found in 7 and GG genotype was found in 17 athletes and GC genotype was found in 6 and GG genotype was found in 19 sedentary groups. As a result of the analysis, there was no statistically significant difference between the athlete and sedentary groups ($p=0.682$). G allele was found to be 23 in athletes and 24 in sedentary people, while C allele was found to

be 8 in athletes and 6 in sedentary people and no difference was found between the ratios ($p>0.05$). When the literature is reviewed, Kurtuluş et al. (2023), who reported findings similar to the present study, investigated the PPARα intron 7 G/C genotypes and allele frequencies among endurance-focused athletes (long-distance runners) and strength/endurance-focused athletes (wrestlers and football players) categorized by sports disciplines, as well as in comparison to non-athletic individuals. The PPARα intron 7 G/C genotype and allele frequencies were found to be similar between the athlete and non-athlete groups ($p>0.05$).

It has been suggested that the C allele of the PPAR-α gene G/C polymorphism has a higher frequency in power sports due to the decrease in beta oxidation and muscle hypertrophy in response to anaerobic performance. In contrast, the higher frequency of the GG genotype in endurance athletes has been associated with increased fatty acid oxidation in skeletal muscle and increased type 1 muscle fiber formation in athletes with the GG genotype (Broos et al., 2013). The main factors important in endurance sports are slow-twitch fibril ratio and maximal cardiac output. These components are under the influence of genetic factors and have been shown to be highly heritable (Simoneau & Bouchard, 1995; Bouchard et al., 1999; Alonso et al., 2014). Ahmetov et al. (2006) suggested that PPARα rs4253778 gene polymorphism may be associated with human performance and as a result of their study, the GG homozygous genotype is more common in endurance athletes, whereas the C allele plays a role in the anaerobic component of physical performance. Although polymorphism studies in athletes have increased, there are not many studies with the relevant polymorphism. It is thought that investigating the distribution of PPARα gene in different branches will contribute to the literature.

As highlighted in Table 3, the distribution of G alleles (23 in athletes and 24 in sedentary individuals) and C alleles (8 in athletes and 6 in sedentary individuals) revealed no statistically significant differences between the groups ($p>0.05$). This comparable distribution of alleles across both groups suggests that genetic variations in the studied alleles may not play a decisive role in distinguishing athletic and sedentary populations. These findings align with previous studies reporting similar results, emphasizing the potential influence of other genetic or environmental factors in athletic performance.

Egorova et al. (2014) reported that the distribution of PPAR genes is higher in Russian male soccer players than in sedentary individuals. In their study, Proia et al. (2014) compared the distribution of PPAR genes in male soccer players and sedentary individuals, finding that

the G and GG allele distribution was more prevalent among soccer players. The researchers suggested that this difference could be attributed to the professional level of the soccer players in the study group. Studies on the PPAR gene consistently show a significant difference in favor of male soccer players compared to sedentary individuals, suggesting that the PPAR gene is associated with endurance and strength.

In current study, the lack of variation in the PPAR gene among female soccer players may be related to the lower physiological adaptation of females to the sport's demands compared to males, given the structural demands of soccer. Due to the limited development of women's soccer in Türkiye, interest from women remains low, leading to a situation where, rather than a selection based on talent, nearly anyone interested can become a soccer player. In current study group, the absence of significant differences may be attributed to the female composition of current study sample and the generally lower endurance levels of female soccer players compared to their male counterparts.

The primary limitation of current study is the sample size. This number of participants may not be sufficient to determine the impact of the investigated polymorphism on soccer performance. Another limitation is the lack of physical or biochemical data from the soccer players involved in the study. The uniformity in age among participants is thought to minimize the estimated effects of environmental factors. Studies involving higher-level athletes with similar gene polymorphisms could support early engagement in sports.

It should be recognized that genetic backgrounds can cause differential effects across various sports disciplines, potentially conferring advantages in athletic performance. Based on these findings, it may be advisable to train elite athletes in specific branches tailored to their genetic profiles.

Developing distinctive projects across different regions nationwide could help normalize and promote women's soccer in Türkiye. While some candidate genes associated with sports are frequently studied, there remain numerous genes with limited research. Therefore, further studies focusing on less-studied genes are needed.

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KATKI ORANI ***CONTRIBUTION RATE***	**AÇIKLAMA** ***EXPLANATION***	**KATKIDA BULUNANLAR** ***CONTRIBUTORS***
Fikir ve Kavramsal Örgü *Idea or Notion*	Araştırma hipotezini veya fikrini oluşturmak *Form the research hypothesis or idea*	Bade YAMAK
Tasarım *Design*	Yöntem ve araştırma desenini tasarlamak *To design the method and research design.*	Faruk BOZYURT
Literatür Tarama *Literature Review*	Çalışma için gerekli literatürü taramak *Review the literature required for the study*	Bade YAMAK
Veri Toplama ve İşleme *Data Collecting and Processing*	Verileri toplamak, düzenlemek ve raporlaştırmak *Collecting, organizing and reporting data*	Faruk BOZYURT
Tartışma ve Yorum *Discussion and Commentary*	Elde edilen bulguların değerlendirilmesi *Evaluation of the obtained finding*	Faruk BOZYURT

Destek ve Teşekkür Beyanı/ *Statement of Support and Acknowledgment*

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Etik Kurul Beyanı/ *Statement of Ethics Committee*

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This research was conducted with the decision of Ondokuz Mayıs University Ethics Committee dated 28.09.2018 and numbered B.30.2.ODM.0.20.08/1721-1920.