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PPAR α gene variation and physical performance in Russian athletes

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Abstract Peroxisome proliferator-activated receptor α (PPAR α) regulates genes responsible for skeletal and heart muscle fatty acid oxidation. Previous studies have shown that the PPAR α intron 7 G/C polymorphism was associated with left ventricular growth in response to exercise. We speculated that GG homozygotes should be more prevalent within a group of endurance-oriented athletes, have normal fatty acid metabolism, and increased percentages of slow-twitch fibers. We have tested this hypothesis in the study of a mixed cohort of 786 Russian athletes in 13 different sporting disciplines prospectively stratified by performance (endurance-oriented athletes, power-oriented athletes and athletes with mixed endurance/power activity). PPAR α intron 7 genotype and allele frequencies were compared to 1242 controls. We found an increasing linear trend of C allele with increasing anaerobic component of physical performance ($P=0.029$). GG genotype frequencies in endurance-oriented and power-oriented athletes were 80.3% and 50.6%, respectively, and were significantly ($P<0.0001$) different compared to controls (70.0%). To examine the association between PPAR α gene variant and fiber type composition, muscle biopsies from m. vastus lateralis were obtained and analyzed in 40 young men. GG homozygotes ($n=25$) had significantly ($P=0.003$) higher percentages of slow-twitch fibers (55.5 ± 2.0 vs $38.5 \pm 2.3\%$) than CC homozygotes ($n=4$). In conclusion, PPAR α intron 7 G/C polymorphism was associated with physical performance in Russian athletes, and this may be explained, in part, by the association between PPAR α genotype and muscle fiber type composition.

Keywords PPAR α – Polymorphism - Fatty acids - Muscle fiber type - Physical performance

Introduction

Peroxisome proliferator-activated receptor α (PPAR α) is a transcription factor that regulates lipid, glucose, and energy homeostasis and controls body weight and vascular inflammation. PPAR α 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 (Braissant et al. 1996). The level of expression of PPAR α 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 PPAR α regulation of gene expression (Russel et al. 2003; Horowitz et al. 2000).

PPAR α regulates the expression of genes encoding several key muscle enzymes involved in fatty acid oxidation (FAO) (Aoyama et al. 1998; Gulick et al. 1994; Schmitt et al. 2003). Chronic electrical stimulation of latissimus dorsi muscle in dogs increased muscle PPAR α content and medium-chain acyl-CoA dehydrogenase gene expression (Cresci et al. 1996). These data suggest that PPAR α may be an important component of the adaptive response to endurance training by transducing physiological signals related to exercise training to the expression of nuclear genes encoding in skeletal muscle mitochondrial FAO enzymes.

Metabolization of carbohydrates and fatty acids provides the primary means for energy production in working skeletal muscle, whereby selection of these substrates depends primarily on exercise intensity (Brooks et al. 1994) and as we suppose, on gene variants involved in regulation of muscle metabolism. Variation in the PPAR α gene influences plasma lipid levels (Flavell et al. 2000; Vohl et al. 2000), cardiac growth (Jamshidi et al. 2002), and risk of coronary artery disease (Flavell et al. 2002).

Cardiac hypertrophy is associated with both decreased PPAR α expression (Barger et al. 2000) and decreased FAO (Allard et al. 1994; Kagaya et al. 1990). Exercise-induced left ventricular (LV) growth in healthy young men was strongly associated with the intron 7 polymorphism of the PPAR α gene. 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 due to influences on cardiac substrate utilization (Jamshidi et al. 2002) - the C allele being associated with reduced PPAR α expression and FAO.

If PPAR α expression is a key regulator of the response to physical training, then one might anticipate genetic variation in the PPAR α gene to be associated with human performance phenotypes. More specifically, one might expect increased PPAR α expression and FAO, and thus the intron 7 G allele, to be associated with endurance performance. C allele carriers, on the other hand, are speculated to be more predisposed to intense anaerobic (power) performance by using mainly glucose in muscle metabolism. We have tested this hypothesis in the study of a mixed cohort of 786 Russian athletes in 13 different sporting disciplines prospectively stratified by performance (endurance-oriented athletes, power-oriented athletes and athletes with mixed endurance/power (acyclic) activity).

We also speculated that the C allele carriage (suggested decreased PPAR α gene activity) would be associated with a reduced proportion of type I (oxidative/slow) fibers than GG homozygosity. To examine the association between PPAR α gene variant and fiber type composition, muscle biopsies from m. vastus lateralis were obtained and analyzed in 40 young healthy men.

Materials and methods

The University of St Petersburg Ethics Committee approved the study and written informed consent was obtained from each participant.

Subjects and controls

Seven hundred and eighty six male and female Russian athletes of regional or national competitive standard were recruited from the following sports: swimming (n=58), track-and-field athletics (n=77), triathlon (n=30), cross-country skiing (n=62), biathlon (n=28), skating (n=72), road cycling (n=63), rowing (n=251), boxing (n=22), ice hockey (n=15), wrestling (n=63), court tennis (n=15) and weightlifting (n=30). The athletes were prospectively stratified into groups according to event duration and distance, covering a spectrum from the more endurance-oriented to the more power-oriented. The first group included middle (MDA) and long distance athletes (LDA), such as 800-1500 m swimmers (race duration 8-15 minutes), triathletes, 3000-5000 m skaters (race duration 4-7 minutes), biathletes, cross-country skiers, road cyclists and rowers with predominantly aerobic energy production. The second group comprised short distance athletes (SDA) (race duration <70 seconds; 60-400 m runners, 500 m skaters, 50-100 m swimmers) and weightlifters with predominantly anaerobic energy production. The third group included athletes whose sports utilized mixed anaerobic and aerobic energy production (court tennis players, wrestlers, ice hockey players and boxers). Sixty-

one athletes were classified as 'outstanding', being at least national representatives; the others were classified as 'average' athletes, being regional competitors with no less than 4 years experience participating in their sport.

Controls consisted of 1242 healthy unrelated pupils (n=534, aged 11-12), students of different St Petersburg Universities (n=535, aged 17-27) and St Petersburg inhabitants (n=173, aged 20-42). The athletes and control groups were all Caucasian Russians, with an equivalent ratio from European and Siberian descent (3:1 in both groups). Further characteristics are presented in Table 1.

Forty healthy men (aged 18-29; height 179.1 ± 0.9 cm, weight 72.8 ± 1.5 kg) gave their informed consent to participate in muscle biopsy study which was reviewed and approved by the Physiological Division of the Russian National Bioethics Committee.

Genotyping

DNA was extracted from mouthwash samples as previously described (Bolla et al. 1995). Genotyping for the intron 7 G/C (refsnp 4253778) variant was performed by polymerase chain reaction (PCR) and restriction enzyme digestion, as previously described (Flavell et al. 2002).

Muscle fiber typing

M. vastus lateralis was chosen for muscle biopsy because of great individual variability of muscle fiber type composition (i.e. 5-90% for type I fiber). Samples of m. vastus lateralis of 40 young healthy men were obtained with the Bergstrom needle biopsy procedure under the local anesthesia with 1% lidocaine solution. Prior to analysis, samples were frozen in liquid nitrogen and stored at $< -80^{\circ}\text{C}$. Serial sections (10 μm) were prepared using a cryostat and microtome at -20°C , with sections then mounted on slides. The immunoperoxidase technique was employed for immunohistochemical identification of myosin isoforms. Antibodies against the slow (MHCs) and fast (MHCf) myosin isoforms were used (clones NCL-MHCf (a+b) and NCL-MHCs (Novocastra Laboratories)). Sections incubated without primary antibodies were to detect nonspecific staining. The antigen-antibody marking was intensified with the Vectastain ABC kit (Vector Labs, CA) to visualize the diaminobenzidine peroxidase reaction.

Fiber distribution was expressed as a ratio of the number of fibers of each type in a section to the total number of fibers. All fibers (no less than 40%) were measured in each section. The cross-sectional area (CSA) was determined for at least 100 fibers of each type using image analysis system QUANTIMET-500 (Leica) outfitted with colour digital video camera JVC TK-1280E (image resolution 720 x 512 pixels with 8 bit/pixel). Sections to compare were prepared and stained all together with the Sigma (USA) reagents.

All analysis was done blind to genotype.

Statistical analysis

Allele frequencies were determined by gene counting. Genotype distribution and allele frequencies between groups of athletes and controls were then compared by χ^2 test. Frequency of the C alleles across the 3 groups with different metabolic demands was compared by χ^2 test for linear trend by using the anaerobic component as the categorical variable. The Spearman's correlation test was applied to the quantitative variables (muscle fiber characteristics). *P* values of < 0.05 were considered statistically significant.

Results

PPAR α intron 7 genotype distributions amongst all athletes and controls were in Hardy-Weinberg equilibrium. 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). No difference was found in C allele frequencies within groups of controls (16.1% for pupils, 16.7% for students, 16.2% for St Petersburg inhabitants). The genotype distribution and allele frequency amongst the whole cohort was similar to that amongst sedentary controls (Table 2).

We found an increasing linear trend of C allele with increasing anaerobic component of physical performance ($P < 0.029$ for linear trend) (Figure 1). Intron 7 C allele frequencies in endurance-oriented and power-oriented events were 10.8% ($P < 0.0001$, comparison with controls) and 27.2% ($P < 0.0001$, comparison with controls), respectively. There was not significant difference in C allele frequencies between the athletes with mixed endurance/power activity and controls ($P = 0.115$). However, genotype distribution in athletes with mixed endurance/power activity also showed significant difference ($P = 0.012$), compared to controls.

In considering individual sporting disciplines, as hypothesized, endurance-oriented athletes had significantly higher percentage of GG genotype (MDA/LDA swimmers (91.7%, $P = 0.021$), cross-country skiers

(88.7%, $P=0.0015$), MDA/LDA skaters (87.9%, $P=0.026$) and triathletes (86.7%, $P=0.048$) compared to controls (70.0%). Biathletes, road cyclists and rowers did not show such significance.

There were significant differences in PPAR α genotype distribution only in ice hockey ($P=0.032$) and court tennis players ($P=0.047$) within the group of mixed endurance/power activity, compared to controls.

In power-oriented events group we found significantly elevated frequencies of GC and CC genotypes, compared to controls, so that C allele frequencies in SDA runners, weightlifters, SDA skaters and SDA swimmers were 23.4% ($P=0.024$), 26.7% ($P=0.034$), 29.5% ($P=0.002$) and 33.8% ($P=0.0002$), respectively.

It is worth mentioning that intron 7 C allele frequency significantly correlated with elite athlete status. Linear trends for increasing allele frequencies were also observed with by 'elite' status for both power-oriented (29.6% of C allele frequency in elite athletes ($n=27$), $P=0.0316$) and endurance-oriented disciplines (92.2% of G allele frequency in elite athletes ($n=34$), $P<0.0001$).

We also investigated the association of PPAR α intron 7 polymorphism with physical performance separately in male and female athletes (Figure 2). Amongst endurance-oriented athletes, C allele frequency in both men ($n=335$, frequency 11.6%, $P=0.004$) and women ($n=156$, frequency 9.0%, $P=0.007$), was significantly different compared to controls. Similarly, in power-oriented events group the strong association of C allele was found both in men ($n=131$, frequency 26.7% vs. controls (frequency 17.1%); $P=0.0006$) and women ($n=49$, frequency 28.6% vs. controls (frequency 15.6%) $P=0.003$).

Interestingly, muscle fiber typing of 40 men showed significant correlation between PPAR α intron 7 polymorphism and muscle fiber specification. Mean percentages of type I fiber in GG homozygotes ($n=25$), heterozygotes ($n=11$) and CC homozygotes ($n=4$) were $55.5 \pm 2.0\%$, $44.7 \pm 2.6\%$ and $38.5 \pm 2.3\%$, respectively ($r=0.55$, $P=0.0002$). Furthermore, mean percentages of type II fibers in GG homozygotes, heterozygotes and CC homozygotes were $48.4 \pm 2.2\%$, $58.1 \pm 3.3\%$ and $61.0 \pm 2.1\%$, respectively ($r=-0.48$, $P=0.0015$). Mean CSA of type I fiber in GG homozygotes was slightly bigger compared to heterozygotes and CC homozygotes ($5479 \pm 274 \mu\text{m}^2$ vs $5122 \pm 520 \mu\text{m}^2$ and $4952 \pm 493 \mu\text{m}^2$, respectively), but this correlation was nonsignificant.

Discussion

This is the first study to demonstrate that variation in the PPAR α is associated with physical performance in athletes and correlated with their elite status. Specifically, the intron 7 C allele seems associated with power-orientated disciplines, and the G allele with endurance performance. Genotype distribution and C allele frequencies in athletes with mixed power/endurance activity were in intermediate position between endurance- and power-oriented athletes, being similar to controls.

Studies to date suggest that the C allele seems associated with reduced PPAR α expression or function. PPAR α activators (fibrates) reduce the incidence of cardiovascular disease (CVD), whilst the intron 7 C allele is associated with increased risk of CVD (Jamshidi et al. 2002). Furthermore we have recently demonstrated that the intron 7 C allele is associated with reduced response to fenofibrate, a PPAR α activator (Foucher et al. 2004). We speculate that the intron 7 polymorphism is in allelic association with an unidentified variant in a regulatory region of the PPAR α gene that affects PPAR α levels, which in turn affect transcriptional activation of PPAR α target genes. Efforts to examine the effect of intron 7 genotype on PPAR α mRNA levels and to identify functional promoter variants are presently underway.

Such findings suggest that the observed associations are mediated through alterations in PPAR α expression. The mechanisms through which such altered PPAR α 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 power-oriented 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. On the other hand, the association of GG genotype with endurance performance might relate to a propensity for increased FAO.

In addition, PPAR α expression is raised in type I (oxidative) rather than type II muscle fibers. However, our data also suggest an allelic association not only with function *within* a fiber type, but with fiber type distribution itself: the G allele was associated with an increased proportion of type I fibers when compared to type II fibers. Such data are intriguing, and suggest a potential influence of PPAR α expression on muscle fiber differentiation. As successful endurance athletes have relatively more slow-twitch than fast-twitch fibers in the trained musculature (and sprinters an excess of fast-twitch fibers), part of the allelic association with performance phenotypes might have been mediated through genotype-associated alterations in fiber type proportion.

Our study does have limitations. The paucity of functional data relating to the PPAR α alleles needs to be addressed with further *in vitro* studies. Further, the association of PPAR α genotype with alterations in muscle

function in response to training is advocated. Our study also lacked biopsy data from elite athletes. Finally, as in all such studies, extension to, and replication within other racial groups is proposed.

In summary, we have shown, for the first time that variation in the PPAR α gene is strongly associated with physical performance in Russian athletes, and with muscle fiber type in controls. Such findings have important implications for our understanding of muscle function in both health and disease.

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Table 1. PPAR α intron 7 genotype distribution of the athletes and controls with sex (frequencies) and age.

	PPAR α intron 7 genotype		
	GG, %	GC, %	CC, %
Athletes			
All, $n=786$	71.5	25.1	3.4
Male, $n=571$	70.8	25.6	3.6
Female, $n=215$	73.0	24.2	2.8
Age, years	26 \pm 7	24 \pm 5	21 \pm 4
Sport experience, years	14 \pm 4	11 \pm 3	10 \pm 3
Controls			
All, $n=1242$	70.0	27.3	2.7
Male, $n=559$	68.9	27.9	3.2
Female, $n=683$	71.0	26.8	2.2
Age, years	18 \pm 2	18 \pm 3	17 \pm 4
Muscle biopsy study			
Male, $n=40$	62.5	27.5	10.0
Age, years	22 \pm 1	22 \pm 1	23 \pm 2

Values are means \pm SE. GG, wild-type homozygote; GC, heterozygote; CC, mutant homozygote.

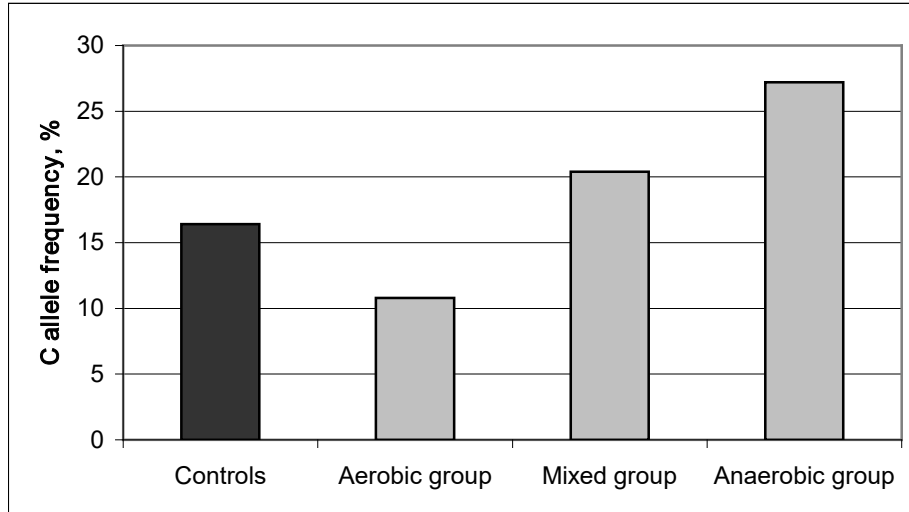


Figure 1. PPAR α intron 7 C allele frequency of 786 Russian athletes and 1242 controls is shown. C allele frequency in controls was 16.4%. By comparison, it was 10.8, 20.4 and 27.2% for predominantly aerobic group ($n=491$), mixed aerobic and anaerobic group ($n=115$), and predominantly anaerobic group ($n=180$), respectively ($P=0.029$ for linear trend).

Table 2. PPAR α intron 7 genotype distribution and frequencies of PPAR α gene C allele in athletes stratified by power/endurance orientation and sporting discipline. Comparison with controls was by χ^2 test.

Group	Sport	n	Genotype			P value	C allele, %	P value
			GG,%	GC,%	CC,%			
Endurance-oriented events	Swimming (800-1500 m)	24	91.7	8.3	0	0.068	4.2	0.023*
	Cross-country skiing	62	88.7	9.7	1.6	0.006*	6.4	0.003*
	Triathlon	30	86.7	13.3	0	0.129	6.7	0.043*
	Biathlon	28	85.7	14.3	0	0.178	7.1	0.063
	Skating (3000-5000 m)	33	87.9	9.1	3.0	0.657	7.6	0.055
	Road cycling	63	79.4	17.5	3.1	0.228	11.9	0.183
	Rowing	251	74.9	23.1	2.0	0.281	13.5	0.113
	All	491	80.3	17.9	1.8	0.0001*	10.8	0.0001*
Events with mixed power/endurance (acyclic) activity	Boxing	22	72.7	22.7	4.6	0.817	15.9	0.933
	Ice hockey	15	73.4	13.3	13.3	0.032*	20.0	0.595
	Wrestling	63	63.5	30.2	6.3	0.199	21.4	0.138
	Court tennis	15	66.7	20.0	13.3	0.047*	23.3	0.308
	All	115	67.0	25.2	7.8	0.012*	20.4	0.115
Power-oriented events	Running (60-400 m)	77	55.8	41.6	2.6	0.025*	23.4	0.024*
	Weightlifting	30	53.3	40.0	6.7	0.107	26.7	0.034*
	Skating (500 m)	39	43.6	53.8	2.6	0.001*	29.5	0.002*
	Swimming (50-100 m)	34	44.1	44.1	11.8	0.0004*	33.8	0.0002*
	All	180	50.6	44.4	5.0	0.0001*	27.2	0.0001*
Totals		786	71.5	25.1	3.4	0.397	16.0	0.725
Controls		1242	70.0	27.3	2.7	1.000	16.4	1.000

* $P < 0.05$, statistically significant differences.

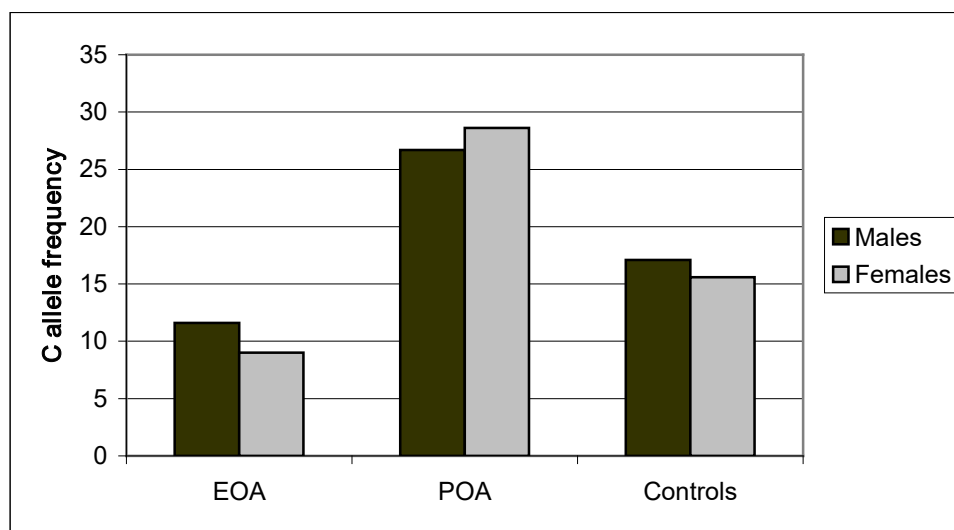


Figure 2. Distribution of PPAR α 7 intron C allele amongst male and female athletes in two event groups and sedentary controls (EOA – endurance-oriented athletes, POA – power-oriented athletes). C allele frequencies in endurance-oriented athletes, both men (frequency 11.6% vs controls (frequency 17.1%); $P=0.004$) and women (frequency 9.0% vs controls (frequency 15.6%); $P=0.007$) were significantly different. Similarly, C allele frequencies were significantly higher both in male (frequency 26.7%, $P=0.0006$) and female power-oriented athletes (frequency 28.6%, $P=0.0033$) compared to controls.