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Polymorphisms of candidate genes determining the clinical and hemostasiological characteristics of endocarditis of various etiology

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ABSTRACT

Aim. To investigate polymorphisms of 18 genes as possible molecular genetic markers of predisposition or resistance to development of non-infective (NE) or infective endocarditis (IE).

Materials and methods. The study encompassed 81 patients with NE and 94 patients with IE. The control group included 225 conditionally healthy people. Polymorphisms of 18 genes were tested using polymerase chain reaction (PCR).

Results. For the first time, a statistically significant relationship was identified between gene polymorphisms and valvular vegetations: for genes in the hemostatic system – rs6025 (1691 G > A) of the *F5* gene (AG genotype), rs1126643 (807 C > T) of the *ITGA2* gene (TT genotype); for folate pathway genes – rs1805087 (2756 A > G) of the *MTR* gene (AG genotype) and rs11697325 (*–8202* A/G) of the *MMP9* gene (AA genotype) and rs2476601 (C1858T) of the *PTPN22* gene (TT genotype). The protective effect of gene polymorphisms was revealed: for the *NOS3* gene (4b / 4b genotype) and G (–572) C of the *IL6* gene (CC genotype). For two polymorphisms, an association with thromboembolic complications in NE was revealed: rs1126643 (807 C > T) of the *ITGA2* gene and rs1799889 (–675 5G > 4G) of the *PAI* (*SERPINE1*) gene. In IE, such an association was detected for the polymorphism rs11697325 (–8202 A/G) of the *MMP-9* gene.

Conclusion. The polymorphisms of candidate genes were revealed, that are associated with the clinical and hemostasiological characteristics of IE and NE. In NE, for the first time, the association with thromboembolic complications was identified for two polymorphisms: rs1126643 (807 C > T) of the *ITGA2* gene and rs1799889 (– 675 5G > 4G) of the *PAI-1* (*SERPINE1*) gene. In IE, such a relationship was detected for one polymorphism – rs11697325 (8202 A/G) of the *MMP-9* gene.

Keywords: endocarditis, candidate genes, single nucleotide polymorphism, hemostasis, thromboembolic complications

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study.

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Полиморфизмы генов-кандидатов, связанные с клинико-гемостазиологическими характеристиками эндокардитов разной этиологии

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РЕЗЮМЕ

Цель. Изучить полиморфизмы 18 генов как возможных молекулярно-генетических маркеров предрасположенности или резистентности к развитию неинфекционного (НЭ) и инфекционного (ИЭ) эндокардитов.

Материалы и методы. Пациенты с неинфекционным (81 человек) и инфекционным (94) эндокардитами; контрольная группа 225 условно здоровых человек. Полиморфизмы 18 генов изучали с помощью полимеразной цепной реакции (ПЦР).

Результаты. Впервые для полиморфизмов генов установлена статистически значимая ассоциация с синдромом вегетаций на клапанах сердца: для генов системы гемостаза – rs6025 (1691 G > A) гена *F5* (AG), rs1126643 (807 C > T) гена *ITGA2* (TT), гена фолатного цикла – rs1805087 (2756 A > G) гена *MTR* (AG), а также rs11697325 (–8202 A/G) гена *MMP9* (генотип AA) и rs2476601 (C1858T) гена *PTPN22* (TT). Выявлена «протективная» роль полиморфизмов: гена *NOS3* (4b/4b) и G (–572) C гена *IL6* (CC). Для двух полиморфизмов обнаружена ассоциация с тромбоэмболическими осложнениями при НЭ – rs1126643 (807 C > T) гена *ITGA2* и rs1799889 (– 675 5G > 4G) гена *PAI1* (*SERPINE1*) и для одного – при ИЭ – rs11697325 (–8202 A/G) гена *MMP-9*.

Заключение. Выявлены полиморфизмы генов-кандидатов, ассоциированные с клинико-гемостазиологическими характеристиками неинфекционного и инфекционного эндокардитов. Впервые при неинфекционном эндокардите для двух полиморфизмов обнаружена ассоциация с тромбоэмболическими осложнениями – rs1126643 (807 C > T) гена *ITGA2* и rs1799889 (–675 5G > 4G) гена *PAI1* (*SERPINE1*) и для одного полиморфизма – при инфекционном эндокардите – rs11697325 (–8202 A/G) гена *MMP-9*.

Ключевые слова: эндокардиты, гены-кандидаты, однонуклеотидный полиморфизм, гемостаз, тромбоэмболические осложнения

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИИТПМ – филиал ИЦиГ СО РАН (протокол № 16 от 03.06.2014).

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INTRODUCTION

Endocarditis is characterized by altered microcirculation (microthrombosis, vascular remodeling, platelet aggregation, fragmented blood flow) and blood rheology with development of disseminated intravascular coagulation [1]. The study of hemostatic system parameters is one of the promising directions for forming a panel of markers for risks of thromboembolic complications in endocarditis [2, 3]. Hyperfibrinogenemia is an independent factor leading to hypercoagulation [4]. Changes in platelet – leukocyte aggregate formation and thrombocytosis are the most important disorders of the hemostasis determining the development of hyperaggregation [5].

Genetic predisposition to endocarditis has been of great interest for scientists and clinicians [6–8]. There are data on the association of single nucleotide polymorphisms in the genes of the hemostatic system and folate pathway with vegetations on the cardiac valvular apparatus [9]. In the sample of 123 patients with IE, an association of a higher risk of IE with the TT genotype of the rs1205 polymorphism in the *CRP* gene was identified, while a decrease in the IE risk was correlated with the GA genotype of the rs1143634 polymorphism in the *IL1B* gene, the GT genotype of the rs3212227 polymorphism in the *IL12B* gene, the AC genotype of the rs1130864 polymorphism in the *CRP* gene, and the G allele of the rs1801197 polymorphism in the *CALCR* gene [10].

The aim of the study was to investigate polymorphisms of 18 genes as possible molecular and genetic markers of predisposition or resistance to development of non-infective (NE) and infective endocarditis (IE).

MATERIALS AND METHODS

The NE group included 81 patients aged 43.0 ± ± 13.9 years: 40 men and 41 women. The IE group consisted of 94 patients: 76 men and 18 women aged 41.0 ± 16.0 years. The control group included 225 conditionally healthy people of the same age.

An inclusion criterion was the presence of endocarditis. The diagnosis of NE was established after IE exclusion according to the Duke criteria [11]: negative blood culture for IE, normal body temperature, and the absence of sonographic signs of infection in the valves. In NE, only thickening and compaction of the valve flaps and minimal regurgitation were detected. In addition, there was no progression of heart disease and other complications of IE, such as tears, ruptures, and perforations of the valve flaps, as well as abscesses. Exclusion criteria encompassed pregnancy and recent acute conditions (trauma, polychemotherapy, surgery).

The informed consent was signed by all the patients in accordance with the ethical principles of the Declaration of Helsinki developed by the World Medical Association “Ethical Principles of Conducting Scientific Medical Research with Human Participation”. The study was approved by the Ethics Committee at the Research Institute of Therapy and Preventive Medicine (Protocol No. 16 of 03.06.2014).

Genomic DNA was isolated from 6–10 ml of venous blood, DNA extraction was performed using phenol – chloroform extraction. Polymorphisms of the genes of the hemostatic system and folate pathway were tested using real-time PCR on test systems manufactured by DNA-Technology (Russian Federation): rs6046 (10976 G > A) of the *F7* gene, rs5985 (103 G > T) of the *F13* gene, rs1800790 (- 455 G > A) of the *FGB* gene, rs1799963 (20210 G > A) of the *F2* gene, rs6025 (1691 G > A) of the *F5* gene, rs1126643 (807 C > T) of the *ITGA2* gene, rs5918 (1565 T > C) of the *ITGB3* gene, rs1799889 (-675 5G > 4G) of the *PAI-1* (*SERPINE1*) gene, rs1801131 (1298 A > C) of the *MTHFR* gene, rs1801133 (677 C > T) of the *MTHFR* gene, rs1805087 (2756 A > G) of the *MTR* gene, rs1801394 (66 A > G) of the *MTRR* gene. The polymorphisms of the *NOS3* (4a / 4b) and *ACE* (rs1799752) genes were tested us-

ing PCR with flanking sequences. The polymorphisms of the *CTLA4* (rs231775), *MMP-9* (matrix metalloproteinase-9) (rs11697325), *PTPN22* (rs2476601), and *IL6* (rs1800795) genes were tested using PCR – restriction fragment length polymorphism (RFLP). Genotyping was performed at the Research Institute of Therapy and Preventive Medicine – a branch of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences.

Statistical analysis was performed using SPSS, version 21.0. The distribution of genotype frequencies of all polymorphisms in the control group complied with the Hardy – Weinberg equilibrium. The odds ratio (OR) was calculated using the Woolf – Haldane analysis, which allows for calculations for a 2 x 2 table for cases when at least one of the table cells has a zero value. Statistical significance of differences in the frequencies of the studied variables in the alternative groups was determined by the $\chi 2$ test with the Yates correction for continuity and by the two-sided Fisher's exact test for 2 x 2 tables. To assess the development of thromboembolic complications, a bivariate logistic regression analysis of its association with gender, diagnosis, and hemostatic system parameters, including standardization by age, was performed. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The average age of the patients did not differ significantly. The majority of patients were young and middle-aged people. In the NE group, the percentage gender distribution was equal; in the IE group, men significantly prevailed, $p = 0.001$. According to the ultrasound findings, the degree of heart valve disorders varied from minor disorders (slight regurgitation, thickening of the valve flaps), which is typical of NE, to severe defects (heart valve stenosis or heart valve disease) in IE.

According to the genotyping findings, at the first stage, the genotype frequencies of the studied polymorphisms were determined in the groups of patients with endocarditis and the control group; then compliance of the genotype frequency distribution with the Hardy – Weinberg equilibrium was evaluated in the control group (using the $\chi 2$ test). In all the studied polymorphisms, the genotype frequency distribution complied with the Hardy – Weinberg equilibrium.

When studying the polymorphisms of 18 candidate genes for endocarditis, we revealed an association with five markers (Table 1). Carriers of the CT and CC genotypes of the rs1126643 polymorphism (807 C > T) in the *ITGA2* gene were identified in 88 % of NE patients and in 73 % of IE patients. For carriers of the TT genotype, the probability of vegetation formation in IE was 2 times higher than in the control group (OR = 2.36, 95% confidence interval (CI): 1.1–5.8, $p = 0.04$). In addition, carrying the A allele in the *MTR* gene doubled the OR for developing both NE and IE (OR = 2.02, 95 % CI: 1.05–3.92, $p = 0.04$), and carrying the AA genotype increased the OR for developing valvular vegetations by more than 2 times (OR = 2.35, 95% CI: 1.35–4.11, $p = 0.02$).

Table 1

Statistically significant parameters of polymorphisms of candidate genes for endocarditis (compared with the control group)

Non-infective endocarditis	Endocarditis	Infective endocarditis
For the *MMP-9* rs11697325 (-8202 A/G) AA genotype		
$p > 0.05$	OR = 1.95; 95% CI: 1.10–3.48, $p = 0.03$	OR = 2.31; 95% CI: 1.11–4.81, *p = 0.03*
For the *PTPN22* rs2476601 (C1858T) TT genotype		
$p > 0.05$	OR = 8.49; 95% CI: 1.67–43.20, $p = 0.006$	OR = 18.56; 95% CI: 3.59–96.01, $p = 0.0002$
Polymorphisms of genes of the hemostatic system		
For the *F5* rs6025 *(1691 G>A)* AG genotype		
occurrence frequency 8.9%, $p = 0.04$	$p > 0.05$	not detected
For the *ITGA2* rs1126643 *(807 C>T)* TT genotype		
$p > 0.05$	$p > 0.05$	OR = 2.36; 95% CI: 1.10–5.80, $p = 0.04$
MTR rs1805087 *(2756 A>G)* polymorphisms of the *MTR* gene of the folate pathway		
For the AG genotype		
OR = 0.47; 95% CI: 0.23–0.98, $p = 0.05$	OR = 0.44; 95% CI: 0.24–0.79, $p = 0.006$	OR = 0.4; 95% CI: 0.18–0.90, $p = 0.027$
For the AA genotype		
OR = 2.22; 95% CI: 1.11–4.45, $p = 0.03$	OR = 2.35; 95% CI: 1.35–4.11, $p = 0.03$	OR = 2.52; 95% CI: 1.19–5.32, $p = 0.02$
G allele		
$p > 0.05$	OR = 2.02, 95% CI: 1.05–3.92, $p = 0.04$	$p > 0.05$

The factor V Leiden mutation in IE was not detected. At the same time, the number of carriers of the factor V Leiden mutation (AG polymorphism) was significantly larger in NE (8.9 % vs. 3.6 % in the control group, $p = 0.04$).

A statistically significant increase in the frequency of carrying the AA genotype of the rs11697325 (-8202 A/G) polymorphism in the *MMP-9* gene was revealed in the group of patients with endocarditis (in 40 % of cases) compared with the control group (in 22% of cases), $p = 0.03$. Therefore, carrying the AA genotype doubled the risk of developing endocarditis compared with the control group, OR = 1.95, 95% CI: 1.1–3.48, $p = 0.03$. When comparing the IE group with the control group, carrying the AA genotype of the rs11697325 (-8202 A / G) polymorphism in the *MMP-9* gene increased the risk of developing endocarditis by more than 2 times, OR = 2.31, 95% CI 1.11–4.81, $p = 0.04$ (Table 1).

PTPN22 expressed on lymphocytes through formation of a complex with C-terminal Src kinase (CSK) suppresses subsequent mediators of T-cell receptor signaling. Substitution of the arginine amino acid residue with a tryptophan one (R620W) excludes the possibility of interaction between PTPN22 and CSK, which leads to impaired feedback regulation of activated lymphocytes [12]. The OR for developing IE in carriers of the TT genotype of the rs2476601 polymorphism (C1858T) in the *PTPN22* gene is significantly higher compared with carriers of the other two genotypes (C / T + C / C), OR = 18.56, 95% CI: 3.59–96.01, $p = 0.0002$ (Table 1).

Excessive production of prothrombin is a risk factor for myocardial infarction and thrombosis, including pulmonary embolism, which often has a fatal outcome [13]. Polymorphism rs1799963, caused by substitution of guanine (G) with adenine (A) at position 20210 of the *F2* gene, leads to its increased expression with a risk of arterial and venous thrombosis [14]. Among our patients with endocarditis, no carriers of the AG polymorphism in the *F2* gene were registered, so the contribution of this polymorphism to the development of vegetations on the cardiac valvular apparatus and thromboembolic complications could not be studied (Table 2).

Table 2

Statistically significant parameters of gene polymorphisms that prevent from development of endocarditis (compared with the control group)

Non-infective endocarditis	Endocarditis	Infective endocarditis
For the *NOS3* 4b / 4b genotype		
OR = 0.44; 95 % CI: 0.22–0.9, $p = 0.03$	OR = 0.47; 95 % CI: 0.27–0.81, $p = 0.006$	$p > 0.05$
For the *IL6* G (- 572) C CC genotype		
OR = 0.28, 95% CI: 0.08–0.96, $p = 0.03$	$p > 0.05$	$p > 0.05$

The *IL6* gene encodes the interleukin-6 (IL-6) protein, which takes part in the development of the immune response, initiating its own phase of reproduction. In the NE group, a decrease in the frequency of the SS genotype was detected, OR = 0.28, 95% CI: 0.08–0.96, $p = 0.03$ (Table 2).

When examining the polymorphisms in the nitric oxide synthase 3 (*NOS3*) gene, the frequency of the 4b / 4b genotype in the *NOS3* gene in the group of healthy individuals was higher than in patients with endocardia, so the presence of the 4b / 4b genotype in the *NOS3* gene reduced the probability of developing NE, OR = 0.44, 95 % CI: 0.22–0.9, $p = 0.03$ (Table 2). According to the data [15], a decrease in the content of nitric oxide disrupts normal vascular function and increases vascular tone and thrombus formation, and a decrease in the NOS3 activity has a protective effect.

According to the logistic regression analysis, the development of thromboembolic complications was revealed 1.5 times more often in IE than in NE, 95% CI: 1.06–3.94, $p = 0.04$. Associations of polymorphisms were revealed: rs1126643 (807 s > T) of the *ITGA2* gene, OR = 2.09, 95% CI: 1.14–3.85, $p = 0.02$ and rs1799889 (- 675 and 5G > 4G) of the *PAI-1* (*SERPINE1*) gene with the development of thromboembolic complications in NE, OR= 4.12, 95% CI: 1.25–13.63, $p = 0.02$; in IE, the rs11697325 (-8202 A / G) polymorphism of the *MMP-9* gene, OR = 3.43, 95% CI: 1.15–12.11, $p = 0.04$.

DISCUSSION

The most significant thromboembolic events in patients with endocarditis in our study were acute stroke and myocardial infarction. Possible associations between the development of thromboembolic complica-

tions and polymorphisms of 18 candidate genes were studied.

Disruption of the folate pathway leads to hyperhomocysteinemia, hypercoagulation, and heart valve thrombosis [16, 17]. Thromboembolic complications were detected 1.5 times more often in IE than in NE, $p = 0.04$. Associations of rs1126643 polymorphism (807 C > T) of the *ITGA2* gene and rs1799889 (-675 5G > 4G) polymorphism of the *PAI-1 (SERPINE1)* gene with the development of thromboembolic complications in NE were revealed; and in IE, such an association was identified for the rs11697325 (-8202 A / G) polymorphism of the *MMP-9* gene.

Studying polymorphisms of candidate genes for endocarditis allowed to identify 5 unfavorable prognostic markers. Platelet receptor genes are likely to be important factors of thrombotic risk upregulation.

In carriers of certain alleles and genotypes of the collagen receptor gene (*ITGA2* 807 C > T), adhesion of platelets to one another and to the vascular endothelium increases, which leads to increased thrombosis [18, 19]. For carriers of the TT genotype, the probability of vegetation formation in IE is twice as high as in the control group.

Disruption of the folate pathway contributes to hyperhomocysteinemia, which can lead to hypercoagulation and heart valve thrombosis [20, 21]. The *MTR* gene encodes the amino acid sequence of methionine synthase, one of the key enzymes of methionine metabolism, which catalyzes production of methionine from homocysteine via its remethylation. We found that carrying the A allele doubled the risk of developing both NE and IE (OR = 2.02, 95% CI: 1.05–3.92, $p = 0.04$), and carrying the AA genotype increased the risk of developing vegetations by more than 2 times.

It is known that in the presence of factor V Leiden mutation at position 1691 in the gene encoding coagulation factor V, adenine is replaced with guanine, so factor V is not cleaved by protein C, a natural physiological anticoagulant, as it happens under normal conditions, but becomes resistant to its action, which leads to an increase in the concentration of factor V in the blood serum. The factor V Leiden mutation has a modifying effect on the *PAI-1* polymorphism in terms of the risk of recurrent thromboembolic complications and increases the risk of venous thromboembolism by 4 times [16]. In the NE group, the number of factor V Leiden mutation carriers was significantly larger (8.9 % vs. 3.6 % in the control group, $p = 0.04$), while in the IE group, the factor V Leiden mutation was not detected.

Some proteins expressed in endocarditis may be used as favorable prognostic biomarkers. The study of protective gene variants revealed associations for two polymorphisms. Prothrombin is a precursor of thrombin and plays an important role in fibrin formation. Excessive production of prothrombin is a risk factor for myocardial infarction and various thromboses, including pulmonary embolism, which often has a fatal outcome [14]. The rs1799963 polymorphism, due to the replacement of guanine (G) with adenine (A) at position 20210 in the *F2* gene, leads to increased gene expression in the A variant. Patients carrying one copy of this allele have a 5-fold increased risk of thrombus formation; and among those with two copies of the 20210A allele, the risk increases by 50 times. Under such conditions, the carriers become prone to earlier or more severe arterial and venous thrombosis, especially if there is a family history of such events [13]. There were no carriers of the A allele among our patients with endocarditis.

The protective effect of the SS minor allele genotype of interleukin-6 (IL-6) in the development of acute coronary syndrome was shown [23]: compared with SS homozygotes, the SG genotype is characterized by a 2.2-fold increase in the risk of developing the disease. In our study, in the NE group, the presence of a protective SS genotype was identified compared with the IE group ($p = 0.03$).

A decrease in the content of nitric oxide disrupts normal vascular function and increases vascular tone and thrombus formation, while an increase in the activity of this enzyme has a protective effect [15]. In the study of *NOS3* gene polymorphisms, the frequency of carrying the 4b / 4b genotype of the *NOS3* gene in the group of healthy individuals was higher than in patients with endocarditis. Therefore, the presence of the 4b / 4b genotype of the *NOS3* gene statistically significantly ($p = 0.006$) reduces the risk of developing IE and NE.

CONCLUSION

We identified polymorphisms of candidate genes associated with clinical and hemostasiological characteristics of NE and IE, which can be used to assess the risk of developing these diseases, as well as for differential diagnosis. For 5 out of the 18 studied gene polymorphisms, an association with the risk of developing endocarditis, namely, with valvular vegetations, was revealed: for proinflammatory genes – rs11697325 (-8202 A / G) of the *MMP-9* gene (AA genotype) and rs2476601 (C1858T) of the *PTPN22* gene (TT

genotype); for genes of the hemostatic system – rs6025 (1691 G > A) of the *F5* gene (AG genotype) and rs1126643 (807 C > T) of the *ITGA2* gene (TT genotype); for the gene of the folate pathway – rs1805087 (2756 A > G) of the *MTR* gene (AG genotype).

For the first time, the protective effect of two gene polymorphisms for developing endocarditis was established: proinflammatory gene polymorphisms of the *NOS3* gene (4b / 4b genotype), G (- 572) C of the *IL6* gene (CC genotype).

In terms of differential diagnosis, for the first time, an association with thromboembolic complications was revealed for two polymorphisms in NE – rs1126643 (807 C > T) of the *ITGA2* gene and rs1799889 (-675 5G > 4G) of the *PAI-1* (*SERPINE1*) gene. In IE, an association with thromboembolic complications was revealed for one polymorphism – rs11697325 (-8202 A / G) of the *MMP-9* gene.

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Authors contribution

Bakhareva Y.S. – examination and treatment of patients, collection and preparation of biomaterial, review of literature, statistical processing of the research results and their interpretation, drafting of the manuscript. Maksimov V.N. – design of the study, provision of material and technical facilities for laboratory tests, interpretation of the results. Ivanova A.A. – preparation of samples with biomaterial, carrying out of PCR. Chapaeva N.N. – differential diagnosis of endocarditis, consultations on research planning. Aidagulova S.V. – review of literature, editing of the manuscript. Voevoda M.I. – consultations on interpretation of the results, design of the study.

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