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# STUDYING THE ROLE OF HEMOSTASIS SYSTEM GENE POLYMORPHISM IN THE MECHANISMS OF ROSACEA DEVELOPMENT

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**Abstract:**

The polymorphism of the genes of the hemostasis system was studied. The study identified genes that are presumably involved in the occurrence and development of rosacea, as well as affecting the severity of the disease. Unfavorable variants of the genes of the hemostasis system, in particular the platelet link: ITGB3: 1565 T>C rs5918, PAI-1: –675 5G>4G rs1799889. The identified loci provide specificity of inflammatory mechanisms in rosacea, and identify potential pathways for therapeutic intervention.

**Keywords:** rosacea, SNP, blood coagulation genes

Rosacea is a chronic inflammatory disease of the skin of the face, manifested by erythema, multiple telangiectasias, papules, pustules, as well as hyperplasia of the connective tissue and sebaceous glands, with exogenous and endogenous provoking factors. The protracted course of the disease, the tendency to relapse, discoloration, skin ulceration and scarring in rosacea have a detrimental effect on the physical, mental and social health of the population. The problems of etiopathogenesis and treatment of patients with rosacea are becoming increasingly important in modern dermatology, despite the long history of studying the incidence and pathomorphogenesis of this dermatosis. In recent years, there has been an increase in the incidence of rosacea up to 5% among other dermatoses. There are numerous works of domestic and foreign researchers devoted to this disease, but the etiology and pathogenesis are still not fully understood. Inheritance, immune dysregulation, microbial invasion, chronic inflammation, vascular hyperreactivity, and other environmental factors are believed by many authors to play an important role in the development of the disease.

An increasing role in the occurrence and development of rosacea in recent years is assigned to the genetic factor. According to the literature, risk factors for rosacea such as heredity, skin type (Fitzpatrick IV ) and specific genetic variants ( ApaI) have already been identified. G / T ), which convincingly indicates a genetic factor of predisposition to this pathological condition. Various studies have identified some genes indicating pathogenic terms such as: intercellular adhesion molecule-1 gene ( ICAM -1) associated with skin barrier function, glutathione S - transferase theta 1 ( GSTT 1) and/or glutathione- S - transferase μ -1 ( GSTM 1) and nucleotide-binding domain, leucine-rich repeat and pyrine domain containing receptor gene 3 ( NLRP 3) associated with the immune system and inflammation, human leukocyte antigen- DR alpha ( HLA - DRA ), butyrophylline-like 2 ( BTNL 2) and trans-transcriptional activator signal transducer ( STAT ) gene, also related to the immune system. Studies based on familial, twin, and regional factors (Celtic and Northern European ancestry) also suggest a genetic component to rosacea. Moreover, genetic research on rosacea has been published every year since 2015 and has been trending to increase with the advent of new technologies such as genome sequencing, omics analysis and other bioinformatics tools used for various studies, including rosacea.

Given the above, there is an obvious need for research to further search for causal genes, paying special attention to the study of their functionality. The study of the genes of the blood coagulation system can give a more complete picture of the causes and mechanism of the development of the disease, and it is also relevant to study their role as potential predictors of the risk of occurrence and severity of rosacea.

**MATERIALS AND METHODS.** The study was conducted on the basis of the Immunogen Research and Diagnostic Center test at the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan . The study group consisted of 27 patients diagnosed with rosacea of varying severity. The control group included 20 apparently healthy subjects who did not suffer from rosacea.

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Genotyping of samples was carried out by the method of polymerase chain reaction in the "real time" mode. To prepare a DNA bank from human peripheral blood , venous blood from the cubital vein, 3-5 ml in volume, was used ; acid ). Blood for further processing could be stored up to 24 hours at a temperature of +4 C. To obtain genomic DNA, a two-stage method of lysis of blood cells was used: 1) obtaining a lysate concentrate of leukocyte cells; 2) further purification of leukocyte mass lysates was carried out by the method of alcohol-salt treatment [ Ausubel , FM, Brent, R., Kingston, RE, Moore , DD, Seidman , JG , Smith, J . A. & Struhl , K. Current Protocols in Molecular Biology - Wiley , New York, 2001.] in a modernized form. In the present study, we adjusted the DNA concentration to 100 ng /µl. Measurement of DNA concentration was carried out on a NanoDrop ™ Lite spectrophotometer ( Thermo Fisher Scientific, USA). Real-time DNA sequence analysis based on Q-PCR HRM technology and PCR detection by microarray electrophoresis. For typing polymorphic variants of the studied candidate genes (Table 1), HRM- qPCR methods ( Stratagene M*3005P, Agilent Technologies, Germany; DT-Prime, Russia) and microarray PCR detection method (MCE 202 MultiNA , Zhimadzu , Japan) were used. ).

Statistical processing was carried out using the JAMOVI version 1.1.9 program. To present qualitative data, both absolute and relative indicators (n, %) were used. To present quantitative data, descriptive statistics were used: mean value ( mean ), standard deviation ( standard deviation ), median ( median ), 25th and 75th percentile. Since the sample was characterized by a predominantly irregular distribution, the Shapiro- Wilk test was used to determine the normality of the distribution of each quantitative trait, and non-parametric tests were used to compare groups - the Mann-Whitney test. To assess the differences in relative values, we used the analysis of X2 contingency tables. The selected critical significance level is 5% (0.05).

**Table 1. Description of the studied genes**

| **Gene** | **SNP** |
|---|---|
| plasma link of hemostasis | |
| F2 - prothrombin<br>(blood clotting factor II) | 20210 G>A |
| F5 - proaccelerin<br>(factor V of blood clotting)<br>Forms the prothrombinase complex, which converts prothrombin to thrombin | 1691 G>A |
| F7 - proconvertin<br>(factor VII of blood clotting)<br>Interacts with factor III, activates factors IX and X - formation of a blood clot | 10976 G>A |
| F13A1 - fibrinase<br>(factor XIII of blood clotting)<br>Participates in the formation of insoluble fibrin. Stabilizes the fibrin clot | 103 G>T |
| FGB - fibrinogen<br>(blood clotting factor I)<br>Forms insoluble protein fibrin at the final stage of blood coagulation | –455G >A |
| platelet link of hemostasis | |
| ITGA2-α2 - integrin<br>(platelet receptor for collagen)<br>Provides interaction of platelets with damaged vessel walls | 807 C>T |
| I TGB3-β3 - integrin<br>(platelet receptor for fibrinogen)<br>Participates in platelet aggregation and adhesion to subendothelial structures | 1565 T>C |
| fibrinolytic component of hemostasis | |
| PAI-1 - serpin<br>(antagonist of tissue plasminogen activator )<br>Limits fibrinolytic activity at the location of the hemostatic plug | –675 5G>4G |

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## RESULTS AND DISCUSSION

The age of patients in the comparison group ranged from 18 to 64 years and averaged 39.1±13.6 years. The age of patients in the control group ranged from 18 to 40 years, the average age was 35.2 ± 9.1. In the comparison group, women accounted for 52% of the examined, men 48%. In the control group, women accounted for 45%, men - 55%. The incidence of chronic cholecystitis among patients in the study group was significantly higher ( $p < 0.05$) than in the comparison group. Clinical data of patients are presented in Table 2.

**Table 2. Clinical characteristics of the examined study and control groups.**

| Parameter | Comparison group | Control group |
|---|---|---|
| Number of examined | 27 | 20 |
| Gender:<br>women men | 14 (52.0%)13 (48.0%) | 9 (45%)11 (55%) |
| Age, year | 39.1 (±13.6) | 35.2 (±9.1) |
| Rosacea subtype :<br>ETPPPP | 12 (44.4%)15 (55.6%) | -- |
| presence of Demodex fol . | 14 (52.0%) | - |
| Status _ localis :<br>erythema ,<br>telangiectasia,<br>papules, pustules | 25 (92.6%)23 (85.2%)13 (48.0%)13 (48.0%) | ---- |
| Localization:<br>cheeks, forehead, chin, wings of the nose | 19 (70.4%)9 (33.3%)4 (14.8%)9 (33.3%) | ---- |
| Pathology of the gastrointestinal tract:<br>gastroduodenitis, cholecystitis, fatty hepatosis<br>H.pilory , diffuse thickening of the liver, chronic pancreatitis, gastritis | 5 (18.5%)6 (22.2%)1 (3.7%)1 (3.7%)1 (3.7%)1 (3.7%) 3 (11.1%) | 2(10%)-----2(10%) |
| Astheno -neurotic condition | 4 (14.8%) | 2 (10%) |
| Diabetes | 2 (7.4%) | 15%) |
| **Mycosis** | **2 (7.4%)** | **-** |

## POLYMORPHISMS OF THE GENES OF THE HEMOSTASIS SYSTEM .

Structurally, in the hemostasis system, there are: a plasma link (coagulation factors and fibrin formation - genes F2, F5, F7, F13, FGB) and a vascular-platelet link (platelet adhesion to the vascular wall, vascular contraction, platelet aggregation, thrombus formation - ITGA2 genes, ITGB3, PAI-1). When studying polymorphisms of 8 genes of the blood coagulation system, namely F2:20210 G>A, F5:1691 G>A, F7:10976 G>A, F13A1:103 G>T, FGB: –455 G>A, ITGA2: 807 C>T, ITGB3: 1565 T>C , PAI-1: – 675 5G>4G, in patients with rosacea, the data presented in Table 3 were obtained. In the study of the plasma hemostasis link in the studied patients, a favorable G / G allele was predominantly detected . Heterozygous and homozygous unfavorable gene variants were found in a small part of the examined.

At the same time, unfavorable gene variants were found in the vascular-platelet link both in the homozygous and in the heterozygous state in a significant part of the subjects. A significant part of the unfavorable variants is found in the ITGB3: 1565 T>C

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and PAI-1: –675 5G>4G genes in comparison with the control group.

**Table 3. Frequency of occurrence of polymorphic alleles of genes of the hemostasis system**

| gene polymorphism | frequency distribution of genotypes | | | | | p |
|---|---|---|---|---|---|---|
| | Genotype | comparison group | | control group | | |
| | | n | % | N | % | |
| F2:20210 G>A rs1799963 | G/G | 27 | 100 | 20 | 100 | 1.0 |
| | G/A | 0 | 0.0 | 0 | 0 | - |
| | A/A | 0 | 0.0 | 0 | 0 | - |
| F5:1691 G>A rs6025 | G/G | 26 | 96.3 | 20 | 100 | 0.9 |
| | G/A | 1 | 3.7 | 0 | 0 | 0.9 |
| | A/A | 0 | 0.0 | 0 | 0 | - |
| F7:10976 G>A rs6046 | G/G | 18 | 66.7 | 7 | 35 | **0.03** |
| | G/A | 9 | 33.3 | 12 | 60 | 0.07 |
| | A/A | 0 | 0.0 | 1 | 5 | 0.24 |
| F13A1:103 G>T rs5985 | G/G | 21 | 77.8 | 13 | 65 | 0.3 |
| | G/T | 5 | 18.5 | 7 | 35 | 0.2 |
| | T/T | 1 | 3.7 | 0 | 0 | 0.24 |
| FGB: -455G >A rs1800790 | G/G | 18 | 66.7 | 15 | 75 | 0.6 |
| | G/A | 8 | 29.6 | 5 | 25 | 0.7 |
| | A/A | 1 | 3.7 | 0 | 0 | 0.24 |
| **ITGA2: 807C >T rs1126643** | C/C | 13 | 48.2 | 17 | 8 5 | 0.25 |
| | C/T | 10 | 37.0 | 2 | 1 0 | 0.6 |
| | T/T | 4 | 14.8 | 1 | 5 | 0.07 |
| **ITGB3: 1565 T>C rs5918** | **T/T** | **22** | **81.5** | **20** | **100** | **0.04** |
| | **T/C** | **5** | **18.5** | **0** | **0** | **0.04** |
| | C/C | 0 | 0.0 | 0 | 0 | - |
| **PAI-1: -675 5G>4G rs1799889** | **5G/5G** | **8** | **29.6** | **13** | **65** | **0.0 2** |
| | **5G/4G** | **eleven** | **40.8** | **6** | **thirt y** | **0.4** |
| | **4G/4G** | **8** | **29.6** | **1** | **5** | **0.03** |

ITGA2-α2: 807 C>T rs1126643 is an integrin , a platelet receptor for collagen, which ensures the interaction of platelets with a damaged vessel wall. Integrin , gene alpha 2 (ITGA2: 807 C>T), encodes GP Ia / IIa ( platelet glycoprotein Ia / IIa ) - among the major integrin receptors, also known as α2β1 integrin , located on chromosome 5q23-31. The ITAG2 gene SNP alters the expression of GP Ia / IIa C807T. GP Ia / IIa is responsible for platelet adhesion to type I collagen. Therefore, GP deficiency Ia / IIa , acquired or congenital, leads to disruption of the platelet adhesion process. The data obtained when compared with the control group was not significant, but there is a tendency ( p = 0.07) for the presence of differences in comparison with the control group.

ITGB3-β: 31565 rs5918 - integrin , platelet receptor for fibrinogen. Participates in aggregation and adhesion of platelets to subendothelial structures. Integrin -β3 ITGB3: 1565 T>C, a common polymorphism in ITGB3 known as human platelet antigen 1 (HPA-1b, PlA2, or rs5918), arises from a single nucleotide substitution at position 1565 in ITGB3 exon 2, resulting in a substitution leucine (PlA1) to proline (PlA2) at residue 33. SNP has been extensively studied as a hereditary risk factor for acute coronary syndrome, and its effect on platelet function has also been studied. The polymorphism could potentially influence the activity of the GPIIb / IIIa complex and could also be associated with thrombosis. According to the above studies, GPIIb / IIIa is considered to be a platelet-to-platelet contact receptor playing an important role in platelet aggregation, and its SNPs are associated with platelet hyperreactivity. As is known, an increase in the aggregation properties of platelets is

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closely related to the state of the vascular wall. The participation of platelets in the hemostasis system in rosacea is determined by their angiotrophic function (the ability to maintain the normal structure and function of microvessels, their resistance to damaging effects, as well as the ability to maintain spasm of damaged vessels by secreting vasoactive substances, such as serotonin, norepinephrine and others). Comparison of the study group with the control group in the ITGB3: 1565 T>C gene revealed significant differences in the T /C genotype ( $p < 0.05$).

Plasminogen activator inhibitor type 1 (PAI-1: -675 5G>4G), also known as protein serpinopeptidase inhibitor branch E member 1 (SERPINE1), belongs to the superfamily of serine protease inhibitors . It plays an important role in the regulation of the fibrinolytic system, rapidly inhibiting tissue-type plasminogen activator ( tPA ) and plasminogen activator urokinase type ( uPA ) which cannot cleave plasminogen to form plasmin. The gene encoding PAI-1 has several polymorphic loci, among which the most commonly studied insertion-deletion polymorphism is 4G/5G, containing either 4 or 5 (4G/5G) guanine bases on the 675 PAI-1 promoter. Various variants of the PAI-1 gene alter the level of PAI-1, which leads to a decrease in plasma fibrinolytic activity. To date, associations between PAI-1 4G/5G polymorphism and the risk of atopic dermatitis, metabolic syndrome, etc. have been studied in various publications. Significant differences were found in the homozygous 4 G /4 G variant compared with the control group ( $p <0.05$), which suggests a decrease in plasma fibrinolytic activity in these patients.

## CONCLUSION:

Thus, rosacea is a polyetiological ( multifactorial ) independent dermatosis with the participation of many pathological reactions in its pathogenesis. The main cause of rosacea is a genetic predisposition that leads to lymphatic vasculopathy . The implementation of heredity is facilitated by various exogenous and endogenous triggers. The study identified genes that are presumably involved in the occurrence and development of rosacea, as well as affecting the severity of the disease. Unfavorable variants of the genes of the hemostasis system, in particular the platelet link, are ITGB3: 1565 T>C rs5918, PAI-1: –675 5G>4G rs1799889.

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