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Left ventricular hypertrophy of arterial hypertension in patients with CHD is associated with polymorphism of the tumor necrosis factor gene

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Abstract

The aim of the study was to study the possible association of the polymorphic variant G (-308) A of the tumor necrosis factor alpha (**TNF**) gene with the structure and function of the myocardium in patients with arterial hypertension (**AH**) and coronary heart disease (**CHD**). Patients were selected for the study from those included in the observation programs of ORACLE I and II (*ORACLE - ObseRvation after Acute Coronary syndrome for deveLopment of trEatment options*). A total of 875 patients with hypertension were examined, with an average age of 62.7 ± 12.57 years (*531 men (60.8%) and 344 women (39.2%)*).

In the examined group, there were 137 (15.8%) patients suffering from type 2 diabetes mellitus, 645 (73.7%) with a history of coronary artery disease before inclusion in the study, 78 (9.0%) suffered a stroke, 519 (59.3 %) had hypertension of 3 severities. The frequency distribution of alleles and genotypes of the **TNF gene** (AA - 7 patients (0.7%), **AG genotype** - 237 patients (27.1%), **GG genotype** - 631 patients (72.1%) differed from those expected by Hardy-Weinberg equation by reducing the frequency of the rare homozygous genotype: chi-square 9.87, p <0.005. In patients with **LVH**, the frequency of the **GG genotype**, *allele G and the frequency of allele A of the TNF gene* were significantly lower. Allele A carriers in the homo- and heterozygous state had a greater **LVMI**, a lower left ventricular ejection fraction, and a ratio of peak rates E and A. In a multifactorial regression analysis, the male gender, the level of systolic pressure, the age of patients, and the presence of genotype allele A of the **TNF gene**.

Key words: arterial hypertension, left ventricular myocardial hypertrophy, tumor necrosis factor, gene, polymorphism.

The tumor necrosis factor alpha (**TNF**) is one of the pro-inflammatory cytokines involved not only in the regulation of inflammation, but also influencing the formation of insulin resistance, activation of the renin-angiotensin system (**RAAS**) and the activation of growth factors. Shown, that increased tumor necrosis factor is associated with an increase in cardiovascular risk. It is known that this factor is associated not only with the formation of the immune response, but also the development of atherosclerosis, endothelial dysfunction, insulin resistance, coagulation disorders. Experimental evidence of the involvement of tumor necrosis factor alpha in the development of left ventricular hypertrophy (**LVH**) in animals has been found [1]. At the same time, **LVH** in patients with cardiovascular diseases is an additional risk factor for adverse outcomes. This determines the interest in the pathogenic mechanisms of LVH development, in particular, associated with the activation of the inflammatory system. The expression of **TNF** is largely determined by genetic factors, one of the variants of which is the **polymorphism G (-308) A**, which is located in the region of the gene promoter. In this regard, the goal of our study was to examine the possible association of the **polymorphic variant G (-308) A** of the tumor necrosis factor **alpha (TNF)** gene with the structure and function of the myocardium in patients with arterial **hypertension (AH)** and coronary heart disease (**CHD**).

Material and research methods

Patients were selected for the study from those included in the observation programs of ORACLE (*ORACLE - ObseRvation after Acute Coronary syndrome for deveLopment of trEatment options*) - ORACLE I (2004-2009) and ORACLE II (2014-2018). A total of 2695 patients who had an episode of acute coronary syndrome were observed in the studies. To assess the association of the parameters of the structure and function of the myocardium and **TNF gene polymorphism**, 875 patients with hypertension were selected, who underwent an echocardiographic study, as well as those who did not tolerate large-focal myocardial infarction and did not have valvar heart disease. *The average age was 62.7 ± 12.57 years (531 men (60.8%) and 344 women (39.2%)), 137 (15.8%) patients had type 2 diabetes, 645 (73.7 %) had a history of coronary artery disease before inclusion in the study, 78 (9.0%) suffered a stroke, 519 (59.3%) had AH 3 severity.*

In an echocardiographic (*EchoCG*) study in M-mode at the level of the chords of the mitral valve from

the parasternal access, the end-diastolic (CCD), the final systolic size (CCP), the thickness of the interventricular septum (TMV) and the thickness of the posterior wall of the left ventricle (TSSL). The mass of the myocardium of the left ventricle (MLM) was calculated *by the formula R. Devereux and N. Reichek, 1977* [2]:

$1.04\times\{(TMZHP+TZSLZh+KDR)^3-KDR^3\}$ -13.6.

The left ventricular myocardial mass index (LVMI) was calculated as the ratio of left ventricular myocardial mass to body surface area (Cornell criterion: 115 g /m^2 was considered the upper limit of normal for men and 95 g / m^2 for women).

The global diastolic function of the left ventricle was assessed by trans-mitral blood flow using pulse wave Doppler echocardiography from the apical access at the four-dimensional position with the position of the control volume at the ends of the mitral valve cusps. The following indicators were determined: the maximum speed of the early (Amax) filling of the LV, the maximum speed of the late (Amax) filling of the LV, their E / A ratio, and the time of isovolumetric relaxation (IVRT) of the LV.

The determination of the alleles and genotypes of the TNF gene was performed using PCR. We used 2.5x PCR-RV reaction mixture containing SynTaq DNA polymerase with antibodies inhibiting the activity of the enzyme (CJSC Syntol, Moscow). Oligonucleotide primers were synthesized by Evrogen (Moscow). Genomic DNA was isolated from whole blood of patients by the method of extraction with a mixture of phenol and chloroform after incubating blood samples with proteinase K in the presence of 0.1% sodium dodecyl sulfate.

Alleles of polymorphic markers were identified using hybridization-fluorescence analysis (TaqMan® analysis) on a Bio-Rad CFX96 C1000 Touch real-time amplifier (Bio-Rad Laboratories, Inc., USA) in 25 µl of the reaction mixture of the following composition: 2.5x Reaction mixture for PCR-RV, 4 pcol of each primer and probe, 25 ng of genomic DNA.

Conditions for amplification of the DNA fragment: pre-denaturation of 95 ° C / 2 min, 95 ° C / 10 s, 60 ° C / 60 s - 40 cycles. The composition of primers and probes are presented in table 1.

Statistical data processing was carried out using the SPSS 23.0 program. For extended indicators, an analysis was made of the distribution and criteria for its compliance with the normal one. Since the distribution of all the studied parameters corresponded to the normal, parametric calculation methods were used for the analysis. For extended variables, the mean values and standard deviation from the mean ($M \pm SD$) were calculated. To assess the significance of differences in the means used t-test. Discrete values were compared by

Table 1

Used primers and probes

Primer	Allele	Sequence
Straight	-	TGGAAGTTAGAAGGAAACAGAC
Back	-	ACACAAGCATCAAGGATACC
Probe 1	G	FAM-CCGTCCCCATGCCC-BHQ1
Probe 2	A	HEX-CCGTCCTCATGCCC-BHQ1

Pearson criterion χ_2. To assess the independence of the influence of various factors on myocardial hypertrophy, logistic regression was used. Parameters that demonstrated statistical significance in a single-factor analysis were included in the multivariate analysis. For all types of analysis, $p < 0.05$ was considered statistically significant.

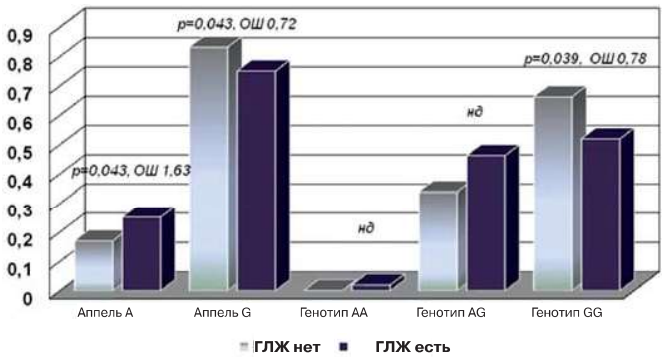
The correctness of the distribution of genotype frequencies was determined by matching the Hardy-Weinberg equilibrium ($pi2 + 2pipj + pj2 = 1$) and was calculated using the Gen Expert Expert software calculator.

Results

The frequency distribution of alleles and genotypes of the TNF gene (AA - 7 patients (0.7%), AG genotype - 237 patients (27.1%), GG genotype - 631 patients (72.1%) differed from those expected by Hardy-Weinberg equation by reducing the frequency of the rare homozygous genotype: chi-square 9.87, $p < 0.005$.

In total, 400 patients in the examined group had LVH. In this group there were more men. Patients with LVH were older in age, had a higher level of systolic blood pressure (BP), more often circulatory insufficiency was recorded. Patients with LVH had a lower level of glomerular filtration rate (Table 2).

Carriers of allele A in the homo and heterozygous state had a greater LVMI, a lower left ventricular ejec-



Legend: On "X": Allele A; Allele G; AA genotype; GA genotype; GG genotype;
The dark blue color marks the existence of: *LVH - left ventricular hypertrophy. The light blue color marks the inexistence of:* LVH - left ventricular hypertrophy.
Figure 1. Frequency distribution of genotypes of the TNF gene in patients with and without LVH.
TNF - tumor necrosis factor alpha;
LVH - left ventricular hypertrophy;

Table 2

Clinical characteristics of patients with dependence on the presence of LVH

Options	Patients without LVH (*n*=475)	Patients with LVH (*n*=400)	*R*
Sex : Male/ Female	278/197 (58/41%)	253/147 (67/33%)	0,046
Age years	58,2±1,35	64,8 ±1,02	0,001
Type 2 diabetes, *n* (%)	66 (14,1)	71 (17,8)	нд
Duration GB (years)	13,1 ±1,29	13,3±1,09	нд
BMI, kg / m^2	28,7±0,47	28,3±0,33	нд
Maximum systolic blood pressure, mm.rt.st.	184,1± 3,3	195,3 ± 2,86	0,01
Maximum diastolic blood pressure, mm.rt.st.	103,5 ±2,17	108,1 ±1,45	нд
Circulatory failure, *n* (%)	197 (42%)	227 (52%)	0,001
GFR (MDRD), ml / min / 1.73 sq.m.	64,8±16,79	60,9±18,78	0,005

LVH - left ventricular hypertrophy; GB hypertensive disease; Arterial blood pressure; GFR-glomerular filtration rate.

Table 3

Parameters of the structure and function of the myocardium of the left ventricle in patients with different genotypes of the polymorphic marker G (-308) A of the TNF gene

Options	GG genotype	GA genotype	AA genotype	*R*
TMZHP, mm	12,13±2,478	11,49±5,187	13,14±7,714	нд
TZSLZH, mm	10,88±1,512	11,07±5,095	11,57±4,434	нд
CRD LV, mm	47,25±4,528	49,55±7,575	50,14±9,139	нд
The diameter of the aortic root, mm	25,87±6,756	28,00±6,565	27,50±6,164	0,037
Diameter PL, mm	38,84±6,119	38,60±5,532	37,50±6,547	нд
EF LV,%	62,67±10,619	58,00±11,650	56,55±11,848	0,001
MLW, g	253,1±59,237	259,3±82,76	270,9±93,19	нд
LVMI, g / m	131,35±31,079	139,74±53,514	151,65±41,313	0,020
E_{max}, m / s	89,10±3,618	71,96±2,114	55,86±17,582	0,021
A_{max}, m / s	76,91±2,2025	77,26±5,122	57,86±10,447	нд
E / A ratio	1,36±0,927	1,04±0,554	0,99±0,356	0,013

TMZH-thickness of the interventricular septum; TZSLZh- thickness of the posterior wall of the left ventricle, LVC LV, of course, the diastolic size of the left ventricle; LP - left atrium; EF LV-left ventricular ejection fraction; MLJ is the mass of the myocardium of the left ventricle; LVMI index of myocardial mass of the left ventricle.

tion fraction, and a ratio of peak velocities E and A (Table 3). Thus, the carriage of allele A in the hetero- and homozygous state was associated with left ventricular myocardial hypertrophy, as well as with the formation

Table 4

Regression analysis of the independence of the association of clinical factors with LVH in patients with hypertension

Factors	Univariate analysis		Multivariate analysis	
	OR [95% CI]	*r*	OR [95% CI]	*r*
Male	2,28[1,66-3,13]	0,001	1,54 [1,02–2,03]	0,048
Age over 60 years	2,62[1,92-3,59]	0,001	1,71 [1,34–2,28]	0,01
CAD> 180 mmHg	2,20[1,57-3,07]	0,002	1,86 [1,22–2,64]	0,002
Circulatory failure	2,44[1,81-3,30]	0,001	1,76[0,96-2,58]	0,053
СКФ (MDRD)<60 мл/мин/1,73 м2	1,76[1,03-2,28]	0,039	1,12[0,86-2,48]	0,086
Carriage of allele A of the polymorphic marker A (-308) G of the gene TNF	2,36[1,09-4,93]	0,005	1,97 [1,12–2,89]	0,003

LVH - left ventricular hypertrophy; Hypertension - arterial hypertension; Systolic blood pressure; GFR-glomerular filtration rate.

of left ventricular diastolic dysfunction and a decrease in myocardial contractility.

Considering the differences in the main clinical characteristics of groups of patients with and without LVH, one-factor and multifactorial regression analysis of the association of clinical factors and the TNF gene genotype with the development of LVH was conducted (Table 4). For inclusion in the regression analysis, extended factors (age and level of MAP) were converted to discrete ones. By age, patients are divided into 2 groups according to the median indicator (60 years). According to the maximum level of blood pressure, patients are divided into groups according to the level corresponding to hypertension of 3 degrees (above and below 180 mm Hg). In a multifactorial regression analysis, the male gender, systolic pressure, age of patients, the presence of the TNF gene A allele in the genotype were independently associated with an increase in LVMI.

The discussion of the results

The possible association between the activation of inflammation processes and the formation of myocardial hypertrophy is actively discussed in the literature. The involvement of TNF in the formation of myocardial hypertrophy is confirmed by a number of clinical and experimental data. In a study on a group of 764 patients with hypertension, it was shown that the levels of TNF and IL-6 are more significant predictors of the development of concentric remodeling and concentric left ventricular hypertrophy than hemodynamic factors, and systolic blood pressure in particular. The level of blood pressure to a greater extent determined the increase in myocardial mass [3].

In patients with hypertrophic cardiopathy, a significantly higher level of tumor necrosis factor, IL-6 and serum amyloid P is registered compared with comparable healthy controls. At the same time, in patients with local myocardial fibrosis, the level of interleukins 1 and 4 is higher, as well as matrix metalloproteinase, which indicates different mechanisms for the development of these diseases [4]. In a group of patients with Fabry disease with a significant increase in the level of TNF, expression of TNF receptors was shown to associate the level of these markers with the severity of myocardial hypertrophy and the formation of diastolic myocardial dysfunction, as well as an increase in BNP and a heart failure clinic [5].

In experiments on mice, it was shown that an increase in the expression of TNF and its receptors may trigger the formation of hypertrophic cardiopathy and myocardial hypertrophy of aortic stenosis [6]. Experimental animals also showed a strong association between the level of cycling TNF and the activation of myocardial fibrosis and kidney processes in animals with elevated blood pressure [7].

The association between the development of myocardial hypertrophy and an increase in the level of tumor necrosis factor is explained by the fact that TNF enhances the effect of angiotensin II on the expression of various growth factors. This mechanism is mediated by the presence of a common signaling pathway for TNF and anti-tensin II. This signaling pathway is also associated with the mechanisms of antioxidant protection and includes mitogen-activated protein kinase, transforming growth factor beta1 and nuclear factor Kappa-B. (MAPK / TGF-β / NF-κB). A similar mechanism of interaction between TNF and RAAS has recently been confirmed in animal experiments [8].

In our study, we did not evaluate the plasma level of TNF, but used a genetic marker in the TNF gene associated with changes in the expression of factor.

The TNF gene is mapped in the chromosomal region 6p23-q12. One of the most interesting polymorphic variants of a gene is the replacement of G (-308) A, which is localized in the promoter of the gene. Associations of the rare A allele of this polymorphic variant with bronchial asthma, psoriatic arthritis and systemic lupus erythematosus are shown [9, 10, 11].

There is a lot of data on the association of the polymorphic marker G (-308) A with the risk of coronary complications [12]. Similar data were obtained, including by our group, in the framework of a multicenter observational study of ORACLE [13]. We also studied the association between the polymorphism of this gene and myocardial changes in patients with aortic stenosis, but no association was found [14].

There is evidence in the literature about the association of the A allele of the polymorphic marker G (-308) A of the TNF gene with the development of gestational hypertension and preeclampsia, obtained on a group of 1,623 pregnant women [15]. Also of interest are data on the association of allele A with the level of systolic blood pressure and the level of insulin in blood plasma in patients with metabolic syndrome, obtained in a meta-analysis that includes more than 800 patients [16]. There are data on the association of the carriage of allele A with the risk of developing diabetic nephropathy [17].

In our study, we showed for the first time the association of the A allele of the polymorphic marker G (-308) A of the TNF gene with the development of myocardial hypertrophy in patients with arterial hypertension and the formation of diastolic and systolic myocardial dysfunction. These data confirm the possible role of the TNF signaling pathway in the formation of myocardial changes and create prerequisites for further research.

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