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Polymorphism of the Vascular Endothelial Growth Factor Gene (*VEGF*) and Aerobic Performance in Athletes

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Abstract—The subject of this study is the frequency distribution of alleles of the vascular endothelial growth factor gene (*VEGF*; the *G*-634*C* polymorphism) in athletes ($n = 670$) and in a control group ($n = 1073$) and the relationships of genotypes with aerobic performance in rowers ($n = 90$). Genetic typing was performed using the analysis of restriction fragment length polymorphism. The frequency of the *VEGF C* allele in the group of endurance athletes ($n = 294$) was significantly higher than in the control group and increased together with increasing sports qualification. In addition, a correlation of the *VEGF C* allele with a high aerobic performance of athletes (according to data on the maximal power and maximal oxygen consumption) and with a substantial contribution to the energy supply of aerobic metabolism (according to the values of maximal lactate content) has been found. It is inferred that the *G*-634*C* polymorphism of the *VEGF* gene is associated with physical performance of athletes and plays a key role in sports selection.

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INTRODUCTION

Enhancement of endurance as a result of systematic aerobic exercise is determined by numerous adaptive responses to training stimuli. These responses include an increase in the number of capillaries around each muscle fiber, which results in improvement of gas and heat exchange and in acceleration of excretion of the products of decomposition of nutrients and their exchange between blood and working muscle fibers. The improvement of the maximal oxygen consumption (V_{O_2max}) as a result of training is mainly determined by an increased maximal blood flow and higher density of muscle capillaries in active tissues [1]. The number of capillaries and its ratio to the number of muscle fibers in leg muscles of an athlete engaged in cyclic sports may be 5–30% and 50% higher, respectively, than in a sedentary person [2–4].

Individual differences in the degree of adaptive changes, such as growth of blood vessels of skeletal muscles and the myocardium, are to a greater extent accounted for by genetic factors that determine the genetic predisposition to performing physical exercises of different intensities and durations [5]. On this basis, an important problem of muscle performance genetics is to identify genetic markers associated with the regulation of the growth of vessels; it may help in settling many questions of optimal sports specialization and correction of the training process.

One of the main factors directly influencing the growth of vessels is vascular endothelial growth factor (VEGF), the expression of which significantly increases under aerobic physical exercise [6, 7]. VEGF is a glycoprotein that binds with the cells of blood and lymphatic vessels and stimulates their proliferation. The effect of VEGF is mediated by its receptors (*VEGFR*1, *VEGFR*2, and *VEGFR*3). The *VEGF* gene is located in chromosome 6 (6p12). The expression of *VEGF* is stimulated by a great number of proangiogenic factors, including the hypoxia-induced factor (HIF) and epidermal (EGF) and fibroblast (FGF) growth factors. In addition, the level of VEGF is influenced by the pH value of the blood and the partial pressure and concentration of oxygen in inhaled air [8].

Among the studied polymorphisms of the *VEGF* gene, of special interest are the variants located in the promoter (regulatory) region. For example, the substitution of cytosine for guanine at position –634 (the *G*-634*C* polymorphism; *rs*2010963) increases the gene activity and, accordingly, determines individual differences in the level of expression [9]. According to data on subjects not engaged in sports, the *VEGF C* allele is associated with a greater increase in the V_{O_2max} level as a result of aerobic physical exercise [10].

In this connection, it would be particularly interesting to estimate the correlation of the *G*-634*C* polymorphism of the *VEGF* gene with the physical performance of athletes. It can be assumed that the frequency of the *VEGF C* allele is higher in endurance athletes, with the *VEGF GC* and *CC* genotypes being associated with higher values of aerobic performance. For testing this

hypothesis, the frequency of the *VEGF C* allele has been determined in athletes engaged in cyclic sports focused on endurance training to some extent. Rowing was chosen as a model for studying physical performance because it is a complex sport that mainly requires endurance (rowers cover a distance of 2000 m 70% at the expense of aerobic metabolism) [11].

The goal of this work was to study the distribution of *VEGF* allele frequencies in athletes engaged in cyclic sports and in a control group of subjects not engaged in sports and to estimate the correlation of *VEGF* genotypes with aerobic performance in rowers.

METHODS

Characteristics of the sample. The study was performed in 670 athletes engaged in cyclic sports (229 women, 20 ± 0.5 years old, and 441 men, 20 ± 0.2 years old).

According to the type of energy supply for a training load, we divided cyclic sports into three groups within which physiological characteristics of the subjects found in training exercises were the same [12]. In addition to signs characterizing training for endurance, quickness, and strength, the power of the work performed during training was taken into account, with division into submaximal, high, and moderate (sprinters and athletes engaged in acyclic sports were excluded from the research). Group I (moderate power; the main energy source is fatty acids and glycogen; the time of competitive exercise performance is over 30 min) included athletes engaged in biathlon, track cycling, ski racing (15–50 km), marathon running, swimming (5–25 km), race walking, and triathlon. Group II (high power; fatty acids and glycogen; 5–30 min) included athletes engaged in rowing, running (3–10 km), speed skating (5–10 km), ski racing (5–10 km), and swimming (800–1500 m). Group III (submaximal power; glycogen and lactate; 45 s to 3–5 min) included athletes engaged in running (800–1500 m), kayakling, speed skating (1–3 km), swimming (200–400 m), and short track speed skating.

At the moment of sampling of biological material for genotyping, 18 athletes were honored masters of sport (HMSs), 60 were international masters of sport (IMSs), 135 were masters of sport (MSs), 210 were candidates for masters of sport (CMSs), and 247 had adult sports rankings.

During a physiological examination, indices of aerobic and anaerobic performance were estimated in 90 rowers, including 56 men (27 CMSs and 29 MSs) and 34 women (13 CMSs and 21 MSs).

The control group consisted of 1073 residents of St. Petersburg, Moscow, and Naberezhnye Chelny (585 women, 18 ± 0.1 years old, and 488 men, 17.6 ± 0.1 years old). The main condition for inclusion of subjects into the control group was the absence of experience in regular sports activity (in questionnaires, the respondents did not indicate any sports qualification).

DNA isolation. DNA samples isolated from subjects by the method of alkaline extraction [13] or sorbent method were used for molecular genetic analysis by means of a DNA-sorb-A kit (Central Research Institute of Epidemiology, Ministry of Health of the Russian Federation) as recommended by the manufacturer, depending on the method of sampling of biological material (washing out or scraping of epithelial cells of the oral cavity).

Analysis of restriction fragment length polymorphism (RFLP). The *G*-634*C* polymorphism of the *VEGF* gene was analyzed using a two-primer system (forward primer, 5'-GTAGCAAGAGCTCCA-GAGAGAAGT-3'; reverse primer 5'-TGGAC-GAAAAGTTTCAGTGCGACG-3') [10]. Amplicons 342 bp in length were hydrolyzed using *BslF I* restriction endonuclease (SibEnzyme). Fragments 249 and 93 bp in length corresponded to the *G* allele, and a fragment 342 bp in length corresponded to the *C* allele. The lengths of restriction fragments of the products were analyzed by electrophoretic separation in 8% polyacrylamide gel followed by ethidium bromide staining and visualization in transmitted UV light using an ETS Vilber-Lourmat transilluminator (France).

Determination of the indices of aerobic and anaerobic capacities in a test with gradually increasing load until failure. The aerobic capacity was determined in a test with an increasing load using a PM3 mechanical rowing ergometer (Concept II, United States). The initial load was 150 W for men and 100 W for women; the step duration was 3 min; the rest break between steps was 30 s. The work was performed until failure (a stroke power decrease by >30 W compared to the preset power; a respiratory quotient >1.1). Since the athletes were sometimes unable to perform the exercise for as long as 3 min at the last step, the following estimated value was taken as the maximal power (W_{max}):

$$W_{max} = W_{n-1} + \frac{(W_n - W_{n-1})t_n}{180},$$

where W_n is the mean power of the last step (W), W_{n-1} is the mean power of the next-to-last step (W), and t_n is the time of work at the last step (s).

Gas exchange indices and heart rate (HR, beats/min) were recorded continuously (during each breathing cycle) throughout the test (MetaMax 3B (Cortex, Germany) and Vmax 229 (SensorMedics, United States) gas analyzers were used). The V_{O_2max} (l/min) was determined by the gas exchange values averaged over the last 30 s of each step of the test.

The lactate content of the blood (La_{max}, μM) was determined electrochemically (Super GL easy, Dr. Mueller, Germany); capillary blood (20 μl) was taken from a finger after each step and immediately after the end of the exercise.

Distribution of the absolute and relative frequencies of *VEGF* genotypes and alleles among athletes from different groups and control subjects

Group	Sport	*n*	*Genotypes*			*C allele*, %	*p*
			GG	*GC*	*CC*		
I	Biathlon	34	16	16	2	29.4	0.43
	Track cycling	110	50	45	15	34.1	0.0025*
	Ski racing, 15–50 km	71	34	30	7	31.0	0.1
	Marathon	5	0	4	1	60.0	0.026*
	Swimming, 5–25 km	21	12	7	2	26.2	0.94
	Race walking	24	11	9	4	35.4	0.12
	Triathlon	29	18	10	1	20.7	0.6
	Total	294	141	121	32	31.5	0.0008*
II	Rowing	192	107	70	15	26.0	0.56
	Running, 3–10 km	5	1	4	0	40.0	0.44
	Skating, 5–10 km	4	3	0	1	25.0	0.97
	Ski racing, 5–10 km	64	28	31	5	32.0	0.071
	Swimming, 800–1500 m	25	14	9	2	26.0	0.94
	Total	290	153	114	23	27.6	0.14
III	Running, 800–1500 m	11	6	3	2	31.8	0.59
	Kayaking	35	20	14	1	22.9	0.86
	Skating, 1–3 km	8	4	4	0	25.0	0.96
	Swimming, 200–400 m	24	9	13	2	35.4	0.12
	Short track speed skating	8	6	2	0	12.5	0.41
	Total	86	45	36	5	26.7	0.57
Total of athletes		670	339	271	60	29.2	0.0026*
Control group		1073	618	384	71	24.5	1.00

* Statistically significant differences between the groups of athletes and the control group ($p < 0.05$).

The data were statistically processed using the GraphPad InStat software. The mean value (*M*), standard error of the mean (±*SEM*), and root mean square deviation (*s*) were determined. The significance of differences in the frequency of alleles between the compared groups was determined using the χ^2 test (for large groups) or Fisher's exact test (for small groups). The groups were compared with respect to quantitative characters (physiological indices) in an unpaired test. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Analysis of the distribution of VEGF genotype and allele frequencies in athletes and control subjects. The following results were obtained upon the analysis of the frequency distribution of genotypes and alleles with respect to the *G*-634*C* polymorphism of the *VEGF* gene in the control group ($n = 1073$) and in the athletes ($n = 670$). The frequency of the *VEGF C* allele in the control group was 24.5%; it was similar in women (25.3%) and men (23.6%). The distribution of genotypes *GG* (57.6%), *GC* (35.8%), and *CC* (6.6%) observed in the control group fit the Hardy–Weinberg equilibrium ($\chi^2 = 0.61$; d.f. = 2; $p = 0.74$) (table).

The *VEGF C* allele frequency in the total group of athletes was significantly higher than in the control group (29.2 versus 24.5%; $p = 0.0026$). The distribution of genotypes *GG* (50.6%), *GC* (40.4%), and *CC* (9%) in the group of athletes also fit the Hardy–Weinberg equilibrium ($\chi^2 = 0.16$; d.f. = 2; $p = 0.93$). The table shows the data on the distribution of *VEGF* genotypes and alleles in athletes with specializations differing in

the type of energy supply. As can be seen in the table, only group I, with mostly endurance training, had a significantly higher frequency of the *VEGF C* allele as compared to the control group (31.5 versus 24.5%; $p = 0.0008$).

The analysis of the distribution of *VEGF* gene alleles between the sexes showed no differences either in the pooled sample of athletes or in athletes of group I (all athletes: women, 29%; men, 29.3%; group I: women, 28.3%; men, 32.9%).

The assessment of the distribution of allele frequencies depending on sports qualification showed that the frequency of the *VEGF C* allele in athletes of group I (long-distance racers) had a tendency towards an increase with increased qualification: it was 26.3% in the MS group ($n = 38$), 28.8% in the IMS group ($n = 40$), and 50% in the HMS group ($n = 10$) ($p = 0.09$).

The higher frequency of the *VEGF C* allele in long-distance racers compared to the control and its increase with increased sports qualification suggests that the *VEGF C* allele favors the development and expression of endurance.

Relationship between physiological indices and VEGF genotypes in athletes. The finding of substantial differences in some physiological indices between rowers of different sexes and qualifications made it necessary to perform separate analyses of the relationship between phenotypes and genotypic data. Hence, 90 athletes were divided into four subgroups (male CMSs ($n = 27$), male MSs ($n = 29$), female CMSs ($n = 13$), and female MSs ($n = 21$)).

The mean W_{max} values were significantly higher in male MSs with the *VEGF C* allele as compared with carriers of the *VEGF GG* genotype (*CC* + *GC*, 401 (31) W; *GG*, 371 (47) W; $p = 0.049$). In addition, the *VEGF C* allele was associated with higher V_{O_2max} values in men (*CC*, 5.5 (0.6) l/min; *GC*, 5.1 (0.5) l/min; *GG*, 4.9 (0.5) l/min; $p = 0.09$) and with a greater contribution to the energy supply of aerobic metabolism in female MSs, which is indirectly evidenced by the minimal values of lactate content upon failure to continue the exercise (*CC*, 4.4 μM; *GC*, 8.4 (0.9) μM; *GG*, 8.7 (1.6) μM; $p = 0.08$).

The results are in agreement with previously published data on 148 volunteers, 50–75 years old, leading a sedentary life [10]. The increase in V_{O_2max} after 24 weeks of aerobic training was significantly higher in carriers of *VEGF* haplotypes with the –634°C allele. The same study showed that the *VEGF C* allele in a culture of human myoblasts was expressed to a greater extent than the *G* allele. The researchers supposed that the functional significance of the *G-634C* polymorphism of the *VEGF* gene is determined by the localization of the polymorphic region at the site of binding of the promoter with various transcription factors regulating the gene activity.

The high expression of the *VEGF C* allele suggests a greater adaptive growth of capillaries in response to aerobic physical exercise. Consequently, carriers of the *VEGF C* allele may have some advantage in increasing the aerobic performance, which has been revealed in this and previous studies. This assumption is supported by the higher frequency of the *VEGF C* allele in the group of long-distance racers compared to the control. This phenomenon underlies the process of sports selection: the alleles that favor the development of endurance and the achievement of high sports results are accumulated in the subgroups of long-distance racers as their sports qualification increases.

CONCLUSIONS

Thus, the *G*-634*C* polymorphism of the *VEGF* gene is associated with physical performance of athletes and plays a key role in sports selection. The results of this research are of both fundamental and applied significance for understanding the molecular mechanisms of adaptation of the cardiovascular system to aerobic exercise, selecting an optimal sports specialization, and professional training of athletes.

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