Influence of motor activity and polymorphism I/D of ACE on the affinity of oxygen for hemoglobin

Albina Dautova[1*], *Evgenia* Khazhieva[2], *Ekaterina* Isaeva[3], *Ilmira* Khabibulinna[3] and *Valentina* Shamratova[3]

[1]Volga Region State University of Physical Culture, Sport and Tourism, Kazan, Republic of Tatarstan, Russian Federation
[2]Republican Clinical Infectious Diseases Hospital, Ufa, Republic of Bashkortostan, Russian Federation
[3]Bashkir State Medical University, Ufa, Republic of Bashkortostan, Russian Federation

Abstract. The influence of rs4646994 polymorphism of the *ACE* gene on the affinity of oxygen for hemoglobin among young men with different levels of physical activity has been studied. 245 young men aged 20-22 years were included in the study. All young men were divided into three groups depending on their motor activity: low (LMA), average (AMA) and high (HMA). $SatO_2$, pO_2, pCO_2, p50 and HbO_2 were analyzed in capillary blood of all examined young men. It was found out that I/I genotype of the *ACE* gene is associated with a decrease in the affinity of oxygen for hemoglobin both in LMA (p=0.022) and in HMA (p=0.000096). The intensification of physical activity among I/D and D/D genotypes is accompanied by an increase in the level of hemoglobin oxygenation in blood, while the I/I genotype with part of HbO_2 does not change depending on motor activity. These features can be explained by the shift of the oxygen dissociation curve to the left among young men with the *D allele genotype, with an increase in physical activity. On the contrary, the I/I genotype of the *ACE* gene have efficient oxygen extraction to tissues, regardless of the level of motor activity compared to the D/D genotype.

1 Introduction

Ensuring energy consumption while physical activities requires the body to mobilize the body's oxygen transport system (OTS). Muscular work can be achieved by various mechanisms: increase of cardiac activity, increase in blood oxygen capacity, increase in the functional activity of the external respiration system [1, 2]. However, increase in oxygen transport to tissues due to a more intensive work of the heart is not always the best solution due to high energy costs but increase in the number of erythrocytes, despite increase in blood oxygen capacity weakens the overall effect of oxygen transport due to a deterioration of rheological situation in the microvasculature [3].

The most recognized adaptive mechanism of modulation of O_2 transport to tissues is considered to be a change in the affinity of hemoglobin to oxygen (in particular, due to

*Corresponding author: dautova.az@mail.ru

Bohr Effect) [4]. So, it is known that in the animal world the genetic determinants of adaptation to different oxygen content realized by varying p50, which corresponds to the O_2 voltage at 50% blood desaturation [4]. Scanning the human genome made it possible to identify the genes responsible for the adaptation of the body's OTS to hypoxia [5]. Thus, the population living in high mountain regions demonstrates unique physiological mechanisms of adaptation to life at high altitude which predetermines interest in the study of genetic markers responsible for these adaptive mechanisms [6, 7].

One of these markers is insertion/deletion (I/D) polymorphism of the angiotensin I-converting enzyme (*ACE*) gene [8, 9]. In a study conducted by David R. Woods with other researches, it was found out that during a rapid ascent to a height (5000 m), climbers with the I/I genotype had a more stable $SatO_2$ value during the ascent than those with the (D/D) genotype. But at the same time during a slow rise to a height, statistically significant differences in $SatO_2$ depending on the I/D *ACE* polymorphism were not found [8].

It is also well known that the I/D polymorphism of the *ACE* gene is a marker of physical performance associated with maximum oxygen consumption (VO_{2max}) [10] and other indicators characterizing such physical qualities as endurance and strength [10. 11]. But at the same time, the question of the influence of I/D polymorphism on the state of the gasotransport function of blood, the hemoglobin profile, and the affinity of hemoglobin to O_2 remains unexplored. Given the above, we suggested that the I/D polymorphism of the *ACE* gene may be associated with the degree of hemoglobin oxygenation and its affinity for O_2. Therefore, the aim of this study was to assess the effect of physical activity of young men and I/D polymorphism of the *ACE* gene on the degree of oxygenation of hemoglobin and its affinity for O_2.

2 Materials and Methods

The study involved 245 young men aged 21±2 years, clinically healthy according to the results of the annual dispensary examination. All participants in the study were informed about the tasks and methods used and gave voluntary written consent to participate in the experiment. The study was conducted in compliance with the Declaration of Helsinki. Based on the survey, we obtained information about the motor activity of students (the volume, nature, intensity and frequency of physical activity both in sports activities and in everyday life). All young men were divided into three groups depending on the level of motor activity. The first group with low physical activity consisted of full-time students (n=151), who according to the questionnaire devoted less than 150 minutes per week to aerobics, swimming or average-intensity walking. The second group (n=44) included young men who spend 150-300 minutes a week on physical activity. The students of this group were engaged in swimming, gymnastics, dancing, running and other activities that require physical effort. The level of their motor activity is characterized as avarage. The third group (n=50) included athletes who at the time of the survey had qualifications of at least 1 adult category in athletics; in this group, training took place 3-4 times a week for 2 hours, which is 360-480 minutes per week. Therefore, their motor activity can be assessed as high.

"RAPIDlab 865" examined capillary blood of all the probationers taking into account such indicators of the oxygen regime as partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), oxygen saturation ($SatO_2$) and parameters hemoglobin blood profile as content of oxygenated Hb (HbO_2) carboxyhemoglobin - (COHb) and O_2 tension at 50% blood desaturation (p50).

DNA isolated from blood leukocytes by phenol-chloroform extraction was used for genetic analysis. The method for determining the *ACE* gene polymorphism consisted in the amplification of specific DNA fragments (polymerase chain reaction) using specific oligonucleotides (sense oligo, 5' CTGGAGACCACTCCCATCCTTTCT 3' and antisense

oligo, 5' GATGTGGCCATCACATTCGTCAGA 3') (Synthol, Russia)). Polymerase chain reaction was performed on a Tertsik thermal cycler (OOO DNA Technology, Moscow). The results of amplification were evaluated by vertical electrophoresis in 7% polyacrylamide gel.

Statistical analysis of the results was carried out using the program Statistica (version 10). The studied samples were tested for the normality of the distribution of quantitative indicators using the Shapiro-Wilk test as well as for the equality of the variances of the studied trait using the Levene test. A multivariate analysis of variance (Factorial ANOVA) was carried out to identify individual as well as combined influence of factors. To identify statistical differences between groups, post hoc comparisons were made using Student's t-test, calculating a new level of critical significance to control for type 1 error. Numerical values in the text are presented as mean (M) and standard error of the mean (m).

3 Results

Dispersion and comparative analysis of hemoglobin profile and blood gases revealed some physiological features in the functioning of the gas transport link among young men with different levels of motor activity depending on the marker of the *ACE* gene. It has been shown that the I/D polymorphic variant of the *ACE* gene affects the hemoglobin profile HbO_2, in combination with motor activity, the partial pressure of oxygen (pO_2). The intensity of motor activity affects pO_2 and pCO_2, the content of HbO_2 and COHb, $SatO_2$, hemoglobin affinity for oxygen (p50) (Table 1).

Table1. Effect of motor activity and the polymorphic variant I/D of the *ACE* gene on hemoglobin oxygenation and its affinity for O_2 according to ANOVA, ($p<0.05$)

Indicator	**Factors**		**Joint influence of factors**
	F1	F2	F1F2
HbO_2, %	0.04	0.00003	
$SatO_2$, %		0.008	
COHb, %			
pCO_2, mmHg		0.01	
pO_2, mmHg		0.001	0.002
p50, mmHg		0.001	

The influence of physical activity is clearly manifested in the I/D genotype. A comparison of the average pO_2 values among young men with different motor activity made it possible to reveal increase in the indicator as the intensity of motor activity increased. Athletes with the I/D genotype, arterial blood oxygen tension had higher (96.2±2.4 mmHg) in comparison with the same genotype of low motor activity (73.9±1.4mmHg) and average motor activity (83.1±2.5 mmHg) ($p=0.0013$ and $p=8.36385E-07$, respectively). Comparison of mean values at the same motor activity level demonstrated lower pO_2 values of high motor activity in the I/I genotype than among individuals with *D allele (I/I: 79.3±5.8 mmHg, I/D: 96.2±2,4 mmHg) ($p<0.05$).

Mean HbO_2 values at different motor activity levels also differed significantly depending on the I/D (*ACE*) polymorphic variant. Thus, the level of HbO_2 in the blood is higher among young men with the D/D genotype (94.3±0.4 %) with hypodynamia, in

relation to I/D of low motor activity (92.9±0.3 %) (p=0.017), with high motor activity – among individuals who also have the *D allele in the genotype (I/D genotype), HbO_2 is higher in comparison with the I/I genotype (I/D: 96.3±0.3 %, I/I: 92.8±1.9 %) (p=0.0003). Thus, the intensification of physical activity of the I/D and the D/D genotypes is accompanied by a statistically significant increase in the level of oxyhemoglobin to the blood, while among young men with the I/I genotype, the proportion of HbO_2 does not change depending on motor activity.

When comparing the mean group values of $SatO_2$ the same holds true to the dynamics of HbO_2. To the greatest extent, the increase of physical activity affects $SatO_2$ as well as HbO_2 of the I/D genotype: with high motor activity, the highest values of the indicator (97.34±0.2 %) are marked in relation to the I/I genotype (95.3± 0.9 %) (p=0.00014). At the same time, low motor activity of the I/D genotype $SatO_2$ was 94.5±0.3 %, which is lower than among representatives of the D/D genotype – 95.5±0.2% (p=0.033), $SatO_2$ in the group of students with low motor activity of the D/D genotype and I/I genotype had no differences. When comparing the groups of young men experiencing average and high physical activity, it can be seen that the D/D and I/D genotypes have higher $SatO_2$ values (p=0.0005).

p50 is considered to be the most stable and independent of all the parameters characterizing the blood oxygen delivery system, the pO_2 value at which 50% of hemoglobin is saturated with O_2. A lower p50 corresponds to a higher Hb-O_2 binding affinity or a "left-shifted" O_2 dissociation curve. On the other hand, a higher p50 corresponds to a lower Hb-O_2 binding affinity and a "right-shifted" O_2 dissociation curve. According to the results of our data the I/I genotype of the *ACE* gene is associated with a decrease in the affinity of hemoglobin for oxygen, both due to physical inactivity and among athletes. Thus, p50 tended to increase with the I/I genotype compared to the D/D genotype, as in the low motor activity group (D/D: 25.4±0.5 mmHg, I/I: 27.4±1.7 mmHg, p=0.022) and high motor activity (D/D: 25.3±0.7 mmHg, I/I: 28.0±1.76 mmHg, p=0.000096)..

We also found out that among young men with the I/I genotype, with a high level of physical activity, the pCO_2 value is higher compared to the D/D and I/D genotypes (D/D: 39.6±1.7 mmHg, I/D: 40.6±1 mmHg and I/I: 45.7±1.8 mmHg) (p=0.03). As for the *D allele (D/D and I/D genotypes), thus increase of motor activity level does not lead to increase in the formation of pCO_2.

4 Discussion

During exercise the increased demand for oxygen is met by increasing muscle blood flow [12] and by improved O_2 unloading from Hb achieved by decreasing Hb-O_2 affinity [13].

Currently, there is ongoing debate about the advantages of higher or lower hemoglobin-oxygen (Hb-O_2) affinity in humans, particularly during hypoxia [14]. A decrease in Hb-O_2 affinity is often observed among humans during acclimatization to altitudes ranging from 2500 to 4500 m, presumably to facilitate O_2 off-loading and protect against tissue hypoxia [15]. For example, evidence suggests that some groups of humans native to high altitude have a greater Hb-O_2 affinity than sea-level residents [16, 17].

According to the results of this study the I/I (*ACE*) genotype makes the hemoglobin affinity for oxygen lower, both among young men with low motor activity levels and among athletes compared to the D/D genotype.

Changes in Hb-O_2 affinity throughout the vasculature optimize both O_2 loading in the lungs and O_2 off-loading to peripheral tissue. For example, byproducts of metabolism (increased temperature, increased CO_2, and lower pH) contribute to a localized decrease in Hb-O_2 affinity in exercising muscle, thereby promoting O_2 off-loading and utilization [3,

18]. Trained individuals have a higher Bohr effect at low SO_2 probably due to elevated 2,3-DPG, which might cause an even greater increase in the arterio-to-venous O_2 difference. The elevated 2,3-DPG in trained individuals might be a consequence of the stimulated erythropoiesis, which decreases red blood cell age. Young red blood cells have an increased metabolic activity, higher 2,3-DPG, and a lower Hb-O_2-affibity than senescent red blood cells [19].

Additionally, a greater accumulation of metabolic byproducts (e.g., lactate and H^+) during high-intensity exercise have been reported in humans with high Hb-O_2 affinity compared to those with normal Hb-O_2 affinity.

At the same time, the study of the association of the I/D polymorphism of the *ACE* gene with the concentration of lactic acid in blood of athletes did not reveal a significant difference between the groups of three types of the genotypes ($p > 0.05$) [20].

Evidence for compromised O_2 off-loading may be seen through compensatory increases in hematocrit resulting in a higher O_2 carrying capacity per unit of blood [21, 22].

In addition to an elevated hematocrit humans with high Hb-O_2 affinity likely develop skeletal muscle adaptations to compromised O_2 off-loading such as a greater percentage of non-oxidative (type II) muscle fibers than their counterparts with normal Hb-O_2 affinity [23]. This assumption is confirmed by the data on the predominance of fast muscle fibers among athletes with the DD genotype, while individuals with the II genotype have predominantly “slow” (oxidative) fibers [24].

Humans with high Hb-O_2 affinity showed smaller increases in erythropoietin production when residing at high altitude [25]. A lesser erythropoietin production during high-altitude acclimatization suggests that O_2 delivery is better preserved among humans with high Hb-O_2 affinity. Assessment of the level of erythropoietin among athletes after staying at a moderate altitude (2200 m) for 48 hours did not reveal a relationship between the D/D genotype and hypoxia-induced erythropoietic response. Changes in erythropoietin levels did not differ depending on the *ACE* gene polymorphism, the genotypes (DD and ID / II) showed a significant increase in erythropoietin levels, as well as hemoglobin concentrations after exposure to moderate altitude [26].

Short-term periods of hypoxia observed during intense physical activity require adjustment of both the cardiovascular and respiratory systems to maintain adequate delivery of O_2 to the cells and tissues of the body [27, 28]. One crucial immediate adjustment in response to hypoxia is increased ventilation which raises alveolar ventilation, increases arterial pO_2 and protects against arterial O_2 desaturation. At a given alveolar pO_2, humans with high Hb-O_2 affinity have similar minute ventilation compared to humans with normal Hb-O_2 affinity. Yet, due to the left-shift denature of their oxygen dissociation curve, those with hi Hb-O_2 affinity have higher arterial O_2 saturation at given alveolar pO_2 [29].

According to scientific sources high VO_{2max} of athletes with the I/I genotype is associated with lower pulmonary ventilation during high-intensity physical activity, which indicates a more economical function of external respiration and a rational way to adapt the respiratory and cardiovascular system to long-term muscular work [30].

This is confirmed by the results of our study: male athletes with the I/I genotype are characterized by lower arterial blood oxygen tension (pO_2) compared to trained individuals with the *D allele in their genotype (I/D genotype).

Thus, the intensification of physical activity of the I/D and D/D genotypes is accompanied by increase in the level of oxyhemoglobin in the blood, while among young men with the I/I genotype the content of HbO_2 does not change depending on motor activity. These features can be explained by the shift of the oxygen dissociation curve to the left among young men who have the *D allele in their genotype since people with a high affinity of Hb for O_2 have a higher arterial O_2 saturation. However, excessive ventilation at the same time increases the O_2 consumption by the respiratory muscles [29]. The I/I

genotype of the *ACE* gene, on the contrary, have an effective extraction of oxygen to the tissues regardless of the level of motor activity compared to individuals with the D/D genotype of the *ACE* gene which possibly causes high aerobic capacity in their carriers.

In its turn increase in the rate of consumption of O_2 by cells leads to an increase in CO_2 release which we demonstrated as increase in the level of motor activity among individuals with the I/I (*ACE*) genotype. So, the proportion of CO_2 increases and pO_2 decreases in the alveoli, the level of oxygenation of blood in the capillaries of the lungs decreases, and $SatO_2$ decreases.

5 Conclusion

Taking into consideration that the aerobic needs of the body are determined by the *ACE* gene of the I/I genotype [31], the lower blood levels of pO_2, HbO_2 and $SatO_2$ that we have identified in individuals with the I/I genotype may indicate at increased oxygen utilization by muscles and be a consequence of a more effective regulation of the affinity of Hb to O_2 under conditions of emerging hypoxia. It should be noted that despite the differences in the oxygen supply to tissues are genetically determined, the phenotypic manifestation is observed only with high motor activity. The cell's need for O_2 which increases during aerobic activity is obviously realized due to the action of a rather mobile mechanism aimed at overcoming oxygen deficiency in cells.

The results of the study expand the understanding of the mechanisms of oxygen supply depending on genetic predisposition, and data on the association of the adaptive capabilities of the oxygen transport system with I/D polymorphism of the *ACE* gene can be taken into account when choosing a sports specialization.

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