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Research Article

ANALYSIS OF THE ROLE OF FOLATE CYCLE GENES IN THE PATHOGENESIS OF ROSACEA

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ABSTRACT

The polymorphism of the genes of the folate cycle 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 folate metabolism genes: MTHFR: 1298 A>C rs1801131, MTHFR: 677C>T rs1801133. An increase in the level of homocysteine in the blood of patients with rosacea turned out to be significant. The identified loci provide specificity of inflammatory mechanisms in rosacea, and identify potential pathways for therapeutic intervention.

KEYWORDS

Rosacea, SNP, folate cycle genes.

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INTRODUCTION

Rosacea is a chronic inflammatory skin disease of unknown etiology. The disease is characterized by recurrent flushing, erythema, papules, telangiectasias, edema, pustules, or a combination of these symptoms. 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 pathophysiology of rosacea is not fully understood, but heredity, 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 data, such risk factors for rosacea as heredity, skin type (Fitzpatrick IV) and specific genetic variants (ApaI G/T) have already been identified, which strongly 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 (ICAM-1) gene associated with skin barrier function, glutathione S-transferase theta 1 (GSTT1) and/or glutathione S-transferase μ -1 (GSTM1) and nucleotide-binding domain, leucine-rich repeat and pyrine domain containing immune system and inflammation-associated receptor gene 3 (NLRP3), human leukocyte antigen-DR alpha (HLA-DRA), butyrophylline-like 2 (BTNL2) 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 genes of the blood coagulation system, the genes of folate metabolism 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 test scientific and diagnostic center 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. The homocysteine level was diagnosed using the ICLA method, the IMMULITE 2000 Xpi device with the appropriate reagents (SIEMENS, Germany).

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; 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

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leukocyte cells; 2) further purification of leukocyte mass lysates was carried out by the method of alcohol-salt treatment [Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. Current Protocols in Molecular Biology- Wiley, New York, 2001.] in an updated form. In the present study, we adjusted the DNA concentration to 100 ng/µl. DNA concentration was measured 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. For the presentation of quantitative data, descriptive statistics data were presented: 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
genes associated with folate cycle disorders	
MTHFR - methylenetetrahydrofolate reductase Decreased functional activity of the enzyme, increased plasma homocysteine	677 C>T
MTHFR - methylenetetrahydrofolate reductase Accompanied not only by a decrease in enzyme activity, but also by an increase in plasma homocysteine concentration and a decrease in folate levels	1298 A>C
MTR - B12 dependent methionine synthase	2756 A>G
MTRR – methionine synthase reductase	66 A>G

RESULTS AND DISCUSSION

The age of patients in the comparison group ranged from 15 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 of the study group was significantly higher ($p<0.05$) than in the comparison group. The concentration of homocysteine in the blood of subjects in the comparison group was also significantly higher ($p<0.001$) compared to the control group. Clinical data of patients are presented in Table 2.

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

Parameter	Comparison group	Control group
Number of examined	27	20
Sex:		
female	14 (52,0%)	9 (45%)
male	13 (48,0%)	11 (55%)
Age, year	39,1 (±13,6)	35,2 (±9,1)
Subtype of rosacea:		
ETS	12 (44,4%)	-
PPS	15 (55,6%)	-
Demodex fol.	14 (52,0%)	-
Status localis:		
erythema	25 (92,6%)	-
teleangiectasia	23 (85,2%)	-
papules	13 (48,0%)	-
pustules	13 (48,0%)	-
Localization:		
cheeks	19 (70,4%)	-
forehead	9 (33,3%)	-
chin	4 (14,8%)	-
wings of the nose	9 (33,3%)	-
Pathology of the gastrointestinal tract:		
gastroduodenitis	5 (18,5%)	2 (10%)
cholecystitis	6 (22,2%)	-
fatty hepatosis	1 (3,7%)	-
H.pilory	1 (3,7%)	-
diffuse thickening of the liver	1 (3,7%)	-
chronic pancreatitis	1 (3,7%)	-
gastritis	3 (11,1%)	2 (10%)

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Astheno-neurotic condition	4 (14,8%)	2 (10%)
Diabetes	2 (7,4%)	1 (5%)
Mycosis	2 (7,4%)	-
Homocysteine (µmol/L)	**12,4 (±6,9)**	**7,1 (±2,03)**

Polymorphisms of the folate cycle genes.

The genes for the folate cycle enzymes are responsible for the activity of methylation enzymes, which are responsible for many enzymatic transformations. The MTHFR gene encoding methylenetetrahydrofolate reductase, one of the key enzymes of the folate cycle, is located at position 1p36.3. The two most common MTHFR gene polymorphisms, 677 C>T (rs1801133) and 1298 A>C (rs1801131), are associated with reduced enzyme activity. Single nucleotide substitutions (SNP) rs1801133 (677C>T) and rs1801131 (1298A>C) in this gene lead to the formation of a thermolabile form of the enzyme, a decrease in its activity and, as a result, an increase in the level of homocysteine in the blood and a decrease in methionine synthesis. Homocysteine is a sulfur-containing amino acid resulting from the metabolism of methionine. Homocysteine is converted to methionine with vitamin B12 and folic acid as cofactors. In the metabolic cycle of homocysteine synthesis, the lack of these vitamin-containing cofactors leads to an increase in homocysteine levels. Hyperhomocysteinemia is associated with various systemic diseases, including cardiovascular, cerebrovascular, and neuropsychiatric conditions. A number of studies have identified potential mechanisms by which homocysteine may contribute to the development of endothelial dysfunction, including platelet activation. Many studies have demonstrated an increased incidence of hyperhomocysteinemia in patients with various inflammatory skin diseases, including acne, vitiligo, psoriasis, and hidradenitis suppurativa. The accumulation of homocysteine causes damage to the vascular endothelium. Homocysteine has both atherogenic and thrombovascular effects, and also has a neurotoxic effect. The level of homocysteine in the comparison group in our study was significantly higher ($p<0.001$) than in the control group (Table 3).

Table 3. Frequency of occurrence of polymorphic alleles of folate metabolism genes.

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gene polymorphism	genotype distribution frequency					P
	genotype	comparison group		control group		
		N	%	N	%	
MTHFR: 1298 A>C rs1801131	A/A	16	59,3	17	85	0,05
	A/C	**8**	**29,6**	**1**	**5**	**<0,05**
	C/C	3	11,1	2	10	>0,05
MTHFR: 677C>T rs1801133	C/C	13	48,1	16	80	>0,05
	C/T	**9**	**33,3**	**1**	**5**	**<0,05**
	T/T	5	18,6	3	15	>0,05
MTR: 2756 A>G rs1805087	A/A	17	63,0	12	60	>0,05
	A/G	9	33,3	8	40	>0,05
	G/G	1	3,7	0	0	>0,05
MTRR: 66 A>G rs1801394	A/A	9	33,3	5	25	>0,05
	A/G	11	40,7	12	60	>0,05
	G/G	7	26,0	3	15	>0,05

When studying the polymorphisms of the genes of the folate cycle, data were obtained that suggest the presence of the influence of the metabolism of folic acid, on the mechanism of development of rosacea. Variants of folate metabolism genes, leading to an increase in the level of homocysteine in the blood, also contribute to the traumatization of the vascular endothelium.

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 folate metabolism genes: MTHFR: 1298 A>C rs1801131, MTHFR: 677C>T rs1801133. An increase in the level of homocysteine in the blood of patients with rosacea turned out to be significant.

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