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Search for Associations between *G/A* Polymorphism of the *EPAS1* Gene and the Maximal Oxygen Consumption in Russian Athletes

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Abstract—This study investigates associations between *G/A* polymorphism of the epithelial PAS domain protein 1 (*EPAS1*) gene (rs1867785) and the maximum rate of oxygen consumption (VO_{2max}) in male Russian athletes. The study engaged 241 male athletes from different sports; the control group of nonathletes included 92 subjects. Increased frequencies of the *AA* and *AG* genotypes of the *EPAS1* gene ($\chi^2 = 14.16$, $p = 0.03$) were found in the cohort of male athletes. The frequencies of these alleles in the subgroups with moderate (*EPAS1*A* 38.1% and *EPAS1*G* 61.9%) and high (*EPAS1*A* 41.8% and *EPAS1*G* 58.2%) VO_{2max} values significantly differed from those in the control group ($\chi^2 = 7.53$, $p = 0.006$ and $\chi^2 = 6.58$, $p = 0.01$, respectively). The higher aerobic capacities are probably associated with the presence of at least one minor *A* allele of the EPAS1 gene in the genome.

Keywords: genetics of sports, *EPAS1*, maximal oxygen consumption, athletes, athlete selection
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One of the main indicators of an athlete's functional state that determines the body aerobic potential is the maximal oxygen consumption (VO_{2max}) [1]. The search for molecular genetic markers associated with this indicator has identified several such genes; one of them is *EPAS1*. The EPAS1 protein is a part of the *hypoxia inducible factor* (*HIF*-1), a heterodimeric transcriptional complex [2]. *HIF*-1 triggers a cascade of molecular responses [3] leading to enhanced angiogenesis, erythropoiesis, some metabolic changes, which results in an increased supply of oxygen to skeletal muscles. *EPAS1* is expressed mainly in the vascular endothelial cells [4], as well as in the epithelial pulmonary cells and cardiac myocytes [5]. EPAS1 quickly degrades in the cell cytoplasm in normoxia, whereas in the case of a decrease in oxygen supply, it is stabilized and translocated into the nucleus [6]. EPAS1 controls the expression of over 100 genes, including the gene of the vascular endothelial growth factor (VEGF), as well as its receptors [7, 8].

Many researchers note that studying the *EPAS1* gene as a potential predictor is promising for successful performance in sports [9–11], in view of its effect on the processes of oxygen supply and consumption. Despite this confirmation, literature data on the effect of the *EPAS1* gene polymorphisms on successful performance in different sports are very scarce and contradictory. A study on the *EPAS1* gene haplotypes characteristic of Australian sprinters and long-distance runners has shown that the *EPAS1* gene (rs1867785) *A* allele characterizes both these groups [9]. An example of female Russian athletes has shown a decrease in the frequencies of the *GA* and *GG* genotypes in the group of long-distance runners, as well as that the *AA* genotype frequency was decreased in the group of sprinters [10]. Thus, there is an unanswered question: Which of the *EPAS1* (rs1867785) gene alleles determines an increased aerobic capacities? No studies on the associations between this polymorphism and the level have been carried out.

The aim of this research was to study the associations between the *G/A* polymorphism of the *EPAS1* gene and the VO_{2max} level in male Russian athletes.

METHODS

The investigated cohort was represented by two groups: 241 athletes representing different sports and a control group of 92 nonathletes. The age of male participants ranged from 18 to 27 years. The subjects were examined in 2009 at the Russian State University of Physical Education, Sport, Youth, and Tourism, Moscow.

The maximal aerobic capacities were determined by the test with gradually increasing treadmill loads (h/p/cosmos Saturn, Germany) only for the group of athletes. The following protocol was used for the load treadmill testing: the first stage had a speed of 2.0 m/s; subsequently, the speed was increased by 0.5 m/s every 2 min. The parameters were measured for 1 min at rest and 1 min during the recovery period. The recorded

Table 1. Mean values of the maximal oxygen consumption (VO_{2max}) for the *EPAS1* genotypes

EPAS1 genotypes	Number	VO_{2max} mL/min/kg ± standard deviation	Minimal VO_{2max} value, mL/min/kg	Maximal VO_{2max} value, mL/min/kg
GG	92	54.2 ± 5.68	38	67
AG	115	54.6 ± 6.76	38	72
AA	34	55.5 ± 5.98	43	73

parameters were averaged every 30 s. The test was performed until failure. During the test, gas exchange indices and heart rate were monitored with a Metalyzer 3B gas analyzer (Cortex, Germany). Data were accumulated and treated using the MetaSoft 3.8 software.

Venous blood samples were used to isolate genomic DNA. Venous blood (5 mL) was sampled into VACUETTE K_3EDTA anticoagulant vacuum test tubes after load tests. Genomic DNA was isolated from venous blood according to the protocol recommended by the Promega (United States). The genotype for every subject participating in the study was determined by the polymorphic system of the *EPAS1* (rs1867785) gene. The genotyping was performed using commercial test systems developed by the Postgenomic and Nanotechnology Innovations, using mini-sequencing with a subsequent detection of products using the matrix assisted laser desorption/ionization–time-of-flight (MALDI-TOF) method.

RESULTS AND DISCUSSION

Hypoxia emerging in skeletal muscles under intense physical loads assists in developing the adaptations that improve oxygen supply and consumption. Individual genetic traits that contribute to the adaptation to hypoxic exposures can thus influence the VO_{2max} level. One of the promising molecular genetic markers controlling the processes involved in oxygen supply is *G/A*, the *EPAS1* gene polymorphism. The frequency analysis in the *EPAS1* genotypes across the group of athletes and the control group of nonathletes has found statistically significant differences. An increased frequency of the *EPAS1 AA* and *AG* genotypes ($\chi^2 = 14.16$, $p = 0.03$) has been detected among the athletes. Thus, in total, the presence of the *EPAS1* minor allele characterizes the genotype of those athletes who achieved a sufficiently high level of sports qualification (candidate master of sports and higher). For example, the frequencies of *EPAS1* alleles in the group of athletes were 38.0% for *EPAS1*A* and 62.0% for *EPAS1*G* versus 25.5% for *EPAS1*A* and 74.5% for *EPAS1*G* in the group of nonathletes ($\chi^2 = 8.\ 63$, $p = 0.003$). The odds ratio was $OR = 1.784$ (95%*CI* 1.221–2.606), which confirms the advantage of the carriers of the *EPAS1* gene minor *A* allele over the *G* allele carriers. Thus, the group of athletes as a whole exhibits selection for the *EPAS1* gene minor *A* allele carriers. This result agrees with the data reported by Henderson et al. [9], which showed the presence of the *A* allele in the genotype of athletes whose competitive activity requires a predominant use of both anaerobic and aerobic metabolisms.

Analysis of associations between the *EPAS1* gene *G/A* polymorphism and the VO_{2max} value has shown no significant relationships. The average VO_{2max} values for the *EPAS1* genotypes are given in Table 1. As seen from the presented data, the mean VO_{2max} values in carriers of different *EPAS1* genotypes are actually the same. However, the carriers of two minor *A* alleles exhibited higher VO_{2max} values.

The group of athletes was subsequently divided into three subgroups according to the VO_{2max} level in the variable load treadmill test (Table 2). The intergroup differences between the frequencies of the tested genotypes were nonsignificant ($\chi^2 = 3.31$, $p = 0.51$). However, there were some trends: e.g., the frequency of *AA* genotype increased from 8.5% in athletes with low VO_{2max} to 14.0% in athletes with high aerobic capacities. The group of subjects with the best VO_{2max} values had the lowest frequency of *GG* genotype carriers. This confirm an enhanced aerobic capacities in the carriers of two minor alleles of the polymorphic system in question.

Our comparison between the frequencies of the gene alleles in question has shown the following significant differences. The allele frequencies in the subgroups with a moderate VO_{2max} (38.1% for *EPAS1*A* and 61.9% for *EPAS1*G*) and the high VO_{2max} (41.8% for *EPAS1*A* and 58.2% for *EPAS1*G*) significantly differ from those in the control group ($\chi^2 = 7.53$, $p = 0.006$ and ($\chi^2 = 6.58$, $p = 0.01$, respectively). There are no differences in allele frequencies between the control group and athletes with low VO_{2max} level (34.0% for *EPAS1*A* and 66.0% for *EPAS1*G*) ($\chi^2 = 1.81$, $p = 0.18$). Higher aerobic capacities are, probably, associated with the presence of at least one *EPAS1* gene minor *A* allele in the athlete's genome. In our view, the presented data allow us to suggest that the *EPAS1* gene *G/A* polymorphism affects the aerobic capacities of athletes. The minor *A* allele of this gene contributes to higher VO_{2max} value and better resistance to hypoxic

Table 2. The estimated distribution of the *EPAS1* genotypes in the tested subgroups by the VO_{2max} level

VO_{2max}	*EPAS1* genotypes			In total
	*EPAS1*AA*	*EPAS1*AG*	*EPAS1*GG*	
Low (35–49 mL/min/kg)	4	24	19	47
Moderate (50–59 mL/min/kg)	24	67	60	151
High (60–75 mL/min/kg)	6	24	13	43
In total for athletes	34	115	92	241

exposures, which can be used in predicting a particular athlete's aerobic capacities. The ratio of the chances for the subgroup of athletes with a high VO_{2max} value, compared to the control group, accounted for 2.099 (*95%CI* 1.221–3.607). Thus, the increased frequency of the *A* allele doubles the chances achieving high VO_{2max} values.

The *EPAS1* gene was identified during a search for genomic regions associated with the VO_{2max} level in nonathletes [12]. The protein product of the *EPAS1* gene realizes a complex control over the processes of adaptation to hypoxic conditions (angiogenesis and remodeling of capillary network, erythropoiesis, hemoglobin concentration, and hematocrit level) [13] and participates in the regulation of catecholamine level [14] and the heart development. The *EPAS1* knockout mouse is characteristic of multiple visceral pathologies and weakened response to oxidative stress [15]. Therefore, the polymorphisms influencing, in any way, the expression of this gene produce a multiple effect on all processes controlled by the EPAS1 protein. The preferable selection of the *EPAS1* gene *A* allele carriers is determined not only by more efficient processes of angiogenesis and erythropoiesis, but also by specific metabolic shifts in skeletal muscles [16, 17]. For example, one of the metabolic adaptations to hypoxia is enhanced glycolysis against a background of a decrease in the oxidative processes in mitochondria [18, 19].

Summing up the discussion, we can conclude that EPAS1 affects the pathway effectively switching the body from a predominant use of the aerobic energy consumption pathway required for long-term exercises to a predominant use of glycolysis that is effective for short-term intense energy outbursts [2, 20]. The investigated *G/A* polymorphism is the *EPAS1* gene intron fragment, which does not affect the amino acid sequence of its protein product. In view of this, it is difficult to conclude about the particular molecular mechanism responsible for the effect of this genetic marker on specific physiological features in athletes. The minor *A* allele carriers may more effectively use capacities of different body systems receiving more oxygen supply. Undoubtedly, the development of aerobic capacities is controlled by a number of genes and their polymorphisms. Therefore, it is expedient to continue studies on associations between other known *EPAS1* gene polymorphisms, as well as other genes, and VO_{2max} values in athletes of different age and gender groups.

CONCLUSIONS

This study has shown genetic selection among athletes representing different sports. Specifically, the frequency of the minor *A* allele in the polymorphic *EPAS1* gene system is higher in the group of athletes, compared with the control group of nonathletes. Furthermore, an increase in the frequencies of the *A* allele and the *AA* genotype correspond to an increase in the aerobic capacities. The data have confirmed an advantage of athletes who are *EPAS1 A* allele carriers over the initial *G* allele carriers. The *EPAS1* gene *G/A* polymorphism can be used when selecting, profiling, and predicting aerobic power in male athletes.

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