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GENETIC ASPECTS OF THE DEVELOPMENT OF MYOCARDIAL REMODELING IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ГЕНЕТИЧЕСКИЕ АСПЕКТЫ РАЗВИТИЯ РЕМОДЕЛИРОВАНИЯ МИОКАРДА У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ 2 ТИПА

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Currently, one of the prognostic criteria for the development of cardiovascular pathology in type 2 diabetes mellitus (DM) is the patient's genetic characteristics. The study involved 258 patients with type 2 DM, of which 50 were carried out genetic testing with the study of polymorphisms of genes AGT:704, AGTR1:1166, GNB3:825, NOS3:–786, NOS3. The presence of remodeling in patients with DM is associated with an increase in the frequency of registration of NOS3:894 gene polymorphism in the form of a combination of mutation homozygotes and heterozygotes by 27.9 %, heterozygous polymorphism of this gene is associated with the identification of an unfavorable version of left ventricular myocardial remodeling concentric hypertrophy (p=0.04). A tendency to an increase in the frequency of the mutation-homozygotes of the gene GNB3:825 by 17.6 % and NOS3:–786 by 26.4 %, as for the heterozygotes of the AGT:704 gene polymorphism by 22.6 % and AGTR1:1166 by 16.7 %.

As a result of our study revealed that the development of structural heart remodeling might be associated with polymorphism of genes encoding NOS3, as well as those responsible for the activity of the ACF: AGT, AGTR1, GNB3. Based on the results of genetic testing, it is possible to assess the prognosis of the disease and rationally select drug therapy, which helps to prevent the development of severe complications of DM.

Keywords: gene polymorphism, type 2 diabetes mellitus, myocardial remodeling

В настоящее время одним из прогностических критериев развития сердечно-сосудистой патологии при сахарном диабете (СД) 2 типа являются генетические факторы. В исследовании участвовали 258 пациентов с СД 2 типа, из которых 50 было проведено генетическое тестирование с изучением полиморфизмов генов AGT:704, AGTR1:1166, GNB3:825, NOS3:–786, NOS3. Наличие ремоделирования у пациентов с СД ассоциировано с повышением частоты регистрации полиморфизма гена NOS3:894 в виде сочетания мутации гомозиготы и гетерозиготы на 27,9 %, гетерозигота полиморфизма данного гена ассоциирована с выявлением неблагоприятного варианта ремоделирования миокарда левого желудочка концентрической гипертрофии (p=0,04). Выявлена тенденция к увеличению частоты мутации гомозиготы гена GNB3:825 на 17,6 % и NOS3:–786 на 26,4 %, со стороны гетерозиготы полиморфизма гена AGT:704 на 22,6 % и AGTR1:1166 на 16,7 %.

В результате исследования выявлено, что развитие структурного ремоделирования сердца может быть связано с полиморфизмом генов, ответственных за фактор эндотелия (NOS3), а также за активность ангиотензин-превращающего фермента: AGT, AGTR1, GNB3. На основании результатов генетического тестирования возможно определение прогноза заболевания и тактики лекарственной терапии, что способствует предупреждению развития тяжелых осложнений СД.

Ключевые слова: полиморфизм генов, сахарный диабет 2 типа, ремоделирование миокарда

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ABP – arterial blood pressure
ACE – angiotensin converting enzyme
ACEI – angiotensin converting enzyme inhibitors
AH – arterial hypertension
ARA II – angiotensin II receptor antagonists
BMI – body mass index
CF – cardiac failure
CVC – cardiovascular complications
DM – Diabetes mellitus type 2
DNA – deoxyribonucleic acid
EchoCG – echocardiography
EDD – end diastolic dimension
EDV – end diastolic volume
EF – ejection fraction
ESD – end systolic dimension
ESV – end systolic volume
iRWT LV – index of relative wall thickness of the left ventricle
iMM LV – index of myocardial mass of the left ventricle
MM LV – myocardial mass of the left ventricle
PCR – polymerase chain reaction
RAAS – renin-angiotensin-aldosterone system
SV – stroke volume

At present day, DM is viewed as an equivalent of the presence of clinically pronounced cardiovascular disease in patients, and it represents the independent risk factor of cardiac insufficiency and cardiovascular complications. It is known that the development of CVC of diabetes is influenced not only by modifiable factors (hyperglycemia, dyslipidemia, AH) but also by individual genetic features, which characterize subject's sensibility to adverse action of pathological factors in DM [1, 2]. Current achievements in genetics are commonly related to the discovery of alleles of various genes, assessment of their frequency, reveal of connections of specific alleles with associated traits, with attempt to show prognostic signs [3]. Research on genetic predisposition to development of cardiovascular pathology in DM is based on study of polymorph candidate genes, which expression products may have significance in pathogenesis of this disease and represent particular interest in terms of prognosis and risk groups' formation [4].

It is known that the primary regulator of vascular tone and blood pressure is RAAS. The RAAS cascade begins with the release of renin in the kidneys, which acts on angiotensinogen, forming biologically inactive decapeptide angiotensin I, which is converted into active angiotensin II by ACE. Since all cascade connections are conditioned by mutual «recognition» of corresponding enzymes, the importance of structural stability of genes encoding them is indisputable. Structural polymorphisms of genes encoding RAAS proteins are currently actively studied [5, 6]. One of these genes is the angiotensin II type 1 receptor gene (AGTR1). The AGTR1 gene is localized on the long arm of the 3rd chromosome (3g21-25). About 20 polymorphisms of this gene are known, the most studied is the 3'UTR site A1166C (rs5186). Thus, in the study of the structural state of this gene, it was found that in position 1166 in the 3' untranslated region, a single-nucleotide (point) replacement of the nitrogenous base of adenine (allele A) by cytosine (allele C) is possible. Polymorphism A>C at position 1166 influences the expression of the receptors because microRNA-155 interacts with a portion of a gene where the specified polymorphic site is localized, modulating the expression of the receptors. However, in the presence of the mutant allele C a similar interaction is absent, thereby increasing the expression and proliferation of smooth muscle cells, endothelial cells, which leads to the pathology of the cardiovascular system and the development of myocardial hypertrophy of the left ventricle [7, 8]. According to the literature, polymorphism of aldosterone synthase gene (CYP11B2 gene) increases the risk of myocardial remodeling [9]. G-protein beta (3)-subunit gene polymorphism primarily affects vascular reactivity and cell growth of cardiomyocytes, allele variant GNB3:825 C>T predisposes to the development of left ventricular myocardium hypertrophy, N0S3:894 gene polymorphism increases the risk of cardiovascular disease [10]. However, the literature data are not always unambiguous. One of the new and promising approaches may be the use of genetic testing methods aimed at identifying genetic risk and predicting various complications of the disease before their clinical manifestations.

Research objective: to study the distribution of frequencies of alleles and genotypes of polymorphic markers complex of candidate genes responsible for the activity of renin-angiotensin-aldosterone system, endothelial vasodilating factor in patients with diabetes and the presence of hypertension, and to evaluate the informativeness of their study as markers of predicting heart disease in type 2 diabetes mellitus.

Material and Methods. The study included 258 patients with DM (group A – 103 patients with different disease history and group B – 105 patients with newly diagnosed DM, the average age was 58.6±7.2 years), of which 50 patients were selected for genetic testing.

Risk factors were clarified during medical history collection: burdened family history of cardiovascular diseases, smoking, body mass index (BMI) was calculated, the study of medical histories, analysis of therapy received by patients, the frequency of registration of diabetes and comorbid pathology (hypertension, obesity), the degree of achievement of target figures of ABP. From medical history, all patients have hypertension: stage I of the disease was observed in 19 (9.5 %), stage II – in 181 (90.5 %), 1 degree of AH – in 93 (46.5 %), 2 degree of AH – in 102 (51 %), 3 degree of AH – in 5 (2.5 %). At the stage of hospitalization, all patients underwent general clinical methods; echocardiography was performed with the assessment of MM, IMM, iRWT LV. Evaluation of the geometric model of the left ventricle was carried out taking into account iMM and iRWT: standard geometry – iMM<115 g/m^2 for men and <95 g/m^2 for women, iRWT<0.42; eccentric hypertrophy – increase in iMM at normal iRWT; concentric remodeling – normal iMM, iRWT>0.42; concentric hypertrophy – increase in iMM, iRWT>0.42 [11].

As part of the study, 50 patients underwent genetic testing. In the blood serum, the polymorphisms in the genes responsible for the activity of ACE were determined: AGT:704, AGTR1:1166, genes regulating intracellular ionic homeostasis – GNB3:825, genes determining the structure of endothelial NO synthase – NOS3:–786, NOS3:894.

During the work, a set of reagents was used to determine the genetic polymorphisms associated with the risk of hypertension by PCR in human DNA preparations derived from peripheral blood. A set of reagents for

PCR amplification of human genomic DNA in real-time was used to estimate the amount of DNA isolated. Genes encoding elements of the renin-angiotensin-aldosterone system (genes of angiotensinogen, receptor to angiotensin II; aldosterone synthetases: AGTR1:1166, AGT:704) were determined in the blood serum, genes regulating intracellular ionic homeostasis (GNB3:825), genes determining the structure of endothelial NO synthase (NOS3:–786, NOS3:894).

Statistical data processing was carried out using the software package Statistica 10.0 (StatSoft, USA). In the examined groups of patients, quantitative signs were presented in the form of M±Sd. The significance of differences of quantitative characteristics was assessed using Student's t-test (for parametric distribution) and the Mann – Whitney test (for non-parametric distribution). The differences were considered significant at $p<0.05$.

Results and Discussion. Comparative characteristic of the presence of structural remodeling in the examined patients was carried out (Table 1). It was found that eccentric hypertrophy was more often registered in patients with newly diagnosed diabetes (group B) vs. patients with the experience of the disease (group A), in which concentric remodeling was more often detected; concentric hypertrophy was registered in groups with the same frequency. This is confirmed by echocardiography data (Table 2), in patients of group A there was an increase in iRWT LV (p=0.000003) along with an increase in ESD (p=0.0002) and ESV (p=0.00006), in group B there was an increase in iMM LV (p=0.003).

Table 1

Comparative characteristics of the presence of structural remodeling in patients included in the study

Indicator	Group A, n=105	Group B, n=103	Chi-square test values
Eccentric hypertrophy	4 (3.8)	14 (13.6)	5.12*
Concentric remodeling	49 (46.7)	16 (15.5)	22.03***
Concentric hypertrophy	13 (12.4)	12 (11.7)	0.00

Note: * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$.

Table 2

Comparative characteristics of echocardiography parameters in the examined patients

Indicator	Group A, n=105	Group B, n=103	P-value
EDD	48.69±0.25	49.42±0.26	none
ESD	32.02±0.27	30.57±0.28	0.0002
EDV	111.27±1.33	114.21±1.39	none
ESV	41.7±0.87	36.97±0.85	0.00006
SV	69.7±0.75	77.58±0.96	0.00000005
EF	62.88±0.54	68.11±0.48	0.000000001
MM LV	181.25±3.16	172.97±2.46	none
iMM LV	89.41±1.56	99.38±1.38	0.003
iRWT LV	0.44±0.01	0.40±0.01	0.000003

The frequency of genes in the group of patients with DM was analyzed depending on the presence or absence of cardiac remodeling. According to the literature [12], the frequency of registration of the normal variant of the eNOS gene was more observed in healthy patients; the heterozygous variant was more common in patients with the development of structural changes of the myocardium (62.2 % and 44.4 %, respectively). As evidenced by the Table 3 data, the presence of remodeling in patients with DM is associated with an increased incidence of NOS3:894 gene polymorphism in the form of a combination of mutations homozygotes and heterozygotes by 27.9 % (p=0.04). There was a tendency to increase the frequency of mutation homozygous gene GNB3:825 by 17.6 % and NOS3:–786 by 26.4 % in the presence of remodeling. A similar trend was observed concerning polymorphism heterozygote of AGT: 704 by 22.6 % and AGTR1:1166 by 16.7 %.

Table 3

Frequency of gene polymorphisms depending on the presence of cardiac remodeling

The name of the gene	Presence of remodeling, n=33	The lack of remodeling, n=17
AGTR1:1166 – homozygous mutation – heterozygote	10 (30.3 %) 0 (0 %) 10 (100 %)	6 (35.3 %) 1 (16.7 %) 5 (83.3 %)
AGT:704 – homozygous mutation – heterozygote	23 (69.7 %) 7 (30.4 %) 16 (69.6 %)	10 (47.1 %) 3 (30 %) 7 (70 %)
GNB3:825 – homozygous mutation – heterozygote	15 (45.5 %) 4 (26.7 %) 11 (73.3 %)	11 (64.7 %) 1 (9.1 %) 10 (90.9 %)
NOS3:–786 – homozygous mutation – heterozygote	28 (84.8 %) 13 (46.4 %) 15 (53.6 %)	15 (88.2 %) 3 (20 %) 12 (80 %)
NOS3:894 – homozygous mutation – heterozygote	**15 (45.5 %)*** 1 (6.7 %) 14 (93.3 %)	**3 (17. 6 %)** 0 (0 %) 3 (100 %)

*$p<0.05$.

The study HyperGEN (Hypertension Genetic Epidemiology Network Study) showed that patients with hypertension and DM have a higher MM LV and are more likely to have a concentric type of heart geometry compared to patients with hypertension without diabetes [13]. In the Table 4 shows the frequency of gene polymorphisms depending on the degree of remodeling. It is revealed that the eccentric hypertrophy is more frequently recorded in the polymorphism of AGTR1:1166 (p=0.0003), AGT:704 (p=0.01) from the side of heterozygote and mutation homozygote of NOS3 gene: 894 (p=0.0001), heterozygote polymorphism of this gene is associated with the identification of an unfavorable variant of left ventricular myocardial remodeling – concentric hypertrophy (p=0.04).

Table 4

The frequency of gene polymorphisms depending on the degree of cardiac remodeling

The name of the gene	No remodeling	Eccentric hypertrophy	Concentric hypertrophy
AGTR1:1166 – homozygous mutation – heterozygote	1 (100 %) 5 (33 %) p=0.0400	0 (0 %) 7 (47 %) p=0.0003	0 (0 %) 3 (20 %) p=1.0000
AGT:704 – homozygous mutation – heterozygote	3 (30 %) 7 (30.4 %) p=0.0894	6 (60 %) 14 (60.9 %) p=0.0128	1 (10 %) 2 (8.7 %) p=0.5000
GNB3:825 – homozygous mutation – heterozygote	1 (20 %) 10 (47.6 %) p=0.0002	4 (80 %) 9 (42.9 %) p=0.0576	0 (0 %) 2 (9.5 %) p=0.1667
NOS3:–786 – homozygous mutation – heterozygote	3 (18.8 %) 12 (44.5 %) p=0.0014	11 (68.7 %) 13 (48.1 %) p=0.3866	2 (12.5 %) 2 (7.4 %)
NOS3:894 – homozygous mutation – heterozygote	0 (0 %) 3 (17.6 %) p=0.0500	1 (100 %) 11 (64.8 %) p=0.0001	0 (0 %) 3 (17.6 %) p=0.0400

According to the literature, the increase of the frequency of occurrence of gene polymorphisms AGTR1: 1166 and GNB3:825 are associated with the insufficient success of pharmacotherapy, which should be taken into account when correcting treatment [12, 14, 15]. It proves that ACE inhibitors and ARA II drugs have a cardioprotective effect [16]. Thus, it can be assumed that the development of remodeling and the success of treatment can affect the genetic characteristics of patients.

Conclusions. In this study, it was found that the development of myocardial remodeling in patients with DM was recorded regardless of the length of the disease, and eccentric hypertrophy was more often recorded in patients with newly diagnosed diabetes. As a result of genetic testing, it was found that the development of structural changes of the heart can be associated with the polymorphism of the gene NOS3:894 in the form of a combination of mutations of homozygote and heterozygote, heterozygous polymorphism of this gene is related to the identification of an unfavorable variant of myocardial remodeling of the left ventricle of concentric hypertrophy.

Thus, the use of genetic testing makes it possible to identify a predisposition to structural myocardial remodeling and, taking into account the personalized approach, to determine the therapeutic and preventive tactics for the management of patients with type 2 diabetes mellitus. At the same time, the development of possible algorithms for the treatment of such patients, taking into account the peculiarities of genetic factors, requires further study.

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