THE PPARA GENE POLYMORPHISM IN TEAM SPORTS ATHLETES

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A Study Design; B Data Collection; C Statistical Analysis; D Manuscript Preparation; E Funds Collection

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Abstract. Peroxisome proliferator-activated receptor α (PPARα) is a transcription factor that regulates lipid, glucose, and energy homeostasis; accordingly, it controls body weight and vascular inflammation. PPARα is expressed at high levels in tissues that catabolize fatty acids (notably liver, skeletal muscle, and heart), and at lower levels in other tissues, including pancreas (Brassiant et al. 1996). The level of expression of PPARα is higher in type I (slow-twitch) than in type II (fast-twitch) muscle fibers (Russel et al. 2003). Endurance training increases the use of non-plasma fatty acids and may enhance skeletal muscle oxidative capacity by the PPARα regulation of gene expression (Russel et al. 2003; Horowitz et al. 2000). PPARα regulates the expression of genes, encoding several

Introduction

Peroxisome proliferator-activated receptor α (PPARα) is a transcription factor that regulates lipid, glucose, and energy homeostasis; accordingly, it controls body weight and vascular inflammation. PPARα is expressed at high levels in tissues that catabolize fatty acids (notably liver, skeletal muscle, and heart), and at lower levels in other tissues, including pancreas (Brassiant et al. 1996). The level of expression of PPARα is higher in type I (slow-twitch) than in type II (fast-twitch) muscle fibers (Russel et al. 2003). Endurance training increases the use of non-plasma fatty acids and may enhance skeletal muscle oxidative capacity by the PPARα regulation of gene expression (Russel et al. 2003; Horowitz et al. 2000). PPARα regulates the expression of genes, encoding several
key muscle enzymes involved in fatty acid oxidation (Aoyama et al. 1998; Gulick et al. 1994; Schmitt et al. 2003). Chronic electrical stimulation of latissimus dorsi muscle in dogs increased its PPARα content and medium-chain acyl-CoA dehydrogenase gene expression (Cresci et al. 1996). These data suggest that PPARα may be an important component of the adaptive response to endurance training, transducing physiological signals (related to exercise training) to the expression of nuclear genes, as well as encoding mitochondrial fatty acid oxidation enzymes in the skeletal muscles. The catabolism of carbohydrates and fatty acids provides primary means for energy production in working skeletal muscle, whereby the selection of these substrates depends primarily on the exercise intensity (Brooks and Mercier, 1994), and gene variants involved in the regulation of muscle metabolism (Ahmetov et al. 2009).

Exercise-induced left ventricular (LV) growth in healthy young men was strongly associated with the intron 7 G/C (rs4253778) polymorphism of the PPARA gene (location: 22q13.31) (Jamshidi et al. 2002). Individuals homozygous for the C allele had a 3-fold greater and heterozygotes had a 2-fold greater increase in LV mass than G allele homozygotes, leading to the hypothesis that the hypertrophic effect of the rare intron 7 C allele is caused by the influences on cardiac substrate utilization. Recently, it was demonstrated that the frequency of the PPARA rs4253778 GG genotype and G allele was higher in 491 Russian endurance-oriented athletes (Ahmetov et al. 2006), 74 elite Israeli endurance athletes (Eynon et al. 2010), 55 elite Polish rowers (Maciejewska et al. 2011) and Polish combat athletes (P = 0.01) (Cięszczyk et al. 2011), compared with controls and/or sprinters. In accordance with the hypothesis, the mean percentage of type I muscle fibre was higher in GG homozygotes than in CC genotype subjects (in a study of 40 physically active healthy men) (Ahmetov et al. 2006). Furthermore, GG genotype was shown to be correlated with high values of oxygen pulse, both in male and female Russian rowers (Ahmetov et al. 2007).

The hypothesis that intron 7 C allele is associated with the hypertrophic effect due to influences on cardiac and skeletal muscle substrate utilization, was supported by the findings that PPARA C allele is over-represented in 180 Russian power-oriented athletes, and associated with an increased proportion of fast-twitch muscle fibres in m. vastus lateralis (Ahmetov et al. 2006).

Sport games are characterized by the involvement of both aerobic and anaerobic metabolic pathways, in which the effort is of intermittent characteristics; and explosive types of movements are repeated over time at high intensity during game situations (Kahn 1999; Stolen et al. 2005). Intermittent activity is determined by high-intensity motion (with energy mostly furnished by the ATP-PC and anaerobic pathways) and low intensity motion (in which the aerobic pathways have the function of active recovery).

Given the role of PPARα in the regulation of metabolism in the skeletal and heart muscles, and the evidence that the PPARA gene variation was linked to athletic performance, we hypothesized that athletes involved in team sports would have different distribution of the PPARA genotypes compared to controls. Therefore, the aim of the present study was to investigate the association between the PPARA gene polymorphism and team-sport athletic status.

Materials and methods

The study was approved by the Ethics Committee of the Kazan State Medical University. Written informed consent was obtained from each participant. The study complied with the guidelines set out in the Declaration of Helsinki.
Six hundred and sixty five male and female Russian athletes of regional or national competitive standard were recruited from the following sporting disciplines: badminton (n = 16), baseball (n = 28), basketball (n = 85), beach volleyball (n = 10), court tennis (n = 33), football (n = 241), futsal (n = 9), handball (n = 24), ice hockey (n = 55), rugby (n = 48), softball (n = 31), table tennis (n = 14), volleyball (n = 53), water polo (n = 18).

Controls consisted of 1,706 healthy unrelated citizens (males and females) of St Petersburg, Moscow, Kazan, Naberezhniye Chelny and Surgut, without any competitive sport experience. The athletes and control groups were all Caucasians.

Genotyping

Molecular genetic analysis was performed with DNA samples, obtained from the epithelial mouth cells by alkaline extraction, or using a DNA-sorb-A sorbent kit, according to the manufacturer’s instruction (Central Research Institute of Epidemiology, Moscow, Russia), based on the method of sample collection (buccal swab or scrape). Genotyping for the PPARA gene rs4253778 G/C (intron 7) polymorphism was performed by PCR on Tercyk multicanal amplificator (DNA Technology, Moscow, Russia) and restriction enzyme digestion, as previously described (Flavell et al. 2002).

Statistical analysis

Genotype distribution and allele frequencies between athletes and controls were compared using $\chi^2$ test. P values of $<$0.05 were considered statistically significant. Bonferroni’s correction for multiple testing was performed by dividing the P value (0.05) with the number of tests where appropriate. Statistical analyses were conducted using GraphPad In Stat software.

Results

PPARA intron 7 genotype distributions amongst controls met Hardy-Weinberg equilibrium (P = 0.456), but not in a whole cohort of athletes (P = 0.0162). Genotype distribution amongst controls was similar to that observed in other reported groups (Jamshidi et al. 2002; Flavell et al. 2002; Flavell et al. 2005).

We found significant (P = 0.0003) differences in the PPARA genotype distribution between the whole cohort of athletes and controls (Table 1). The frequency of PPARA C allele was significantly higher in athletes compared to controls (20.5 vs 16.4%, P = 0.0009). Considering individual sporting disciplines, only football (24.3%; P < 0.0001) and softball (25.8%; P = 0.047) players had significantly higher frequencies of PPARA C allele compared to controls. However, after Bonferroni correction for multiple testing, the associations of PPARA C allele with team-sport athletic status remained statistically significant only in the integral group of athletes and football players.

Discussion

This is the first study to demonstrate that the variation in the PPARA gene is associated with a team-sport athlete status. Specifically, we have shown that the frequency of PPARA C allele was significantly higher in the whole cohort of athletes and particularly, in football players compared to controls, suggesting that anaerobic rather than aerobic metabolism may be crucial for the game performance in team sports. However, it must be taken into account that the dominant contribution (aerobic or anaerobic) of metabolic pathway may be different for each team.
sports discipline, and dependent upon the position on the field, tactical defensive and offensive properties of the team, characteristics of the game itself, and other factors.

Studies to date suggest that the C allele seems to be associated with the reduced PPARα expression or function. PPARα activators (fibrates) reduce the incidence of cardiovascular disease (CVD), whilst the intron 7 C allele is associated with the increased risk of CVD (Jamshidi et al. 2002). We speculate that the intron 7 polymorphism is in allelic association with an unidentified variant in a regulatory region of the PPARα gene that affects PPARα levels, which in turn affect transcriptional activation of PPARα target genes.

Such findings suggest that the observed associations are mediated through alterations in the PPARα expression. The mechanisms, through which such altered PPARα activity influence athletic performance remain speculative, and further in vitro and in vivo studies of gene function are advocated. However, we might speculate that the association of the C allele with game performance relates to a propensity to skeletal muscle hypertrophy and a facilitation of glucose utilization (rather than fatty acid oxidation), in response to anaerobic exercise. In addition, we have recently shown that PPARα C allele was associated with an increased proportion of type II fibers in m. vastus lateralis of healthy men. Since successful strength and power athletes have relatively more fast-twitch than slow-twitch fibers in the trained musculature, part of the allelic association with performance phenotypes might have been mediated through the genotype-associated alterations in fiber type proportion. Indeed, accumulating evidence suggests that the intron 7 C allele of the PPARα gene has an advantage for power-oriented athletes. Recent study demonstrated that the PPARα C allele is over-represented in 180 Russian power-oriented athletes (Ahmetov et al. 2006). Furthermore, in the study of 193 Lithuanian athletes Ginevičienė et al. (2010) has shown that male athletes with PPARα CC/GC genotypes had significantly higher muscle mass and single muscular contraction power (measured by vertical jump test) than GG homozygotes. The frequency of the PPARα C allele was also

<table>
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<tr>
<th>Table 1. PPARα intron 7 genotype distribution and frequencies of PPARα gene C allele in athletes involved in sport games and controls</th>
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<td>Sport</td>
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<td>All athletes</td>
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<td>Controls</td>
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* Statistically significant differences between athletes and controls (a Bonferroni corrected α level was set at 0.0033).
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significantly higher in Lithuanian power-oriented athletes and athletes with mixed aerobic/anaerobic activity (n = 80) in comparison with 250 controls (Ginevičienė et al. 2010). In addition, the male carriers of the PPARα gene C allele amongst Russian middle-school aged children demonstrated better results of handgrip strength testing than GG homozygotes (Ahmetov et al. 2013).

In conclusion, our study provides evidence for the association between the PPARα gene G/C polymorphism and team-sport athlete status. Although more replication studies are needed, the preliminary data suggest an opportunity to use the analysis of PPARα polymorphism along with other gene variations and standard phenotypic assessment in team sports selection.

References


