Peroxisome Proliferator-Activated Receptor Alpha (PPARα) rs4253778 Polymorphism in a Turkish Soccer Player Cohort

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Abstract

Aim: Peroxisome proliferator-activated receptor alpha gene (PPARA) is one of the important gene determining athletes endurance capacity. The product of the gene functions in the regulation of lipid metabolism, in mostly heart and skeletal system, and maintaining glucose homeostasis and biogenesis in mitochondria. In this study, we aimed to determine the allelic distribution of PPARA rs4253778 polymorphism in a Turkish soccer player cohort. Methods: We enrolled 22 professional soccer players and 68 sedentary controls for the study. Buccal cells were used for DNA sources, and real- time PCR methodology was followed for genotyping. GG genotypes were superior in both groups, and this was statistically significant when we compare the genotypes between groups. Result:When we consider the alleles, G allele was found more frequently in players, and in controls. When we compare two groups, we determine a statistically significant difference, indicating the effect of G allele in soccer physiology. Conclusion: Our findings suggest that PPARA rs4253778 polymorphism is an effective genetic factor in soccer physiology, and should be considered as an important genetic biomarker in sports genetics.

Keywords: : Soccer, Athlete, PPARA, Sport Genetics, Polymorphism

Studies to date determined that genetic endowment that we inherit have important effects on athletic performance (Ulucan et al, 2014). In athletic performance, the genetic factors effecting cardiovascular or muscular systems, ethnicity, and the environmental factors like nutritional habits and stress levels all have combined impact (Corak et al., 2017). When it comes to identify the genetic effects, most studies focussed on the polymorphisms in alpha- actinin-3 gene (ACTN3) and angiotensinogen converting enzyme gene (ACE) (Eroglu et al., 2018). In addition to these, there are several genomic regions that have effects on injury risk, the ability of muscles to heal, or behavioral activities.

Peroxisomes are special organelles inside the cell, whose main function is to metabolize the fatty acids. Some hormones, lipid- metabolizing drugs and lipid molecules effects peroxisome proliferation or reduction by binding to steroid hormone receptor superfamily members, called peroxisome proliferator-activated receptors (PPARs). PPARs exist in three different forms, PPAR-alpha (PPARa), PPAR-beta/delta (PPARb/d), and PPAR-gamma (PPARc) (Brassiant et al., 1996). PPARA is a transcription factor and highly expressed in heart, liver, kidney, and skeletal muscles and has a restricted expression in pancreas. During long-term fasting or energy deprivation, free fatty acids bind to PPARAs, enhance hepatic fatty acid oxidation and production of ketone bodies to avoid hyperglycemia (Leone et al., 1999).

The gene responsible for peroxisome PPARa is PPARA. PPARA is located at 22q13.31. It has a functional polymorphism (rs4253778, GVC) in intron 7. G allele is associated with higher gene expression, therefore effecting oxidation of fatty acids in various tissues. Gene is activated after its ligand binding, and promotes uptake, utilization, and catabolism of fatty acids (Desverge et al., 1999). On the other hand, C allele is associated with lower expression rate, and it is related with coronary artery disease due to elevated plasma lipid levels (Flavell et al., 2002; Jamshidi et al., 2002). Some studies also reported that endurance training can increase PPARα levels, therefore increase the activation of mitochondrial pathway to oxidize fatty acids (Eynon et al., 2010). Ahmetov et al. (2006) reported the association of the polymorphism with muscle fibers; GG homozygotes had significantly higher percentages of slow-twitch fibers than CC homozygotes., which can be considered as an predisposition to endurance metabolism.

In the present study, we hypothesized that G allele and GG genotype will be more frequent in soccer players, and aimed to determine the prevalence of PPARA rs4253778 polymorphism in professional Turkish soccer players.

METHOD

Subjects

22 of the Karsıyaka Football team players, aged between 18-23, were recruited to the study after they were informed about the aim of the study, and they signed the voluntary agreement forms explaining the study protocol. The study, which is in agreement with the principles of Helsinki II Declaration, was approved by a local Ethical Committee. All the players had a training section of 5 times a week, and a total of 10 hours/week. Needless to say, with the additional league and cup matches, they had an average of 14 hours/week physical activation.

DNA Isolation

1μl of peripheral blood samples, which was taken from players during their routine biochemical examination, was used for DNA isolation. Commercially available PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, Calif., USA) was used for isolation. The procedures were conducted according to the provided manufacturers' instructions, with small modifications. Briefly, 200μl of Binding Buffer was added to blood samples. 20μl Protein Kinase K and 20μl RNAse was then added to the blood- binding buffer mixture. All was incubated at 58°C for 10 minutes. After incubation, 200μl ethanol was added and mixed by vortexing for 5 seconds. After the mixture was transferred to the filtered tube, centrifuged for 3 minutes at 14,000rpm, 500μl Wash Buffer 1 was added and centrifuged again for 3 min. at 14,000rpm. 120μl of Elution Buffer was added and incubated 1 min.

Genotyping

Real- time PCR (Applied Biosystem, USA) was used for PPARA s4253778 genotyping. Commercially provided Taqman SNP Genotyping Assay (Applied Biosystem, USA) kit was used for PPARa rs4253778 genotyping process. A total of 20 μl PCR mixture contained 10-25 ng genomic DNA and spesific primers for G and C alleles were used in Real- time PCR genotyping.

Statistical Analysis

Chi-Square analyses between groups were performed by using SPSS (version 20.0 for Windows, SPSS, Chicago, IL, USA). Relationships yielding $p < 0.05$ values were considered to be significant.

RESULTS

All the samples in our cohort were successfully genotyped. In soccer player cohort, GG genotype (63,6%) was observed higher than GC (36,4%) and CC (0%) genotypes. We detected no CC genotypes in soccer players. G allele was counted as 36 (81.8%) and C as 8 (18.2). For the control group, like player cohort, GG genotype and G allele were superior to other genotypes, and C allele, respectively. 40 (59%) individual had GG, 24 (35%) had GC and 4 (6%) had CC genotypes. G allele was counted as 104 (76%) whereas C allele counted as 32 (24%). Table 1 lists all the genotypic results of our soccer cohort.

When we statistically compared two groups, there was a statistically significant difference in the terms of genotypes (p=0.01), and in the terms of alleles (p=0.039) between players and control group (not shown in the table).

Table 1. PPARα rs4253778 polymorphism genotype and allele distribution of soccer players and sedentary control group.

Study Group	Genotypes			Alleles	
	GG	GC	CC	G	C
Soccer Players	14	8	0	36	8
Percentage	63,6	36,4	-	81,8	18,2
Control Group	40	24	4	104	32
Percentage	59	35	6	76	24

DISCUSSION and CONCLUSION

PPARα is a transcription factor having functions in regulation of lipid and glucose metabolism, and also in energy homeostasis. Soccer has unique requirements, during a standard 90-minute game, players run an average of 10-12 km. Besides, they also jump, kick, tackle and sprint many times. All of these context need a mixed aerobic and anaerobic metabolism. Therefore, it is important to have information about the genetic parameters effecting aerobic and anaerobic metabolism. When we compare PPARα rs4253778 polymorphism between professional soccer players and sedentary controls, we can consider this polymorphism as an important genetic biomarker for soccer players. In our cohort, GG genotype and G allele was significantly more frequent in players. 63,6% of the soccer players had GG genotype, and G allele was counted in 81,8% of the players. This is one of the first report analyzing this polymorphism in Turkish soccer players and the datas obtained from the study gave us some clues about the effect of PPARA rs4253778 in soccer players.

Before, Proia et al. (2014) reported similar findings to our results. In their study, they analyzed 60 Italian soccer players and 30 healthy controls, and reported that GG genotype and G allele was significantly more frequent in players. Like our study, Akçamlı et al. (2018) analyzed 64 Turkish soccer players and showed the superiority of GG genotype and G alleles in their cohort. Our study is in agreement with the results of the studies, indicating the importance of the given polymorphism in soccer players.

In a study including Polish rowers, it has been reported that rowers have significantly higher rates of G allele and GG genotype compared to sedentary controls (Maciejewska et al., 2011). Another study in Israelian top-level endurance athletes, Eynon et al. (2010) found a higher frequency of GG genotype, when they compared athletes to 240 healthy controls. They also concluded that this polymorphism may belong to a group of several genetic variations that influence endurance performance. In addition to these, Ahmetov et al. (2009) reported that some important polymorphisms, including the PPARα G allele, were overrepresented in a 1,423 Russian athletes cohort compared to 1,132 controls. Unlike these results, Ginevičienė et al. (2010) examined the same SNP in a 193 elite

Lithuanian athlete cohort (endurance, mixed sports, speed/power, and team sports), and reported that C allele was more common in the athlete group than in the general population, and also added that CC genotype was associated with muscle mass and explosive power. Different populations or athletes from

different disciplines may be the reason for these results, but we do not have enough datas to speculate for the incompatible results.

The main limitation of our study is the sample size. Although soccer is one of the most popular sport, and soccer players are much more higher in number than the other players, it is hard to find a cohort with the same or similar environmental conditions. Our players are the members of the same football club, and they have the same training sessions. Another concern that we may have is the genetic heterogeneity of the subjects. Although all the players have Turkish ancestry, it is mostly hard to confirm this biologically. But besides these, this report will be the one of the first papers giving information about the polymorphism.

Conclusion

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 GG genotype and G allele of PPARA rs4253778 in determining the athletic performance of soccer players. But these datas should be supported with more studies to have more precise results.

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