ATHLETIC MEDICINE

Effect of *HIF1A* Gene Polymorphism on Human Muscle Performance

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Allele distribution of hypoxia-inducible factor gene (*HIF1A*; Pro582Ser polymorphism) was studied in power-oriented athletes and controls practicing no athletics; genotype relationships with muscle fiber composition were studied in speed skaters. Genotyping was carried out by PCR. The composition of muscle fibers was evaluated by the immunohistochemical analysis of *m. vastus lateralis*. The incidence of *HIF1A* Ser allele was significantly higher in weight-lifters than in controls (17.9 *vs.* 8.5%; p=0.001) and increased with athletic skill improvement. A relationship between *HIF1A* Ser allele and predominance of fast-twitch muscle fibers was shown (Pro/Ser 46.2 (13.8)%, Pro/Pro 31.4 (8.2)%; p=0.007). Hence, *HIF1A* Pro582Ser polymorphism is associated with muscle activity in humans.

Key Words: *HIF1A; polymorphism; muscle fibers; muscular activity*

Energy supply to human skeletal muscles during performance of highly intense exercise under anaerobic conditions is realized at the expense of the phosphagenic and glycolytic systems. Sprint and weight lifting are power-oriented athletics, in which the result is determined by high anaerobic potential (high reserves of ATP, creatine phosphate, and glycogen; high concentration and activities of glycolysis and phosphagenic system enzymes; predominance of fast-twitch muscle fibers in skeletal muscles, *etc.*).

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor regulating the expression of genes providing cell adaptation to hypoxia. These genes are involved in glycolysis (aldolase, lactate dehydrogenase, phosphofructokinase, pyruvate kinase, phosphoglycerate kinase genes), glucose transport (GLUT family glucose transporter genes), and angiogenesis (erythropoietin, vascular endothelium growth factor, VEGF, and VEGF receptor genes) [2,6,8,11].

HIF-1 is a heterodimer consisting of two subunits: HIF-1a and HIF-1b. The expression of HIF-1a and level of this protein depend on oxygen concentration and partial pressure in the blood; its activity increases in hypoxia [5,9] and hence, the expression of these genes also increases. HIF-1a is synthesized everywhere [12]; its high expression in fast glycolytic muscle fibers *vs.* slow fibers is worthy of note [7].

The Pro582Ser polymorphism was detected in *HIF1A* gene; it consists in cytosine substitution with thymine in exon 12, which leads to substitution of proline with serine in position 582 of the protein [4]. It was shown that this rare substitution (Ser allele carriership) increases transcription activity of the gene allele, stability of HIF1A protein, and hence,

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increases the hypoxic resistance of cells (high glycolytic potentialities, high angiogenesis) [10].

These data suggest that *HIF1A* Ser allele carriers are more liable to weight-lifting athletics than Pro/Pro homozygotes. The relationship between *HIF1A* Ser allele and great volume of fast muscle fibers is hypothesized as a mechanism underlying this liability.

We studied allele distribution of *HIF1A* gene in power-oriented athletes and in non-athletes and evaluated the relationship between genotypes and muscle fiber composition in skaters.

MATERIALS AND METHODS

The study was carried out in athletes specialized in weight lifting (n=53; 39 men and 14 women aged 22.5±0.6 years) and all-round speed skating (n=21; 14 men and 7 women aged 20.5±0.5 years) of different qualification (23 candidate masters of sports (CMS), 46 masters of sports (MS), and 5 International Class Masters of Sports (ICMS)) and 920 non-athletes (control group; 529 women aged 18.1± 0.1 years and 391 men ages 18.6±0.2 years). Biopsy of the skeletal muscles (*m. vastus lateralis*) was carried out in the group of skaters.

The examined subjects were informed about conditions of the experiment and gave written consent to voluntary participation. The experiment was approved by the Ethic Committee of St. Petersburg Institute of Physical Culture and by Physiological Section of Russian National Committee for Biological Ethics.

DNA specimens for molecular genetic analysis were isolated by alkaline extraction [3] or by the adsorbent method (according to the instruction for DNA-Sorb-A kit (Institute of Epidemiology, Ministry of Health of the Russian Federation) depending on the method of biological material collection (washing or scraping off the oral epithelial cells). *HIF1A* gene Pro582Ser polymorphism was detected by the double primer PCR method (forward primer: 5'-GACTTTGAGTTTCACTTGTTT-3'; reverse primer: 5'-ACTTGCGCTTTCAGGGCTTGCGGAACT GCTT-3'; LITECH Company). Restriction of amplicons (197 b. p.) was carried out using *NmuCI* enzyme (Fermentas). Restriction product lengths were analyzed by electrophorests in 8% PAAG with subsequent staining with ethidium bromide and visualization in transmitting ultraviolet light.

Specimens of muscle tissue for evaluation of muscle fiber composition in skaters 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 cryostate at -20°C. Immunochemical detection of myosin heavy chain isoforms was carried out by the immunoperoxidase method. Antibodies to slow and fast myosin chains (clones NCL-MHCs and NCL-MHCf (a+b); Novocastra Laboratories) were used. The antigen-antibody label was intensified by Vectrastain ABC kit (Vector Labs), visualized by the diaminobenzidine peroxidase reaction. All fibers (200-300) in each section were measured by QUANTIMET-500 image analysis system (Leica) with a JVC TK-1280E digital color video camera.

The data were statistically processed using χ^2 test and unpaired t test. The mean (M), error of the mean (±SEM), and standard deviation (s) were calculated. The differences were considered significant at $p<0.05$.

RESULTS

The incidence of *HIF1A* Ser allele in the control group was 8.5%. The distribution of Pro/Pro (83.5%), Pro/Ser (16%), and Ser/Ser (0.5%) genotypes in the control sample conformed to the Hardy—Weinberg equilibrium (χ^2=0.39; df=2, p=0.82).

The incidence of *HIF1A* Ser allele in all athletes was significantly higher than in the control group (16.2 *vs.* 8.5%; p=0.0018). *HIF1A* Ser allele predominated in the group of weight-lifting athletes (17.9%; p=0.001) in comparison with the control (Table 1).

The distribution of weight lifters into groups with consideration with athletic qualification showed linear relationship between *HIF1A* Ser allele incidence (14.7% (CMS)→18.8% (MC)→25% (ICMS) and qualification of these athletes: the incidence of the allele was minimum in CMS and maximum in ICMS (Table 1). This regularity was observed in weight lifters irrespective of the gender.

These differences in the incidence of *HIF1A* Ser allele in athletes (weight lifters) and controls and increased incidence of this allele in athletes of higher qualification reflect natural process of athletic selection.

The percentage of fast muscle fibers in the *m. vastus lateralis*, essential for manifestation of the speed and power potential, amounted to 40.6% in the skaters (*vs.* 8.8% in the control). Analysis of the relationship between *HIF1A* gene polymorphism and percentage of myofibrils detected an association of *HIF1A* Ser allele with high percentage of fast myofibrils: the Pro/Ser genotype carriers had a higher percentage of fast muscle fibers in comparison with the Pro/Pro genotype carriers (46.2 (13.8)% *vs.* 31.4 (8.2)%; p=0.007).

Our data on *HIF1A* Pro582Ser polymorphism association with liability to weight-lifting athletics

TABLE 1. Distribution of *HIF1A* Genotypes and Alleles in Athletes and Controls

Group	*n*	Genotypes, %			Ser allele, %
		Pro/Pro	Pro/Ser	Ser/Ser	
Control	920	83.5	16.0	0.5	8.5
CMS weight-lifters	17	70.6	29.4	0	14.7
MS weight-lifters	32	62.5	37.5	0	18.8*
ICMS weight-lifters	4	50.0	50.0	0	25.0
All weight-lifters	53	64.2	35.8	0	17.9**
Skaters	21	76.2	23.8	0	11.9
All athletes	74	67.6	32.4	0	16.2***

Note. *$p<0.005$, **$p<0.01$, ***$p<0.0018$ compared to the control.

are in line with experimental findings on mouse cell cultures, which demonstrated a relationship between *HIF1A* Ser allele and high transcription activity of the gene and high level of glycolysis. The *HIF1A* Ser allele in this case can be regarded as an allele favoring the development and manifestation of the speed and power capacities.

This assumption is supported by the detected relationship between the *HIF1A* Ser allele and predominance of fast muscle fibers in the *m. vastus lateralis* of skaters. It seems that the expression of *HIF1A* can modify the determination of myofibril composition, which is linked with the relationship between the number of various myofibrils and predominance of this or that type of metabolism [1].

Hence, the results indicate an association of Pro582Ser polymorphism of hypoxia inducible factor gene (*HIF1A*) and human muscle performance and point out the important role of this factor in adaptation of skeletal muscles to exercise in an anaerobic mode.

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REFERENCES

1. I. I. Ahmetov, I. V. Astratenkova, A. M. Druzhevskaya, *et al.*, *Ros. Fiziol. Zh.*, **92**, No. 7, 883-888 (2006).
2. R. E. Airley and A. Mobasheri, *Chemotherapy*, **53**, No. 4, 233-256 (2007).
3. M. K. Bolla, L. Haddad, S. E. Humphries, *et al.*, *Clin. Chem.*, **41**, No. 11, 1599-1604 (1995).
4. S. C. Clifford, D. Astuti, L. Hooper, *et al.*, *Oncogene*, **20**, No. 36, 5067-5074 (2001).
5. L. E. Huang, J. Gu, M. Schau, and H. F. Bunn, *Proc. Natl. Acad. Sci. USA*, **95**, No. 14, 7987-7992 (1998).
6. N. V. Iyer, L. E. Kotch, F. Agani, *et al.*, *Genes Dev.*, **12**, No. 2, 149-162 (1998).
7. D. F. Pisani and C. A. Dechesne, *J. Gen. Physiol.*, **126**, No. 2, 173-178 (2005).
8. G. L. Semenza, B. H. Jiang, S. W. Leung, *et al.*, *J. Biol. Chem.*, **271**, No. 51, 32,529-32,537 (1996).
9. C. H. Sutter, E. Laughner, and G. L. Semenza, *Proc. Natl. Acad. Sci. USA*, **97**, No. 9, 4748-4753 (2000).
10. K. Tanimoto, K. Yoshiga, H. Eguchi, *et al.*, *Carcinogenesis*, **24**, No. 11, 1779-1783 (2003).
11. G. L. Wang, B. H. Jiang, E. A. Rue, and G. L. Semenza, *Proc. Natl. Acad. Sci. USA*, **92**, No. 12, 5510-5514 (1995).
12. C. M. Wiener, G. Booth, and G. L. Semenza, *Biochem. Biophys. Res. Common.*, **225**, No. 2, 485-488 (1996).