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Association of a *PPARD* Polymorphism with Human Physical Performance

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Abstract—Peroxisome proliferator-activated receptor δ (PPARδ) regulates the expression of genes involved in lipid and carbohydrate metabolism. To examine the association of the functional +294T/C polymorphism of *PPARD* with human physical performance, the distribution of *PPARD* alleles and genotypes was studied in a cohort of athletes ($N = 1256$), stratified by specialization and skill level, and in controls ($N = 610$). The frequency of *PPARD* allele C (with a higher transcriptional activity compared to allele T) in the group of endurance-oriented athletes ($N = 898$) was significantly higher than in controls (18.3% vs. 12.1%, $P < 0.0001$). Moreover, in the group of endurance-oriented athletes participating in cyclic sports, the frequency of allele C increased with growing skill level. Thus, *PPARD* allele C was associated with predisposition to endurance performance.

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Key words: physical performance, physical qualities, fatty acid oxidation, regulatory gene, *PPARD*

INTRODUCTION

In the recent years, much research has been devoted to peroxisome proliferator-activated receptors (PPARs), which regulate the expression of most genes involved in lipid and carbohydrate metabolism. The family includes PPARα, PPARγ, and PPARδ, encoded by *PPARA*, *PPARG*, and *PPARD*, respectively.

Since a wide range of pleiotropic biological effects is associated with the PPAR family, the most attention has been paid to *PPARD* mapping to chromosome 6. This gene is equally actively expressed in both adipose tissue and skeletal muscles, primarily in slow twitch muscle fibers [1]. PPARδ participates in regulation of genes involved in fatty acid oxidation, cholesterol metabolism, thermogenesis, embriogenesis, regenerative and inflammatory processes, and carcinogenesis [2].

The effects of PPARδ are mediated through its consecutive binding with ligands, the retinoid X receptor (RXR), a coactivator, and a PPAR-responsive element of a target gene promoter. The PPARδ ligands include saturated and polyunsaturated fatty acids, synthetic and endogenous eicosanoids, and the agonists GW742 and GW501516, which are currently undergoing extensive clinical trials in patients with obesity, type II diabetes mellitus, and atherosclerosis [3]. Fasting and physical exercise increase the circulating levels of endogenous PPARδ ligands and up-regulate the *PPARD* expression, thus triggering adaptation to physical exercise in the skeletal muscle [4, 5].

PPARD overexpression increases the oxidative potential of muscle cells and down-regulates the intramuscular triglyceride accumulation in rodents [6]. Moreover, VP-PPARδ, a ligand-independent product of a modified gene variant, causes a constant activation of the target genes and, as a consequence, a twofold increase in the portion of slow twitch (oxidative) muscle fibers in mice. Model mice are capable of covering without preliminary training nearly twice as long distances as their wild-type counterparts and maintain a normal body weight when fed a high-fat diet [7]. A similar effect is produced by administration of GW501516 [7, 8].

In human, the best clinically studied *PPARD* polymorphism is a single nucleotide substitution in the untranslated region of exon 4 (+294T/C; rs2016520). Mutant allele C has a 39% higher transcriptional activity in comparison to allele T. This may be due to the acquisition of a new binding site for transcription factors up-regulating the *PPARD* expression, e.g., Sp-1 [9]. In carriers of *PPARD* allele C, glucose uptake by muscle cells is slightly increased, whereas the high density lipoprotein level and body mass index are reduced [9, 10].

The increased transcriptional activity of *PPARD* allele C suggests that this allele enhances lipid catabolism and, to a certain degree, reduces the risk of obe-

Table 1. Grouping of major sports

Group, number of subjects	Exercise characteristics (1. Type/; 2. Quality/; 3. Power)	Major energy source	Event duration	Sports
I, 308	1. Cyclic 2. Endurance 3. Moderate	Fatty acids and glycogen	>30 min	Biathlon, road cycling, cross-country skiing 15–50 km, swimming 5–25 km, triathlon
II, 220	1. Cyclic 2. Endurance 3. High	Fatty acids and glycogen	5–30 min	Rowing, speed skating 5–10 km, cross-country skiing 5–10 km, swimming 800–1500 m
III, 81	1. Cyclic 2. Speed and endurance 3. Submaximal	Glycogen and lactate	45 s to 3–5 min	Running 800–1500 m, canoeing, speed skating 1–3 km, swimming 200–400 m, short track
IV, 289	1. Acyclic 2. Agility, speed, strength, and endurance 3. Varying	ATP, creatine phosphate, glycogen, and lactate	Unspecified	Basketball, boxing, wrestling, volleyball, all-round speed skating, mountain bike racing, table tennis, modern pentathlon, shooting, tennis, football, fencing, ice hockey
V, 52	1. Acyclic 2. Agility and strength 3. Maximal	ATP, creatine phosphate, and glycogen	Unspecified	Alpine skiing, ski jumping, artistic gymnastics
VI, 141	1. Acyclic 2. Strength and speed 3. Maximal	ATP, creatine phosphate, and glycogen	1–60 s	Bodybuilding, powerlifting, weightlifting
VII, 165	1. A. Cyclic/B. Acyclic 2. Speed and strength 3. Maximal	ATP, creatine phosphate, and glycogen	10–45 s	A. Running 100–400 m, speed skating 500–1000 m, swimming 50–100 m; B. Throwing, long jump

sity. Evidently, these remarkable data are of considerable importance for the research of the molecular mechanisms of energy supply for human muscular performance.

The purpose of the present work was to investigate the possible association between the +294T/C *PPARD* polymorphism and predisposition to physical performance with different energy supply types in athletes of various specializations.

EXPERIMENTAL

Cohort characteristics. The study involved 1256 Russian athletes of various specializations and skill levels (females, 19.1 ± 0.2 years, N = 373; males, 19.7 ± 0.1 years, N = 883). Biological samples were obtained from volunteer students of Olympic reserve colleges of St. Petersburg and at training gatherings in Moscow and St. Petersburg.

Depending on the workout energy supply type, all sports were divided into seven groups with similar physiological characteristics of the training exercises. In addition to the type of the target physical qualities (endurance, speed, strength, agility, and their combinations), two other factors were taken into consideration. The power of the training exercises was estimated as maximal, submaximal, high, moderate, or varying, and the type of exertion was classified as cyclic (cyclic sports) or acyclic (acyclic sports).

Table 1 shows the division of all sports studied into seven groups (I–VII) according to the above criteria. The groups differ in type of physical exercise (cyclic or acyclic), in energy expenditure, and in the type of developing sports qualities. The major energy sources supporting a particular workout type are also listed.

Group VI includes bodybuilding, which is a power exercise system aimed at increasing the muscular and lowering the fat mass rather than a classical sport. In group VII, including cyclic sports with a maximal power output and speed and strength development, running for 400 m, speed skating for 500–1000 m, and swimming for 100 m are characterized by an intermediate (maximal–submaximal) power.

At the time of sample collection, the athlete cohort included 386 elite athletes (26 Olympic, world, or

Europe championship winners, 77 participants of Olympic games and world and Europe championships (POWE), and 283 athletes of the national competitive standard (NCS)), 417 sub-elite athletes (SA), and 453 non-elite athletes (NA).

The control group comprised 610 individuals, citizens of St. Petersburg (females, 21.5 ± 0.3 years, N = 387; males, 22.8 ± 0.5 years, N = 223). The major criterion for inclusion in the control group was a lack of regular practice in any sport (respondents did not mention any sports rank in the questionnaires).

DNA extraction from epithelial mouth cells. Molecular genetic analysis was performed with DNA samples obtained by alkaline extraction [11] or using a DNK-sorb-A sorbent kit according to the manufacturer's instruction (Central Research Institute of Epidemiology, Russia), depending on the method of sample collection (buccal swab or scrape).

RFLP analysis. The +294T/C polymorphism of the untranslated region of *PPARD* exon 4 was detected by PCR–RFLP (forward primer, 5'-CATGGTATAGCACTGCAGGAA-3'; reverse primer, 5'-CTTCCTCCTGTGGCTGCTC-3'). The 269-bp amplicons were digested with *Bsc*4I (SibEnzyme), producing 167- and 102-bp fragments for allele C and a 269-bp fragment for allele T. The restriction products were resolved by PAGE in 8% gels, stained with ethidium bromide, and visualized in UV light, using an ETS Vilber-Lourmat transilluminator (France).

Statistical methods. Statistical analysis was performed using Statistica 6.0 and GraphPad InStat software. For the age data, mean values (M) and standard errors of the mean ($\pm m$) were calculated. Differences in allele and genotype frequencies between the groups and the correspondence of the genotype distributions to the Hardy–Weinberg equilibrium were evaluated using the χ^2 test (for large samples) and Fisher's exact test (for small samples). Differences were considered significant at $p < 0.05$.

RESULTS

We analyzed the allele and genotype frequency distributions of the +294T/C polymorphism of *PPARD* exon 4 in athletes (N = 1256) and controls (N = 610). In the control group, the frequency of allele C was 12.1% and did not differ between men (11.9%) and women (12.3%). The distribution of genotypes TT (76.9%), TC (22.0%), and CC (1.1%) obeyed the Hardy–Weinberg equilibrium ($\chi^2 = 0.31$; df = 2, P = 0.85) (Table 2).

In athletes, the frequency of *PPARD* allele C was significantly higher than in controls (17.4% vs. 12.3%; $P < 0.0001$). The distribution of genotypes TT (68.6%), TC (27.9%), and CC (3.4%) agreed with the Hardy–Weinberg equilibrium ($\chi^2 = 0.46$; df = 2, P = 0.79). Table 2 shows the *PPARD* genotype and allele frequency distributions in athletes with different energy supply types. Only in groups I–IV, which include endurance-oriented sports, was the frequency of allele C significantly higher than in the control group: in groups I–IV taken together (N = 898) the frequency of *PPARD* allele N was 18.3%, $P < 0.0001$. Moreover, all groups and sports displayed a common trend to a decreasing frequency of allele C with a diminishing endurance component, which depends on the aerobic potential of the body and its ability towards maximal fatty acid utilization.

Certain differences in *PPARD* allele frequency distribution were revealed between the sexes. The frequency of allele C was higher in males in groups I (21.7% vs. 14.5%, P = 0.035), II (18.2% vs. 15.6%), III (21.2% vs. 19.0%), and IV (18.0% vs. 16.7%). In general, the frequency of allele C in female athletes (N = 373, 15.7%) was slightly lower than in male athletes (N = 883, 18.1%) but higher than in the control group (12.3%, P = 0.06). In male athletes, the frequency of allele C was significantly higher in comparison to controls in both the total sample (18.1% vs. 11.9%, P = 0.002) and in individual endurance-oriented groups (I–IV).

When the allele frequency distributions were evaluated with respect to the skill level in the total sample of athletes participating in cyclic endurance-oriented sports (groups I–III), the frequency of allele C was 17.3% in the NSC + SA group (N = 323) and 25.0% in the POWE group (N = 30); i.e., it was higher in top-level athletes (P = 0.1). Furthermore, differences in the frequency of allele C were most pronounced between high- and top-level long-distance athletes of group I: 13.7% in the NSC + SA group (N = 102) vs. 33.3% in the POWE group (N = 15); P = 0.013.

DISCUSSION

Results of numerous studies indicate a genetic predisposition to human physical performance [12]. However, detection of genetic markers associated with physical activity still remains an intricate problem of human genetics. The main difficulty is caused by the fact that muscle performance is regulated by a large number of polymorphic genes, each exerting only a minor effect on the overall physical performance.

The novelty of the present study primarily lies in the right choice of a candidate gene polymorphism whose allele frequencies differ between athletes and controls. This is a polymorphic regulatory gene *PPARD*, which coordinates the expression of dozens of genes involved in lipid and carbohydrate metabolism and, thus, in the regulation of energy supply for muscle performance [5–7]. It has been supposed that the effect of the functional +294T/C polymorphism is

Table 2. *PPARD* genotype and allele frequency distributions in athletes and controls

Group	Sports	*N*	Genotype, %			Allele C, %	*P*
			TT	TC	CC		
I	Biathlon	32	68.8	31.2	0	15.6	0.4
	Road cycling	108	67.6	27.8	4.6	18.5	0.015*
	Cross-country skiing, 15–50 km	119	63.0	33.6	3.4	20.2	0.0017*
	Swimming, 5–25 km	21	66.7	23.8	9.5	21.4	0.09
	Triathlon	28	71.4	21.4	7.1	17.9	0.2
	Total	308	66.2	29.5	4.2	19.0	0.0001*
II	Rowing	189	70.4	26.5	3.2	16.4	0.036*
	Speed skating, 5–10 km	4	75.0	0	25.0	25.0	0.25
	Cross-country skiing, 5–10 km	10	50.0	40.0	10.0	30.0	0.29
	Swimming, 800–1500 m	17	70.6	17.6	12.0	20.6	0.18
	Total	220	69.5	25.9	4.5	17.5	0.0057*
III	Running, 800–1500 m	5	60.0	40.0	0	20.0	0.34
	Canoeing	35	54.3	40.0	5.7	25.7	0.003*
	Speed skating, 1–3 km	8	62.5	37.5	0	18.8	0.43
	Swimming, 200–400 m	25	72.0	28.0	0	14.0	0.66
	Short track	8	62.5	37.5	0	18.8	0.4
	Total	81	61.7	35.8	2.5	20.4	0.006*
IV	Basketball	20	65.0	30.0	5.0	20.0	0.14
	Boxing	22	72.7	27.3	0	13.6	0.81
	Wrestling	82	68.3	28.0	3.7	17.7	0.06
	Volleyball	6	50.0	33.3	17.0	33.3	0.049*
	All-round speed skating	62	74.2	24.2	1.6	13.7	0.57
	Mountain bike racing	10	60.0	30.0	10.0	25.0	0.08
	Table tennis	4	25.0	75.0	0	37.5	0.06
	Pentathlon	17	47.1	47.1	5.9	29.4	0.007*
	Shooting	24	75.0	20.8	4.2	14.6	0.6
	Tennis	15	80.0	20.0	0	10.0	1.0
	Soccer	10	50.0	50.0	0	25.0	0.09
	Fencing	5	60.0	40.0	0	20.0	0.34
	Ice hockey	12	75.0	25.0	0	12.5	1.0
	Total	289	67.8	29.1	3.1	17.6	0.002*
V	Alpine skiing	13	61.5	30.8	7.7	23.1	0.12
	Ski jumping	10	60.0	30.0	10.0	25.0	0.09
	Artistic gymnastics	29	75.9	20.7	3.4	13.8	0.7
	Total	52	69.2	25.0	5.8	18.3	0.09
VI	Bodybuilding	57	73.7	24.6	1.8	14.0	0.55
	Powerlifting	26	73.1	23.1	3.8	15.4	0.5
	Weightlifting	58	67.2	31.0	1.7	17.2	0.14
	Total	141	70.9	27.0	2.1	15.6	0.13
VII	Running, 100–400 m	104	75.0	25.0	0	12.5	0.9
	Speed skating, 500–1000 m	22	63.6	31.8	4.5	20.5	0.1
	Throwing	5	60.0	40.0	0	20.0	0.35
	Swimming, 50–100 m	31	87.1	6.4	6.5	9.7	0.7
	Long jump	3	33.3	66.7	0	33.3	0.16
	Total	165	74.5	23.6	1.8	13.6	0.45
All athletes		1256	68.6	27.9	3.4	17.4	<0.0001*
Control group		610	76.9	22.0	1.1	12.1	1.0

* $P < 0.05$, significant difference between athletes and controls.

comparable to the cumulative effect of polymorphisms in the genes regulated by *PPARD*. An increased expression of allele C in comparison to the wild-type allele [9] suggested that the difference in transcriptional activity of the alleles accounts for the individual variation in fatty acid utilization at physical exertion. Based on this, we assumed that carriers of *PPARD* allele C are predisposed to endurance-oriented events or physical activity that utilizes fatty acids as the major energy source.

To test this hypothesis, we selected representative cohorts of athletes of various specializations and skill levels and a control group to investigate the potential differences in *PPARD* allele frequencies. We supposed that a higher frequency of a particular allele in athletes with certain metabolic characteristics (with different types of energy supply to the muscles) in comparison to controls indicates sports selection and that this allele itself can be considered a marker associated with a certain physical trait (endurance, speed, or strength).

Analysis of the *PPARD* genotype and allele frequency distributions demonstrated that the frequency of allele C in endurance-oriented athletes (groups I–IV) is significantly higher than in control individuals who do not practice any sport. Moreover, in long-distance athletes (groups I–III), the frequency of allele C increased with increasing skill level. Both observations agree with the genetic concept of selection in sports, reflecting the accumulation of the allele favorable for endurance in the group of long-distance athletes.

Thus, our results suggest that carriers of *PPARD* allele C, associated with an increased expression of the transcription factor, are predisposed to endurance performance. At the same time, these results should be considered preliminary since there is no data on the association of the *PPARD* polymorphism +294T/C with the efficiency of fatty acid utilization. The relationship of the *PPARD* genotypes with human capacity for aerobic and anaerobic work also requires further investigation.

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