

DEPENDENCE OF AEROBIC PERFORMANCE OF ATHLETES ON POLYMORPHISM OF GENES

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^A Study Design; ^B Data Collection; ^C Statistical Analysis; ^D Manuscript Preparation; ^E Funds Collection

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Abstract. The adaptation of an athlete to systematic physical exercise has been shown to be determined by a combination of great many genes. The aim of our study was to investigate the dependence of the aerobic capacity parameters in sport on the set of gene polymorphisms. Cardio-respiratory system (CRS) adaptation reactions to exercise of 72 endurance athletes were assessed using the gas analysis. The analysis of the obtained results has shown both single and combined effect of the gene polymorphisms on the aerobic capacity. The impact of 6 polymorphisms on the aerobic performance level was analyzed: T⁻⁷⁸⁶→C polymorphism of the promoter of eNOS gene as well as ACE I/D polymorphism, Pro/Ala polymorphism of PPARG gene, G/C polymorphism of PPARA gene, Pro582Ser polymorphism of HIF1 α gene, and Ala203Pro polymorphism of PPARGC1B. It was found that a single impact on the HR_{max} providing ACE I/D polymorphism. Individual influence of ACE gene accounts for 2% of this index dissipation. Results showed that there is a dependence between the amount the maximum volume of consumed oxygen (VO₂max) from the set of gene polymorphisms. Cumulative impact of these polymorphisms in the combination with the individual parameters (gender; qualification; kind of sport) stipulates 71% of dispersion of VO₂max value.

Key words: sport selection, molecular-genetic markers, aerobic performance, gene polymorphisms

Introduction

Introduction The current level of sports results reaches the limits of physiological capabilities of a human. World record results can be attained only when the individual sports abilities are combined with the correctly managed pedagogical process, which, in its turn, puts forward the process of the search of the genetically gifted athletes (Platonov 2005).

In kind of sports, where competitive activities are mainly based on aerobic mechanisms of ATP synthesis, sports results depend on the physical aerobic capacities, the measure of which is the scope, intensity, and maximum period of time required for work performance (Mishchenko and Suchanowsky 2010).

The generally accepted criterion for assessment of cardiorespiratory system productivity (CRS) and maximum aerobic performance of the organism applicable for assessment of the level of athletes physical capabilities is the amount of maximum consumed O_2 (VO_{2max}). Direct or indirect dependence of sports results on the type of aerobic metabolism and maximum aerobic energy capacity is typical for the majority of kinds of sports, so, in assessing the level of functional capacities of the athletes' organism testing of the maximum aerobic performance is of major importance. The general element of tests intended to determine maximum aerobic capacity is based on the necessity to attain the level of O_2 consumption under physical loads with the incrementally growing intensity lasting for 12–16 minutes till the moment when the maximum VO_{2max} is achieved.

Dependence of a person's aerobic capacity level on the hereditary traits was discovered in the 70–80s of the last century. The adaptation of an athlete to systematic physical exercise has been shown to depend on an individual's inherited properties (Bouchard et al. 1999). These properties are determined by a combination of great many genes polymorphisms (Williams and Folland 2008; Bray et al. 2009; Timmons et al. 2010).

Rapid progress in the development of methods for the molecular genetics in sports for the last 12 years allows us to identify the genotype of a person with high aerobic performance (Rankinen et al. 2010). Recent research helped to find out that the increase in VO_{2max} in the course of a 20-week training program by 47% depends on the hereditary traits (Bouchard et al. 2010). Based on the today's knowledge, aerobic capacities are determined by the combination of a great number of gene polymorphisms. As of today, the genetic map of physical activity of a person contains more than 200 genes, polymorphisms of which are associated with the development and demonstration of physical qualities, as well as small morpho-functional and biochemical characteristics that change under various physical loads (Bray et al. 2009). By today, there is no exact answer to the question as to the number of polymorphisms that have direct effect on demonstration of stamina under intensive physical loads and on aerobic performance of athletes and that are required for diagnostics of aerobic capacity of athletes. A number of authors suggests that the model containing 11 polymorphisms that account for 23% of differences in the growth of VO_{2max} demonstrated by volunteers subjected to endurance training (Timmons et al. 2010). Research conducted under the program "Heritage Family Study" has revealed that 39 polymorphisms are associated with the growth of VO_{2max} , 21 of which account for 49% maximum oxygen consumption variability in the course of the training process (Bouchard et al. 2010). People who had 9 favorable alleles of VO_{2max} polymorphisms have improved by $221 \text{ ml} \cdot \text{min}^{-1}$, and in case of people who had more than 19 favorable alleles, the growth of maximum oxygen consumed had an average value of $604 \text{ ml} \cdot \text{min}^{-1}$.

The conducted analysis of scientific reference literature has allowed the following polymorphisms be attributed to genetic markers that determined aerobic capacity of athletes: the angiotensin I-converting enzyme (ACE) insertion/deletion (I/D) gene polymorphisms (Puthuchearry et al. 2011), T-786C gene polymorphism of endothelial NO-synthase (eNOS) (Gómez-Gallego et al. 2009), Pro/Ala gene polymorphism of the peroxisome proliferator-activated receptor γ (PPARG) (Ahmetov et al. 2009), intron7 G/C polymorphism of the peroxisome proliferator-activated receptor α gene (PPARA) (Ahmetov et al. 2006; Eynon et al. 2010), Pro582Ser (C/T) gene polymorphism of the hypoxia- inducible factor-1 α (HIF1A) (Döring et al. 2010), Ala203Pro gene polymorphism of the peroxisome

proliferator-activated receptor gamma coactivator 1 β (PPARGC1B) (Ahmetov et al. 2009). There is a need for elaboration of recommendations on usage of molecular and genetic markers in particular sports. In our research we investigated the dependence of the aerobic capacity parameters in sport on the gene polymorphisms. The impact of 6 polymorphisms on the aerobic performance level was analyzed. Cumulative impact of these polymorphisms in the combination with the individual parameters (gender; qualification; kind of sport) stipulates 71% of dispersion of $VO_2\text{max}$ value.

Methods

Maximal oxygen consumption of 72 endurance athletes was determined. There were 23 athletes classified as "elite", 33 athletes were classified as "sub-elite", 16 athletes were classified as "non-elite".

This study was pre-approved by the Ukrainian National Academy Of Sciences Bogomoletz Institute of Physiology Biomedical Ethics Committee, Kiev, Ukraine, and all subjects were fully informed of the risk and benefits involved in participation before providing their written consent to participate.

Experimental base of the laboratory for "Theories and methodics of sport preparation and reserve capacities of athletes" of the NDI NUFVSU was used to study indicators of athletes's physical capabilities and reaction of the cardiorespiratory system (CRS), the degree of acidimetric shifts under boundary (maximum) and standard physical loads that allow to determine aerobic capacity of an organism.

Endurance of aerobic mechanisms of energy supply for physical exercises was characterized by the maximum aerobic endurance – maximum level of oxygen consumption ($VO_2\text{max}$) and capacity of loading (W_{max}) under test loads with incremental endurance lasting for 14–20 minutes till the moment of "refusal to work", as well as intensity of loading at the level of anaerobic threshold (W_{Thr}). Treadmill "Laufband" (Germany) and rowing ergometer Concept – II (USA) were used for loading. Tests were conducted after one day of rest by applying a standard dietary pattern and water-intake regime. Athletes were informed on the test program and gave their permission to conduct such tests.

Nonstop measurements of gas exchange and CRS reaction to physical loads were conducted on a real time basis (breath by breath) by applying the ergospirometric complex "MetaMax3B" (Cortex, Germany). Lung ventilation (V_E), respiratory frequency (f_T), respiratory capacity (V_T), concentration of CO_2 and O_2 in expiratory ($F_{E}O_2$, $F_{E}CO_2$) and alveolar air (F_AO_2 , F_ACO_2), O_2 (VO_2), CO_2 generation (VCO_2), gas exchange ratio ($RQ = VCO_2/VO_2$), ventilation equivalents for O_2 ($EQO_2 = V_E/VO_2^{-1}$) and for CO_2 ($EQCO_2 = V_E/VCO_2^{-1}$), oxygen pulse ($O_{2\text{-pulse}} = VO_2/HR$). Taking into account the fact that measurements were taken in the open system, external respiration indices were reduced to BTPS conditions, and gas exchange – to STPD conditions. Registration of exercise heart rate (HR, beats per minute $^{-1}$) was conducted by applying "Sport Tester Polar" (Finland).

DNA preparation and SNP analysis. Genomic DNA was isolated from oral epithelial cells following a standard protocol according to the manufacturers instructions (DiatomTM DNA Prep (Biokom, Russia)). The T⁻⁷⁸⁶→C polymorphism of the promoter of eNOS gene as well as I/D polymorphism of ACE gene, Pro/Ala polymorphism of PPARG gene, G/C polymorphism of PPARA gene, Pro582Ser polymorphism of HIF1 α gene, and Ala203Pro polymorphism of PPARGC1B gene were identified using the method of polymerase chain reaction (PCR), with a subsequent analysis of the restriction length fragments.

Statistical analysis of research results was carried out by applying the SPSS ver.17.0 software package.

Data regarding gas exchange indexes were analyzed for normal distribution with the help of Shapiro-Wilk test. Homogeneity of variances were analyzed with Levine test with the following application of analysis of variance (ANOVA). In case of heterogeneity Brown-Forsythe test was used instead.

To discover functional links between polymorphisms of genes and gas analysis indices, multiple regression analysis method was applied with the resulting linear polynomial curve models with regard to independent parameters.

Results

Influence of 6 polymorphisms on the level of aerobic performance was analyzed with the resulting linear polynomial curve models with regard to independent parameters.

Analysis of the structure of the constructed regression equations by taking into account the models multicollinearity allows to make the following conclusions. Model that determines amounts of $VO_2\text{max}$ in terms of the body weight depending on polymorphisms of genes-candidates is composed of 17 regressors. The dissipation rate that is explained by this model equals 0.71. Statistically significant influence on the level of $VO_2\text{max}$ depends on the athletes' gender (23.36%) and kind of sports (15.76%).

The above facts are well-known in muscular activity physiology and are easily explainable. The remaining 60.9% are contributed by factors that are represented by the variety of gene polymorphisms combination.

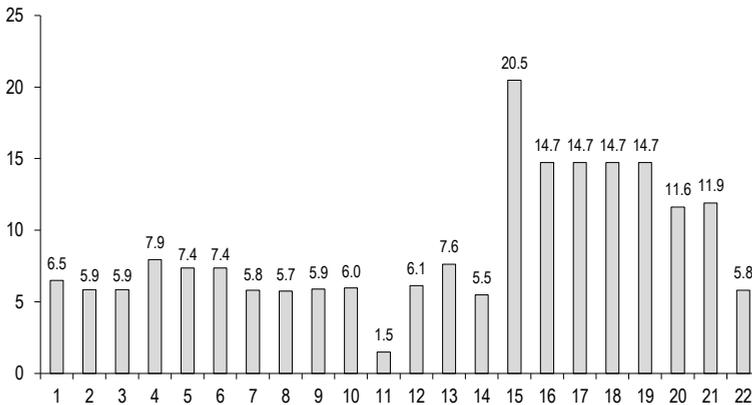
Presence of T/T genotype with regard to T/C polymorphisms of eNOS genes in combination with high qualification of a sportsman results in high values of $VO_2\text{max}$.

Combinations of polymorphisms of PPARA and PPARG, ACE and PPARA genes explain the same degree of index value dissipation ($\approx 6\%$). Individual influence of ACE gene accounts for 2% of this index dissipation.

To avoid the influence of gender, kind of sports, and sporting qualification factors on the CRS reaction characteristics under physical loads, groups of athletes belonging to the same gender (feminine) and the same event (rowing), as well as of the same age and the same sporting qualification (sub-elite) were formed. These sportswomen were subjected to multiple tests (from 6 to 14 times each) in the course of three years. As a result of such selection, this group demonstrated frequent changes in gene polymorphisms occurrence. The selection became homogeneous with reference to polymorphisms of PPARG, PPARA, and PPARGC1B genes. Based on examination results, regressives model was constructed and it demonstrates the relation between polymorphisms of genes and the level of $VO_2\text{max}$. The share of dissipation explained by the model equals 41%. Statistically probable influence on the relative value of maximum oxygen consumption was demonstrated by the following indices: 1) T/C polymorphisms of eNOS gene promoter. Individual influence of this factor explains 35% of $VO_2\text{max}$ dissipation with regard to the body weight with reference to this selection; 2) I/D polymorphisms of ACE gene in combination with the characteristics of the functional state at the time of the sportsman's training accounts for 5% of the index dissipation. This model proves that polymorphic variant of eNOS gene has a more significant effect on the amount of the consumed oxygen than on the functional state of the sportsman, and does not depend on the period and micro-cycles of preparation test conducted.

Stability of CRS reaction characteristics was accessed by applying the coefficient of variation (V) that is one of the dissipation factors that allows to compare various indices. Variability analysis of basic cardiorespiratory system reaction characteristics of sportswomen (Figure 1) specializing in rowing allows to assert that the more stable index is exercise heart rate at maximum levels of VO_2 , by taking into account the fact that tests have been conducted

several times. Exercise heart rate coefficient (V_{HR}) equals 1.5%. With reference to the mathematical statistics on sports indices, the coefficients of variation of which do not exceed 10–15% indicate stable homogeneous values, so the following indices are regarded as stable: gas exchange rate – VCO_2/VO_2 , ($V = 5.5\%$), relative level of maximum oxygen uptake ($VO_{2max} \cdot kg^{-1}$, $V = 5.7\%$) and absolute level of maximal oxygen uptake (VO_{2max} , $V = 5.8\%$), exercise heart rate (HR) at the level of anaerobic metabolism threshold (HR_{Th} , $V = 5.8\%$), maximum work performance intensity (W_{max} , $V = 5.9\%$, $W_{max} \cdot kg^{-1}$, $V = 5.9\%$), maximum level of CO_2 (VCO_{2max} , $V = 5.9\%$, $VCO_{2max} \cdot kg^{-1}$, $V = 6.0\%$), oxygen effect of cardiac cycle (“ O_2 -pulse” or VO_2/HR , $V = 6.1\%$); time required to attain VO_{2max} (t_{min} , $V = 6.5\%$), maximum level of lung ventilation ($V_{E_{max}}$ and $V_{E_{max}} \cdot kg^{-1}$, $V = 7.4\%$), ventilation equivalent to O_2 (EQO_2 , $V = 7.6$), respiratory frequency at the level of VO_{2max} (f_T , $V = 7.9\%$).



1 – t_{min} ; 2 – W_{max} ; 3 – $W_{max} \cdot kg^{-1}$; 4 – f_T ; 5 – $V_{E_{max}}$; 6 – $V_{E_{max}} \cdot kg^{-1}$; 7 – VO_{2max} ; 8 – $VO_{2max} \cdot kg^{-1}$; 9 – VCO_{2max} ; 10 – $VCO_{2max} \cdot kg^{-1}$; 11 – $HR \cdot VO_{2max}^{-1}$; 12 – $V \cdot O_2 \cdot HR^{-1}$; 13 – EQO_2 ; 14 – $V \cdot CO_2 / V \cdot O_{2load}$; 15 – t_{Th} ; 16 – W_{Th} ; 17 – $W_{Th} \cdot Bt \cdot kg^{-1}$; 18 – $V_{E_{Th}}$, $l \cdot min^{-1}$; 19 – $V_{E_{Th}} \cdot kg^{-1}$; 20 – VO_{2Th} ; 21 – $VO_{2Th} \cdot kg^{-1}$; 22 – HR_{Th} .

Figure 1. Variation of coefficient values of cardiorespiratory system reaction characteristics at multiple tests of sportswomen specializing in rowing (%)

The most stable parameters of the cardio-respiratory system reactions of the athletes in the repeated testing included: HR_{max} ($V = 1.5\%$), VCO_2/VO_2 , ($V = 5.5\%$), $VO_{2max} \cdot kg^{-1}$ ($V = 5.7\%$), VO_{2max} ($V = 5.8\%$), HR_{Th} ($V = 5.8\%$), W_{max} and $W_{max} \cdot kg^{-1}$ ($V = 5.9$).

We presume that the specified CRS reaction characteristics to physical loads of aerobic nature are, in a greater degree, determined by genetics when compared with other CRS reaction characteristics that have higher variability. Almost all cardiorespiratory system reaction characteristics registered at the level of anaerobic metabolism threshold, except HR_{Th} , are characterized by high variability, i.e. they depend on the state the sportsman is in at the time of the test, as well as on the level of fitness.

Construction of multiple regressive models for other aerobic possibilities for athletes allows to discover that interaction between polymorphisms of PPARG and eNOS genes statistically, very likely, has an effect on the level of lung ventilation ($V_{E_{max}}$) ($p = 0.040$); which demonstrates responsiveness of the athlete’s cardiorespiratory system to physical loads. Another model proves that polymorphisms of eNOS gene have an effect on the oxygen

ventilation equivalent (EQO_2) ($P = 0.046$); which demonstrate efficiency of lung ventilation for O_2 utilization from the air (Figure 2).

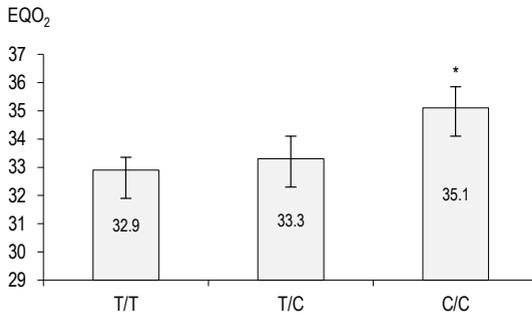


Figure 2. Dependence of ventilation equivalent value of oxygen on polymorphism of eNOS gene: T/T – athletes with genotype T/T, T/C – athletes with genotype T/C, C/C – athletes with genotype C/C, * – statistical reliability of differences between T/T and C/C – genotypes at level of $P < 0.05$

To understand separate influence of gene polymorphisms on CRS reaction under test loads, single-factor dispersion analysis method was applied. This method helped to determine that polymorphisms of ACE gene, probably, have an effect on the efficiency of lung ventilation for utilization of O_2 demonstrated by the value of oxygen ventilation equivalent (EQO_2) while performing under incrementally increasing loads ($p = 0.020$). The highest values of EQO_2 that demonstrate the least efficiency of lung ventilation for utilization of O_2 were typical for athletes with I/I genotype. The average-team index of athletes with I/I genotype was exceeding the same index of the group with I/D genotype by 11.5%. No difference was discovered in values of oxygen ventilation equivalent in groups of athletes with I/D and D/D genotypes.

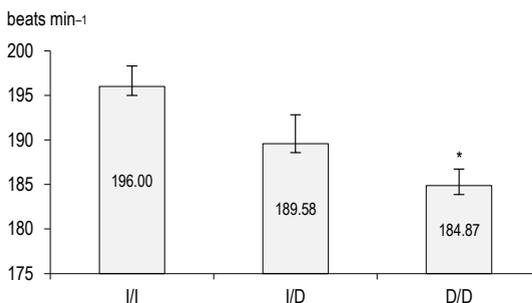


Figure 3. Heart rate of athletes with different genotype of I/D ACE gene polymorphism: I/I – athletes with genotype I/I, I/D – athletes with genotype I/D, D/D – athletes with genotype D/D, * – statistical reliability of differences between I/I and D/D – genotypes at level of $P < 0.05$

Besides, it was discovered that the factor of I/D polymorphism, very likely, has an effect on the value of HR_{max} that is believed to characterize the aerobic capacity ($p = 0.029$) (Figure 3). The highest level of HR_{max} under hard physical work was demonstrated by qualified athletes with I/I genotype, their indices are exceeding similar level of athletes with D/D – genotype by 6,5%. The tendency was discovered as to the demonstration of a higher level of VO_2max by athletes with I/I genotype and its reduction in the athletes with the higher than usual number of D-alleles (I/D and D/D genotypes). Thus, I-allele of I/D polymorphism of ACE gene is associated with the maximum aerobic capacity. This factor is quite understandable by taking into account the fact that the protein product of this gene – angiotension-converting enzyme (ACE) participates in vasomotor reactions and influences metabolism of cardiac muscle. I/D polymorphism of the studied gene is not structural, but influences the level of this gene expression. People with D/D genotype demonstrated maximum levels of ACE, and people with I/I genotype – two times less, and heterozygote – medium (Montgomery et al. 1999).

By applying the method of the single-factor dispersive analysis it was discovered that the factor of polymorphisms of PPARA gene, most likely, has an effect on both the absolute ($p = 0.04$) and relative value of work intensity ($p = 0.009$) being performed at the level of anaerobic threshold. In certain kinds of sports, where aerobic endurance is the main requirement for the functional fitness and special work capabilities of qualified athletes, intensity of work performance at the level of anaerobic metabolism threshold is the best indicator of a high grade athletes' fitness growth compared with the maximum aerobic capacity (MacDougall et al. 1991). Among carriers of G-allele, the lowest level of work intensity being carried out at the level of anaerobic metabolism threshold is typical for athletes with G/G genotype (209.4 ± 4.8 W). Absolute value of W_{Thr} in the athletes with this genotype was lower by 20.3% compared with the similar index of the athletes with G/C genotype. W_{Thr} with regard to the body weight of the athletes with G/G genotype equaled (3.05 ± 0.12) $W \cdot kg^{-1}$, whereas athletes with G/C genotype – (3.67 ± 0.19) $W \cdot kg^{-1}$. Thus, it may be stated that G/C polymorphisms of PPARA gene are associated with the intensity of physical work at the level of anaerobic metabolism threshold. This condition can be explained by the fact that the specified gene controls activity of genes that participate in lipid exchange and carbohydrate metabolism, and the rate with which anaerobic-aerobic metabolism threshold is being achieved depends on the intensity of these processes.

Athletes-carriers of the T-allele of C/T polymorphisms HIF-1 α were characterized by more reduced indices of VO_2max , reduced effect of cardiac cycle (with regard to "O₂-pulse") and high indices of the ventilation equivalent with regard to O₂ that characterizes the reduced efficiency of lung ventilation compared with the athletes with C/C genotype, but no probable differences in basic characteristics of CRS reactions amount athletes with different genotypes in terms of C/T polymorphism of HIF 1A gene were discovered. By taking into account the fact that the main functional role of the HIF 1 α transcription factor is to provide for the adequate adaptation of the organism to hypoxia, and qualified athletes who, in the course of endurance exercises, are subjected to the effects of training load hypoxia, absence of the probable difference may be explained by the fact that with the rising level of adaptation and fitness of athletes individual differences in their respiratory system sensitivity to hypoxia become even. But the tendency of VO_2max level reduction, worsening of cardiac cycle and lung ventilation efficiency of the athletes-carriers of T-allele makes allows to assert that availability of T-allele results in the reduction of cardiorespiratory system reaction efficiency when physical work is being performed at maximum aerobic capacity. Very likely that this is the reason for fact that the athletes with C/C – genotype (Döring et al. 2010) who are known for their endurance prevail when selected for endurance sporting events.

Discussion

The analysis of the obtained results has shown both single and combined effect of the gene polymorphisms on the aerobic capacity in the sports with the prevailing development of endurance. In analyzing genetic aptitude to go in for endurance sports, special attention should be paid to polymorphisms of genes that control metabolic limits of fats and carbohydrates, as well as genes, the expression products of which can influence the processes that limit aerobic performance and have a pleiotropic effect.

Though many studies demonstrated the exceptional significance of the maximal oxygen uptake to achieve the high sport results in the endurance sports, the necessity of VO_2max high level is overestimated (Platonov 2005). Each sport should have its own criteria of the aerobic capacity evaluation. It is necessary to focus on the sport-specific molecular-genetic markers.

In different sporting events that require demonstration of endurance, competitive activities pose their requirements to the level and balance of factors in the structure of the functional fitness. In case of rowing, 70% of the distance is covered for the account of aerobic metabolism (Hagerman 1984). This being the case, the balance of aerobic and anaerobic metabolism in the rowing is distributed as 70% to 30%, and in case of long distance cross-country skiing, the balance of aerobic and anaerobic metabolism is distributed as 95% to 5%. Certain polymorphisms may play key role in the performance of intensive physical work attributed to a certain sporting event that requires demonstration of various endurance aspects, but is of no importance for any other event of the same classification group. That is why, each sporting event requires specific criteria developed for the assessment of aerobic capacities and may be oriented on specific molecular-genetic markers.

The received data allowed to affirm that availability of C-allele T⁻⁷⁸⁶ → C polymorphism of eNOS gene in the homozygous state contributes to a greater reduction of lung ventilation efficiency during long, intensive physical loads at maximum aerobic capacity. The received results are clearly explained by the fact that C-allele contributes to the reduction of eNOS gene activity, and insufficiency of endothelial nitric oxide synthase that takes place in this case is the reason for synthesis reduction, release of nitric oxide and dysfunction of vessel endothelial (Dosenko et al. 2006).

Conclusions

1. Analysis of the received results testified influence of gene polymorphisms on aerobic capacity in the endurance sports. One of the aerobic performance components is aerobic intensity that is characterized by the amount of the maximum oxygen consumption and depends on the type of the set of 6 polymorphisms in combination with individual indices (gender, classification, sporting event). These factors account for 71% the VO_2max dissipation.

2. Association of single polymorphisms with various characteristics of aerobic capacities of the qualified athletes' organisms has been established: I/D polymorphism of ACE gene associated with the maximum aerobic intensity, T/C polymorphisms of eNOS gene associated with the efficient lung ventilation required for utilization of O_2 from the air, G/C polymorphisms of PPARA gene associated with physical working capacity at the level of anaerobic metabolism threshold. The received result may be used for creation of the system of molecular-genetic diagnostics of young athletes's aptitude to go in for sports with the prevailing anaerobic mechanisms of energy supply.

Acknowledgments

This work was supported by Postdoctoral grant of National University of Physical Education and Sport of Ukraine.

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Cite this article as: Drozdovska S.B., Lysenko O.M., Dosenko V.E., Ilyin V.N. Dependence of Aerobic Performance of Athletes on Polymorphism of Genes. *Central European Journal of Sport Sciences and Medicine.* 2015; 9 (1): 65–73.

