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# ASSOCIATION OF POLYMORPHISMS OF THE *PPAR* FAMILY GENES AND *UCP2* GENE WITH ECHOCARDIOGRAPHY INDICES IN ATHLETES

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Development of myocardium hypertrophy in athletes due to systematic physical training is multifactorial process vastly genetically determined. In this work, we studied DNA polymorphism and cardiac hypertrophy in professional athletes under physical activity with aerobic mode of energy supply. We have found that a group of athletes with hypertrophic alteration of the myocardium was characterized with increased frequency of the G/A gene polymorphism of *PPARGC1A* gene comparing to a group of athletes without pronounced changes of heart structure. The correlation between some polymorphism of genes and indices of echocardiography study was established: the correlation between G/C polymorphism of the *PPARA* gene and the middle septum thickness; correlation between Pro/Ala polymorphism of the *PPARG* gene and the left ventricular end-diastolic volume. By the method of binary logistic regression, a model for prognosis of effect on hypertrophy was established – 68.2 % including two polymorphisms: *PPARG* and *UCP2*.

**Key words:** DNA polymorphism, myocardium hypertrophy, echocardiography, adaptation to physical load.

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## АСОЦІАЦІЯ ПОЛІМОРФІЗМУ ГЕНІВ РОДИНИ *PPAR* І ГЕНА *UCP2* ІЗ ПОКАЗНИКАМИ ЕХОКАРДІОГРАФІЇ У СПОРТСМЕНІВ

Розвиток гіпертрофії міокарда у спортсменів у відповідь на систематичні фізичні навантаження – це мультифакторне явище, де одним із чинників виступає спадковість. Для цього було досліджено участь ДНК-поліморфізмів у процесах формування гіпертрофії міокарда у спортсменів під впливом систематичних фізичних навантажень аеробного характеру енергозабезпечення. Група спортсменів з ознаками вираженої гіпертрофії міокарда вірогідно відрізняється від контрольної частотою зустрічі G/A поліморфізму гена *PPARGC1A*, алель А, якого здійснює вплив на енергетичний метаболізм кардіоміоцитів та сприяє розвитку вираженої гіпертрофії міокарда. Встановлено низку асоціацій поліморфізмів генів з показниками ехокадіографічного дослідження серця: асоціація G/C поліморфізму гена *PPARA*з товщиною міжшлуночкової перегородки; асоціація Pro/Ala поліморфізму гена *PPARG* з показниками кінцево-діастолічного об'єму. Методом бінарної логістичної регресії створена модель з класифікаційною здатністю – 68,2%, до якої входять 2 поліморфізми: *PPARG* та *UCP2.*

**Ключові слова:** ДНК-поліморфізми, гіпертрофія міокарда, ехокардіографія, адаптація до фізичних навантажень.

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The main differences between physiological athlete's heart and pathological heart are the economizing of functions at rest and moderate loads, the maximum efficiency of the heart when performing high-power exercise, which is achieved due to the ability to increase stroke volume and stabilize it against heart rate limits [3]. Additionally, the main features of a healthy heart are considered to be the reversibility of the hypertrophy process and the absence of fibrosis.

Most experts in sports medicine and cardiology agree that psycho-emotional and excessive physical activity in athletes, especially in sports with endurance, lead to the development of pathological myocardial hypertrophy followed by dystrophy [3, 6]. To date, this condition is insufficiently studied, not officially recognized and has many names: "stress cardiomyopathy", "cardiomyopathy due to chronic physical exertion of athletes", "myocardial dystrophy due to physical overexertion" and others. A review of the International Classification of Diseases (WHO, 1995) found that this pathology is called "cardiomyopathy with secondary involvement of the myocardium under the influence of physical and stress overload."

Regulation of myocardial mass depends on the interaction of physical activity, cardiac growth factors and individual genetic characteristics. Despite the features of anatomical and electrical remodeling, which are close to pathological, the "athlete's heart" is characterized by a normal or subnormal level of cardiomyocytes' functioning [10]. Some researchers claim that 60 % of left ventricular myocardial mass

variability depends on genetic factors [8, 11]. Physiological hypertrophy in response to exercise differs from pathological one in stimuli, structure and molecular profile [9].

Although qualitative and quantitative indicators of the severity of myocardial hypertrophy have been sufficiently studied and classified [2, 11], discussions on the mechanisms of hypertrophy formation and the possibility of transforming physiological hypertrophy into pathological under the influence of physical activity are still going on. Although physical activity is one of the most reliable means of preventing a number of cardiovascular diseases, and a high level of physical activity is strongly associated with a decrease in these diseases incidence, excessive exercise can be a factor in provoking their development. In addition, the risk of their development includes genetic factors that explain the heterogeneity of the exercise impact on the risk of cardiovascular disease [7, 10].

Hereditary predisposition to the development of severe myocardial hypertrophy in athletes in endurance sports, as a consequence of adaptation to systematic intense physical activity, mainly aerobic energy is polygenetic, due to a combination of gene polymorphisms and the level of their genes' expression. The list of such genes includes genes of proteins-regulators of metabolic networks.

It has recently been found that the presence of certain alleles in the genes that control the synthesis of proteins regulating metabolic networks may be an adverse factor for athletes, under the influence of which the process of pathological stress-induced myocardial modeling in athletes is activated. Despite the fact that the list of genes affecting the development of the cardiovascular system's diseases and their unfavorable alleles is growing exponentially every year, the final contribution of gene polymorphisms to the probability of these pathologies in athletes has not been determined. These, according to various authors, include genes of the renin-angiotensin system, genes of the heart muscle's structural proteins, genes of growth factors and others. In this work, we study the genes affecting the energy metabolism of cardiomyocytes: PPRA− α-receptor gene and PPARG− γ-receptor gene activated by peroxisome proliferators [5]. PPARGC1A – α-coactivator of γ-receptors activated by peroxisome proliferators and *UCP2*-gene of the type II separating protein.

**The purpose** of the work was to study the role of gene polymorphism in the molecular genetic mechanisms of adaptation and pathological processes in the myocardium during prolonged intense exercise.

**Materials and methods.** The study involved 50 athletes who specialized in sports with the predominant requirements for endurance (long-distance running, rowing). Echocardiographic examination revealed signs of pronounced hypertrophy in 25 athletes, so all athletes were divided into two groups: 1) athletes without signs of myocardial hypertrophy or with mild myocardial hypertrophy (group N); 2) athletes with signs of pronounced hypertrophy - group G (n=25).

The division into groups occurred according to the indicators MM (myocardial mass), IMM (myocardial mass index), LVEFV % (left ventricular ejection fraction). The group with hypertrophy included athletes with an MM of more than 170 g [2] and an index of LVEDV (end-diastolic volume of the left ventricle) if it was higher than 145 ml.

DNA was isolated from the buccal epithelium using a set of reagents "Test-Rapid-Genetics" ("DNA-Technology", Russia).

600 μl of lysis solution was added to the sample tube. The tubes were centrifuged at 13,000 rpm for 1 min. The supernatant was removed. Add 300 μl of Test-Rapid reagent was added to the precipitate, vortexed for 5-10 seconds and the tubes were placed into thermostat at 98° C for 10 minutes. The tubes were centrifuged for 3 minutes at 13,000 rpm. The DNA was in the supernatant and couls be be used directly to perform a polymerase chain reaction.

The kit permits the isolation of high molecular weight DNA from fresh biological material (40-50 thousand pairs of high purity nucleotides (OD260 / 280nm 1.6-2.0) .In the process of DNA isolation, the recommendations given in the commercial kit were followed and manipulated according to the following protocol.

The composition of the reaction mixture included: PCR buffer, $MgCl_2$, dNTP (x10), primer (table 1), Dream-Tag-polymerase, the volume was adjusted with deionized water. DNA was added to the reaction mixture. Determination of gene polymorphisms was performed using a 7500 Fast Real-time PCR device (Applied Biosystems, USA).

The obtained results of samples' population analysis were statistically processed using Excel software. The probability of the difference in mean values was determined by Student's t test. The probability of differences in the samples distribution was determined by the criterion $\chi 2$ (Pearson). Values of $p<0.05$ were considered reliable.

Table 1

**The sequence of primers to determine gene polymorphism**

| Name | Primer |
|---|---|
| *PPARA* | 5'− ACAATCACTCCTTAAATATGGTGG − 3' |
| *PPARG* | 5'− GCCAATTCAAGCCCAGTC− 3' |
| *PPARGC1A* | 5'− GTGGGGCTTTGTCAGTGAAT−3' |
| *UCP2* | 5′-GGCCAGTGCGCGCTACGG-3′ |

The method of binary logistic regression was used to assess the dependence of myocardial hypertrophy factor on gene polymorphisms. To create a mathematical model using the method of binary logistic regression, we used one of the leading statistical packages SPSS ver. 20.0.

One-way analysis of variance was used to determine the associations of polymorphisms with indices of the cardiovascular system's state. Echocardiography was checked for normalcy using the Shapiro-Wilk test. The homogeneity of the dispersions was analyzed using the Levine test followed by analysis of variance (ANOVA). In the case of heterogeneity of variances, modifications of the analysis of variance were performed (Brown-Forsyth and Welch tests) [1].

**Results of the study and their discussion.** Association of gene polymorphism included predisposition to development of myocardial hypertrophy in athletes, who specialize in kinds with a predominant development of endurance. At the previous stage of scientific work, by analyzing the scientific literature, a list of candidate genes, whose protein products are involved in the processes of adaptation to exercise, was established, and polymorphisms can lead to changes in physicochemical properties of these proteins, their functional activity, and as a consequence, to changes in morpho-functional parameters of the body.

Markers were selected based on their proven functional significance and the possibility of their detection. The list of these polymorphisms, which can serve as molecular genetic markers of the adaptation processes' course in the body, included polymorphisms of genes encoding proteins, which were involved in the processes of adaptation to physical activity in the heart.

Group I included those genes that encode proteins that were structural proteins of myofibrils, were transcription factors of gene networks, affected the cardiovascular system and had a pleiotropic effect. Because it is known that adaptation to exercise causes an increase in the level of glycolysis in cardiomyocytes, our list also included genes that affected fat and carbohydrate metabolism in the myocardium. Fat metabolism genes are markers of heart function, because the main source of myocardial energy is fat oxidation.

At the second stage of the study, the presence of associations of candidate gene polymorphisms with indices of echocardiographic examination of the heart in athletes who specialized in endurance sports was studied. In our work we studied the polymorphisms presented in table 2.

Table 2

**Characteristics of the polymorphisms studied**

| No. | Gene | Polymorphism | | Type | Chromosome | Localization | SNP ID |
|---|---|---|---|---|---|---|---|
| | | nucleotide form of the record | amino acid form of the record | | | | |
| 1 | *PPARA* | $G^{2528}$→C | – | – | 22q13.31 | 7 intron | rs4253778 |
| 2 | *PPARG* | $C^{34}$ →G | $Pro^{12}$→Ala | mis unpreserved. | 3p25 | exon | rs1801282 |
| 3 | *UCP2* | C/T | Ala/Val | – | 11q13 | 55 protein location | rs660339 |
| 4 | *PPARGC1A* | G/A | $Gly^{482}$→Ser | Protein coding | 4p15.2 | exon | rs8192678 |

Analysis of the distribution of genotypes and alleles was performed by $Pro^{12}$ → Ala *PPARG* gene polymorphism. The frequency distribution of genotypes by polymorphism of the *PPARG* gene in the control group corresponded to the Hardy-Weinberg distribution (p=0.96); whereas in the group with hypertrophy the distribution was different (p=0.01), which may indicate a possible influence of this factor on the phenotype, which had not yet been confirmed statistically due to a small sample. There was a high frequency of athletes with the Ala / Ala genotype (8 %) among athletes with signs of myocardial hypertrophy. In our previous studies in the group of athletes who specialized in endurance, the frequency of Pro / Pro-genotype bull was higher than that in the control group by 12.5 % ($p<0.05$).

Analysis of distribution of genotypes and alleles by $G^{2528} \rightarrow C$ polymorphism of the *PPARA* gene suggests that in the group of athletes with hypertrophy the frequency of the G allele is by 11 % higher than in individuals without hypertrophy.

Distribution by Ala/Val polymorphism of the *UCP2* gene. In the group of athletes with hypertrophy there is a low frequency of the Ala allele (20 %) and a low frequency of the Val allele (p=0.045). C/T polymorphism of the *UCP2* gene causes the replacement of Ala by Val at the 55 position of the UCP2 protein, which leads to increased metabolic efficiency of muscle activity, a tendency to obesity with low physical activity. It is obvious that the increased frequency of Val-allele is the result of sports selection.

The key function of PPARGC1A – 1α is to link all transcription factors to nuclear receptors. Indirect action of PPARGC1A-1α may alter muscle composition (white fast-twitch fibers may turn into red slow-twitch fibers), increase insulin secretion, and reduce body fat. The A-allele is associated with manifestations of metabolic syndrome. Analysis of the distribution of alleles by G/A polymorphism of the *PPARGC1A* gene showed that in athletes with myocardial hypertrophy, the frequency of genotype G/G is by 19 % higher than in the group without hypertrophy, and the frequency of A allele is by 23.5 % higher (p=0.02).

Application of the one-factor analysis of variance (ANOVA) allowed to establish interrelation between polymorphisms and indicators of echocardiographic research of heart. Among the most informative indicators of the heart were considered: LVMM, LVMMI, EDV, EF %, EDV/ LVMM.

The association of G/C polymorphism of the *PPARA* gene with the thickness of the interventricular septum (p=0.042). There is a tendency to decrease the thickness of the septum with decreasing frequency of the C allele, which coincides with the trend established in the analysis of the genotypes' frequency in groups with and without myocardial hypertrophy. In athletes with G/G – genotype LVMM was 1.03±0.02 cm, in athletes with G/C genotype – 0.98±0.06 cm, and in athletes with C/C genotype – 0.72±0.30.

Thus, it can be confirmed that the G-allele of the G/C polymorphism of the *PPARA* gene contributes to an increase in the thickness of the interventricular septum. The obtained fact can be explained by a role of the protein encoded by the specified gene in activation of a network of the genes responsible for a carbohydrate metabolism, and consequently for energy supply of a myocardium during muscular work.

The association of Pro/Ala polymorphism of the *PPARG* gene with left ventricular end-diastolic volume (FDV) (p=0.005) was established. In carriers of the Pro/Pro genotype, the value of EDV was 126.04±5.65 ml, and in carriers of the Pro/Ala genotype – 96.70±6.64 ml. Interestingly, athletes in endurance sports there was high EDV – an index of adequate adaptation. Consequently, the decrease in EDV in athletes carrying the Ala-allele was a sign of the disadvantage of this allele for this kind of sport.

Application of the binary logistic regression method permitted to build a model of the cumulative effect of polymorphisms on the indices of myocardial hypertrophy in athletes. Binary logistic regression is used when the response variable is dichotomous in nature (i.e. it can take only two values – hypertrophy/no hypertrophy), and independent variables can be both quantitative and categorical. This method is very widely used in medicine, because the design of most medical studies often fits the possibility of using binary logistic regression, its results are quite easy to interpret, and the method itself is implemented in most statistical programs. The obtained model has a high classification capacity - 68.2 %. The model includes 2 polymorphisms: *PPARG* and *UCP2.*

According to this model and OR indices (Exp (B)), the *PPARG* gene polymorphism genotypes PP/Ala and Ala/Ala, Pro/Ala increase the risk of developing severe myocardial hypertrophy, and the Ala/Val and Val/Val, Ala/Val *UCP2* gene polymorphisms reduce this risk.

The PRO-allele of the *PPARG* gene may contribute to the development of high physical performance in sports with a predominant manifestation of endurance, as it is involved in the regulation of genes associated with fat accumulation, differentiation of adipocytes and myoblasts, and insulin sensitivity. *PPARG* plays its role in glucose and fat homeostasis, adipogenesis.

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The *UCP2* gene (11q13) is known to be responsible for the transport of fatty acids and to separate oxidative phosphorylation. Increased expression of this gene, which accompanies aging and is caused by total ischemia of the heart, causes mitochondrial dysfunction [4].

The frequency of A-allele distribution in the world population is within the range of 30-40 % and is associated with a decrease in the expression of this gene. In athletes, the A-allele is much less common than in the general population, and the G-allele is associated with increased aerobic performance of athletes. The $Gly^{482}$ → Ser polymorphism is considered a predictor of endurance. Obviously, athletes carrying the A/A genotype are prone to anaerobic exercise, which causes their inadequate adaptation in the form of excessive myocardial hypertrophy and transition of cardiomyocytes to carbohydrate metabolism, while the G-allele promotes adequate adaptation to exercise.

## Conclusion

Association of gene polymorphisms with echocardiographic examination of the heart was established by one-way analysis of variance: the association of G/C polymorphism of the *PPARA* gene with interventricular septal thickness (p=0.042) shows that the G allele contributes to the increase in interventricular septal thickness; there is an association of Pro/Ala *PPARG* gene polymorphism with left ventricular volume on the end-diastolic image (p=0.005).

The group of athletes with signs of severe myocardial hypertrophy probably differs from the group without these signs by the frequency of G/A polymorphism of the *PPARGC1A* gene, – and the frequency of the A-allele is by 23.5 % higher (p=0.02) than the frequency of the G-allele, which can indicate its ability to affect the energy metabolism of cardiomyocytes and promote development of severe hypertrophy. The binary logistic regression method was used to create a model with a classification capacity of 68.2 %, which includes 2 polymorphisms: *PPARG* and *UCP2*.

The study of gene polymorphisms that can affect their activity will permit to recognize a high predisposition to development of myocardial hypertrophy, to perform early non-invasive diagnosis of pathological and pre-pathological conditions of the myocardium.

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