SPORTS MEDICINE

PPARG Gene Polymorphism and Locomotor Activity in Humans

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The distribution of *PPARG* gene allele frequencies (Pro/Ala polymorphism) was studied in sportsmen specialized in speed and force athletics. A relationship between genotypes and human muscle transverse section area was evaluated. The *PPARG* Ala allele was significantly more incident in athletes than in controls, the incidence increasing with higher athletic qualification. A hypertrophic effect of *PPARG* Ala allele on muscle fibers was detected. Hence, the *PPARG* Pro12Ala polymorphism is associated with human motor activity.

Key Words: *PPARG; polymorphism; muscle fibers; motor activity*

Peroxisome proliferator-activated receptor-γ (PPARγ; encoded by *PPARG* gene) is a transcription factor regulating the expression of genes involved in lipid and carbohydrate metabolism [6]. The Pro12Ala polymorphism detected in *PPARG* is a C34G substitution in exon *B*. The capacity of the product of the expression *PPARG* Ala allele to activate the responsive promotors [2] is low, which determines tissue hypersensitivity to insulin and enhanced glucose utilization [3]. Meta-analysis of 30 cases indicates that Ala allele carriers have higher body weight index (BWI) than Pro/Pro homozygotes [4].

From these data, we hypothesize that *PPARG* Ala allele carriers are more liable to the speed and force athletics than Pro/Pro homozygotes, because their muscles better utilize glucose due to hypersensitivity to insulin. The anabolic effect of insulin on skeletal muscles is known; in addition, insulin amplifies the force potential.

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We studied frequency distribution of *PPARG* gene alleles in athletes specialized in speed and force sports and in controls and detected a relationship between genotypes and area of human muscle fiber transverse section.

MATERIALS AND METHODS

The study was carried out in 260 athletes (176 men and 84 women aged 21.6±0.6 years) specialized in speed and force athletics: sprinters (60-400 meter runners; *n*=117), skate sprinters (500-1000 meters; *n*=36), discus, javeline, and hammer throwers (*n*=17), swimming sprinters (50-100 meters; *n*=32), and weight lifters (*n*=58) of different qualification. The group included 67 athletes of different categories, 65 candidate masters of sports, 83 masters of sports, 30 masters of sports of international rank, and 15 honored masters of sports. A group of 46 physically active men took part in the study for evaluation of histomorphological parameters of muscle fibers; BWI=22.9 (2.8) kg/m^2. Control group consisted of 1073 subjects (585 women aged 18.0±0.1 years

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and 488 men aged 17.6±0.1 years). All participants were informed about the conditions of the experiment and gave written consent to participation. The experiment was approved by the physiological Section of Russian National Committee for Biological Ethics.

Specimens of DNA for molecular genetic analysis were isolated by alkaline extraction [1] or adsorbent method (according to the instruction on the use of DNA-sorb-A kit; Central Institute of Epidemiology). The method of DNA isolation depended on the method of biological material collection (oral epithelial cell washing off or scraping). The *PPARG* gene Pro12Ala polymorphism was evaluated [5]. The PCR was carried out using a two-primer system (direct primer: 5'-GCCAATTCAAGC CCAGTC-3'; inverse primer: 5'-GATATGTTTGCA GACAGTGTATCAGTGAAGGAATCGCTTTCCG-3'; Litech Firm). Restriction of amplicons (270 b.p.) was carried out using *Bsh 1236I* enzyme (Fermentas). Restriction product lengths were analyzed by electrophoretic separation in 8% PAAG with subsequent staining with ethidium bromide and visualization in transmitting UV light.

In order to evaluate the composition of muscle fibers, specimens of muscle tissue were collected from *m. vastus lateralis* by needle biopsy after Bergstrem and frozen in liquid nitrogen. Serial transverse sections (10 μ) were sliced in a cryostat at -20°C. Immunohistochemical detection of myosin heavy chain isoforms was carried out using the immunoperoxidase method. Antibodies to slow and fast myosin chains were used (NCL-MHCs and NCL-MHCf (a+b); Novocastra Laboratories). The antigen-antibody label was intensified by Vectrastain ABC kit (Vector Labs), visualized by diaminobenzidene peroxidase reaction. The cross-section area (CSA) was measured in at least 100 fibers of each type using Quintimet-500 image analysis system (Leica) with a JVC TK-1280E color digital videocam.

The data were statistically processed using χ^2 test and paired test. The means (*M*), errors of the means (*SEM*), and standard deviations (*s*) were calculated. The differences were considered statistically significant at $p<0.05$.

RESULTS

The frequency of *PPARG* Ala allele in the control was 15.1%. The distribution of Pro/Pro (71.6%), Pro/Ala (26.4%), and Ala/Ala (2%) genotypes in the control sample corresponded to the Hardy—Weinberg law ($\chi^2=0.47$; df=2, $p=0.79$).

The frequency of Ala allele in the total group of athletes was significantly higher than in controls (23.8 *vs.* 15.1%; $p<0.0001$). The distribution of athletes into groups practicing different sports showed that Ala allele was significantly more incident in comparison with the controls only in skate sprinters (31.9%; $p=0.0002$), throwers (32.4%; $p=0.012$), and weightlifters (25.9%; $p=0.003$) (Table 1).

The distribution of athletes into 5 groups by their qualification showed a linear relationship of Ala allele frequency and qualification: qualified athletes of different categories (16.4%)→candidate masters of sports (16.1%)→masters of sports (30.7%)→masters of sports of International rank (35.0%)→honored masters of sports (36.7%); $\chi^2=16.1$; $p<0.0001$. The frequency of Ala allele was minimum in athletes of different categories and candidate masters of sports and maximum in masters of sports of International rank and honored masters of sports. This regularity was observed in athletes irrespective of specialization and gender.

The detected significant differences in the frequency of *PPARG* Ala allele in athletes engaged in different athletic types aimed at development of speed and force and controls reflect the process of athletic selection: alleles (*PPARG* Ala) favoring the

TABLE 1. Distribution of Genotype and *PPARG* Alleles in Athletes

Athletics type	*n*	Genotypes			Ala allele, %	*p*
		Pro/Pro	Pro/Ala	Ala/Ala		
Control	1073	769	283	21	15.1	1.00
Running, 60-400 m	117	79	29	9	20.1	0.06
Skating, 500-1000 m	36	15	19	2	31.9	0.0002*
Throwing	17	9	5	3	32.4	0.012*
Swimming, 50-100 m	32	21	9	2	20.3	0.34
Weight lifting	58	31	24	3	25.9	0.003*
All athletes	260	155	86	19	23.8	<0.0001*

Note. *Statistically significant differences between athletic groups and control ($p<0.05$).

development of speed and force and high athletic results accumulate in athletes with increasing their qualification, while the incidence of alleles (*PPARG* Pro) limiting physical performance decreases.

Analysis of the relationship between *PPARG* gene polymorphism and muscle fiber CSA revealed an association of *PPARG* Ala allele with greater CSA of slow muscle fibers, in other words, with their more pronounced hypertrophy (Pro/Pro: 5103 (1049) μ^2; Pro/Ala: 5836 (1049) μ^2; p=0.02). In addition, the Pro/Ala genotype carriers had greater fast muscle fiber CSA (Pro/Pro: 5608 (1246) μ^2; Pro/Ala: 6402 (1195) μ^2; r=0.26, p=0.07) than the carriers of the Pro/Pro genotype.

Clinical data, indicating an association of *PPARG* Ala allele with hypersensitivity to insulin [3] suggest higher anabolic effect of insulin on muscle tissue and hence suggest that Ala allele carriership can render advantages for sprinters, throwers, and weight lifters. This hypothesis was confirmed in our study by the analysis of correlations between *PPARG* Pro12Ala polymorphism and muscle fiber CSA: Ala allele was associated with a larger CSA for slow (significant correlation) and fast muscle fibers (correlation at the level of a trend). The results of previous meta-analysis indicating significantly higher BWI in carriers of Ala allele [4] indirectly confirm the hypertrophic effect of Ala allele.

Hence, the results indicate an association between *PPARG* Pro12Ala polymorphism and liability to the development and manifestation of the speed and force qualities. This assumption is supported by the detected relationship between *PPARG* Ala allele and larger size of *m. vastus lateralis* muscle fibers in physically active men.

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