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Molecular And Genetic Mechanisms Of Cardiovascular Pathology In Patients With Rheumatoid Arthritis

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Abstract

Annotation. Rheumatoid arthritis is a disease where hereditary predisposition is associated with the activation of innate immunity, the HLA system, cytokine networks and others. The formation of substances in the body that have pro- and anti-inflammatory effects can be influenced by single nucleotide polymorphisms of a variety of genes, being one of the most frequent causes of changes in their sequence. Given the fact that the inflammatory process plays a significant role in the development of RA and atherosclerosis, also single-nucleotide polymorphisms may be involved in the development and progression of these diseases, which should be taken into account when identifying groups of high cardiovascular risk. **Purpose:** Based on the analysis of molecular genetic features, to identify structural and functional changes in the cardiovascular system, as well as to optimize early diagnosis of cardiovascular pathology in RA patients. **Materials and methods.** 100 patients of the main group and 30 individuals of the control group were examined for VEGF gene C936T and MMP 9 gene C1562T. Among them, there were 80 females (80%) and 20 males (20%). The median age was 48 years [41; 54] and the disease duration was 5 years [3; 8]. The control group included 30 practically healthy individuals comparable in age to the sample of RA patients. There were 21 (70%) women and 9 (30%) men. The mean age of women was 42.67±7.64 years. **Results.** The allele frequency of VEGF C936T (rs3025039) gene differs in different population groups, therefore it is considered necessary to conduct area-based studies. Heterozygosity in Caucasoid and Mongoloid populations varies from 12.5% to 42% worldwide; in a genotype study of the Uzbek population in Samarkand region, heterozygosity was 63%. The high degree of heterozygosity for this polymorphism makes it the most promising for further analysis of associations with the development of multifactorial diseases. **Conclusions.** The molecular genetic markers, allele T and genotype TT of VEGF 936 (rs3025039) and MMP9 1562 (rs3918242) genes detected in RA patients are associated with increased cardiovascular risk in rheumatoid arthritis.

Keywords: rheumatoid arthritis, molecular genetic features, cardiovascular disease, vascular endothelial growth factor, matrix metalloproteinase-9.

Introduction.

The course of atherosclerotic vascular lesions worsens in RA patients against the background of chronic inflammation, which causes a high mortality rate [1, 2]. The development of both RA and cardiovascular pathology is influenced by complex interactions between genetic changes in the organism and environmental factors. Single nucleotide polymorphisms (SNPs) located in the promoter regions of genes can influence the level of expression to a significant extent, resulting in the formation of predisposition to a particular response to a stimulus. To date, one of the urgent tasks of preventive and personalized medicine is the search for genetic markers in RA.

Among the factors of RA immunopathogenesis, one of the most important can be considered the impairment of synovial angiogenesis, which affects the degree of vascularization of the extracellular matrix and synovial membrane. It should be noted that endothelial dysfunction, which develops during pathological angiogenesis in patients with RA, plays an important role in the development of cardiovascular changes [3, 4]. Vascular endothelial growth factor (VEGF) is one of the mediators of angiogenesis regulation, which play an angiogenic, inflammatory, and destructive role in both RA and CVD [4, 5].

Matrix metalloproteinase-9 (MMP9 or gelatinase B) like MMP2 are involved in the degradation of type IV collagen. MMP9 is frequently expressed by leukocytes and its precursors. High concentrations of MMP9 in the blood are observed in the area of accumulation of large numbers of foam cells and atherosclerotic plaque. The development of coronary artery pathology, as well as the severity of their lesions, was associated with the MMP9 C-1562T polymorphism (rs3918242). In addition, this polymorphism was associated with the stiffness of the arterial wall, so patients with arterial hypertension and the presence of the T allele had a high risk of complications from the cardiovascular system [6, 7, 8].

Purpose of the study: Based on the analysis of molecular genetic features, to identify structural and functional changes in the cardiovascular system, as well as to optimize early diagnosis of cardiovascular pathology in RA patients.

Materials and methods:

100 patients of the main group and 30 individuals of the control group were examined for VEGF gene C936T and MMP 9 gene C1562T. Among them, there were 80 females (80%) and 20 males (20%). The median age was 48 years [41; 54] and the disease duration was 5 years [3; 8]. The control group included 30 practically healthy individuals comparable in age to the sample of RA patients. There were 21 (70%) women and 9 (30%) men. The mean age of women was 42.67±7.64 years.
Patients were selected from 3 groups for the study:
Group 1 - 38 RA patients without RF (risk factors): women - 30 (78.9%), men - 8 (21.1%), median age was 43 years [34; 48], disease duration - 4 years [2.5; 6].
Group 2 - 34 RA patients with RF: women - 28 (82.4%), men - 6 (17.6%), median age was 44 years [40; 52.25], disease duration - 6 years [3.25; 7.25].
Group 3 - 28 RA patients with CVD (cardiovascular disease): women - 22 (78.6%), men - 6 (21.4%), median age was 49.5 years [43.25; 54.26], disease duration - 6 years [5; 8].

Results:

The allele frequency of VEGF C936T (rs3025039) gene differs in different population groups, therefore it is considered necessary to conduct area-based studies. Heterozygosity in Caucasoid and Mongoloid populations varies from 12.5% to 42% worldwide; in a genotype study of the Uzbek population in Samarkand region, heterozygosity was 63%. The high degree of heterozygosity for this polymorphism makes it the most promising for further analysis of associations with the development of multifactorial diseases.

Conclusions.

The molecular genetic markers, allele T and genotype TT of VEGF 936 (rs3025039) and MMP9 1562 (rs3918242) genes detected in RA patients are associated with increased cardiovascular risk in rheumatoid arthritis.

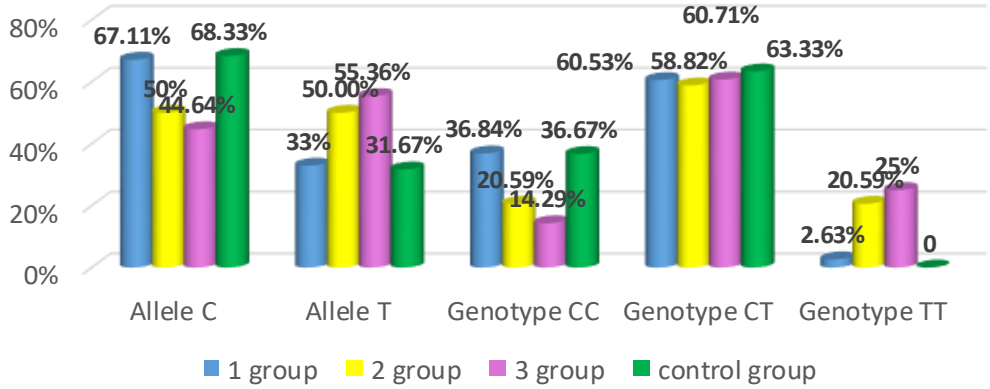

Figure 1: Prevalence of VEGF C936T (rs3025039) alleles and genotypes

The prevalence of the C allele was almost identical (Figure 1) in group 1 and control patients (67.1% and 68.3%, respectively). A statistically significant prevalence of the T allele was observed in groups 2 and 3 of the study (50% and 55.36%, respectively). There is a tendency to decrease the occurrence of CC genotype in patients from groups 2 and 3 (20.59%, 14.29%). In the groups of RA patients and controls, heterozygosity of 60% and 63.3%, respectively, was close in frequency. The appearance of homozygous TT mutant genotype of VEGF C936T gene, which was absent in the control group, was also noted in the groups of RA patients.

Table 1. Differences in the frequency of allelic and genotypic variants of ST polymorphism in VEGF C936T (rs3025039) gene in the group of RA patients and control group

Alleles and genotypes	Number of alleles and genotypes examined				χ2	p	RR	95 %CI	OR	95%CI
	1 group - RA patients without RF		Control group							
	n	%	n	%						
C	51	67,1	41	68,3	0,0	0,90	1,0	0,53 - 1,82	1,0	0,46 - 1,95
T	25	32,9	19	31,7	0,0	0,90	1,0	0,46 - 2,27	1,1	0,51 - 2,18
C/C	14	36,8	11	36,7	0,0	0,99	1,0	0,43 - 2,37	1,0	0,37 - 2,72
C/T	23	60,5	19	63,3	0,1	0,90	1,0	0,41 - 2,21	0,9	0,33 - 2,38

Analysis of VEGF gene polymorphism at position +936 among patients of group 1 and control groups (Table 1) revealed no statistically significant differences (C allele - 67.1% vs. 68.3%, p=0.90; T allele - 32.9% vs. 31.7%, p=0.90; CC genotype - 36.8% vs. 36.7%, p=0.99; ST genotype - 60.5% vs. 63.3%, p=0.90).

Table 2. Differences in the frequency of allelic and genotypic variants of ST polymorphism in VEGF C936T gene (rs3025039) in the group of RA patients with FR and control group

Alleles and genotypes	Number of alleles and genotypes examined				χ2	p	RR	95%CI	OR	95%CI
	2 group - RA patients with RF		Control group							
	n	%	n	%						
C	34	50,0	41	68,3	4,4	0,05	0,7	0,39 - 1,37	0,5	0,23 - 0,95
T	34	50,0	19	31,7	4,4	0,05	1,4	0,61 - 3,08	2,2	1,05 - 4,42
C/C	7	20,6	11	36,7	2,0	0,20	0,6	0,16 - 1,92	0,5	0,15 - 1,35
C/T	20	58,8	19	63,3	0,1	0,80	0,9	0,37 - 2,3	0,8	0,3 - 2,27

Analysis of VEGF gene polymorphism at position +936 showed (Table 2) statistically significant prevalence of C allele in the control group (68.3% vs. 50%; p=0.05, OR=0.5; 95%CI=0.23 - 0.95), with a corresponding increase of T allele (50.0% vs. 31.7%; p=0.05, OR=2.2; 95%CI=1.05 - 4.42) in group 2 of RA patients compared to healthy controls. A statistically significant decrease in the occurrence of homozygous CC variant was found in the group of RA patients compared to healthy controls, however, without statistical significance (20.6% vs. 36.7%; p=0.20, OR=0.5; 95%CI=0.15 - 1.35). Evaluation of the prevalence of heterozygous variant TT showed no significant difference between RA patients and healthy controls (58.8 % vs. 63.3 %; p=0.80, OR=0.8; 95%CI=0.3 - 2.27).

Table 3: Differences in the frequency of allelic and genotypic variants of ST polymorphism in the VEGF C936T (rs3025039) gene in the group of RA patients with CVD and the control group

Alleles and genotypes	Number of alleles and genotypes examined				$\chi 2$	p	RR	95%CI	OR	95%CI
	3 group - RA patients with CVD		Control group							
	n	%	n	%						
C	25	44,6	41	68,3	6,6	0,03	0,7	0,31 - 1,37	0,4	0,18 - 0,79
T	31	55,4	19	31,7	6,6	0,03	1,5	0,7 - 3,36	2,7	1,26 - 5,66
C/C	4	14,3	11	36,7	3,8	0,10	0,4	0,07 - 2,19	0,3	0,08 - 1,01
C/T	17	60,7	19	63,3	0,0	0,90	1,0	0,33 - 2,77	0,9	0,31 - 2,59

In group 3, as well as in group 2, a statistically significant lower frequency of allele C (Table 3) compared to the control group (44.6% vs. 68.3%; p=0.03, OR=0.4; 95%CI=0.18 - 0.79), but a higher frequency of allele T (55.4% vs. 31.7%; p=0.03, OR=2.7; 95%CI=1.26 - 5.66). Significant differences from healthy controls were obtained for patients with RA of group 3 when detecting the frequency of TT genotype, which was reflected in an increase in the proportion of homozygous TT variant. In the group of patients a decrease in the occurrence of homozygous CC variant was detected (14.3% vs. 36.7%; p=0.10, OR=0.3; 95%CI=0.08 - 1.01), and no statistically significant differences were found in heterozygous ST variant (60.7% vs. 63.3%; p=0.90, OR=0.9; 95%CI=0.31 - 2.59).

Taking into account the fact that the prevalence analysis showed a high frequency of heterozygous genotype ST of VEGF 936 in patients with RA, we can speak about the presence of predisposition to RA in carriers of this genotype.

The association of different genotypes with clinical and laboratory indices revealed that carriers of T allele of VEGF 936 gene have higher index of RA activity according to DAS28 (5,0±2,3 vs. 4,5±1,21).

The association of VEGF C936T polymorphism with OSA CMR thickness was determined: CC - 0.89±0.06 mm, CT -0.92±0.09, TT - 1.03±0.05.

The analysis of genotype frequencies in VEGF 936 position of the promoter region of the gene revealed that carriers of VEGF C936C genotypes had seropositive variant of RA in 24% (n=6) of cases, carriers of VEGF 936 CT genotypes had seronegative variant in 11.66% (n=7) of cases and carriers of VEGF 936TT genotypes had no seronegative variant of the disease.

Patients with VEGF 936TT genotype showed high inflammatory activity in relation to patients with VEGF 936CC genotype ($p<0.05$).

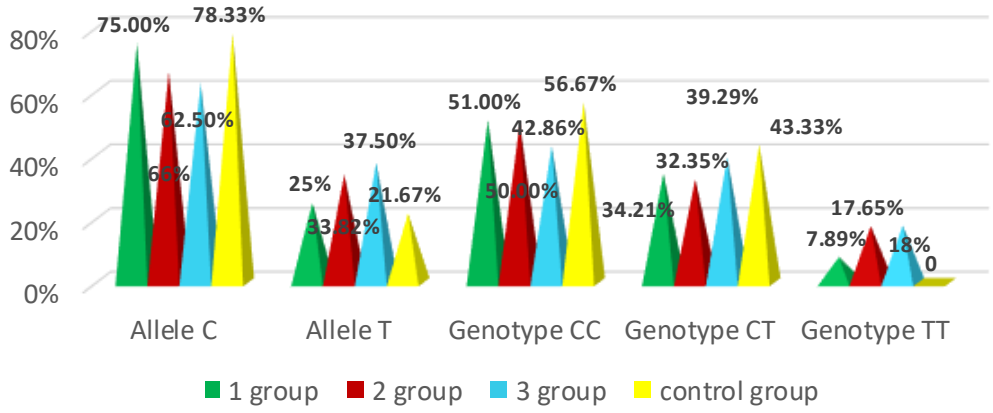

Figure 2: Occurrence of allelic and genotypic variants of MMP9 C1562T (rs3918242) polymorphism in the study groups

When the MMP9 C1562T (rs3918242) gene was studied, the prevalence of the C allele (Figure 2) was almost the same in group 1 patients and controls (75% and 78.33%). The CC genotype was observed less frequently in group 3 patients (42.86%), and the highest percentage of heterozygosity was observed in the control group (43.33%). The absence of homozygous mutant TT variant was characteristic of the control group, and its high percentage of occurrence was observed in patients of groups 2 (17.65%) and 3 (18%).

Table 4: Differences in the frequency of allelic and genotypic variants of ST polymorphism in the MMP9 C1562T (rs3918242) gene in the group of RA patients and the control group

Alleles and genotypes	Number of alleles and genotypes examined				χ2	p	RR	95%CI	OR	95%CI
	1 group - RA patients without RF		Control group							
	n	%	n	%						
C	57	75,0	47	78,3	0,2	0,70	1,0	0,5 - 1,85	0,8	0,37 - 1,85
T	19	25,0	13	21,7	0,2	0,70	1,0	0,42 - 2,62	1,2	0,54 - 2,69
C/C	22	57,9	17	56,7	0,0	0,95	1,0	0,44 - 2,37	1,1	0,4 - 2,77
C/T	13	34,2	13	43,3	0,6	0,50	0,8	0,32 - 1,94	0,7	0,25 - 1,82

No statistically significant differences were obtained in the distribution of genotypes of the C1562T (rs3918242) polymorphism of the MMP-9 gene (Table 4) between group 1 patients and control group individuals.

Table 5. Differences in the frequency of allelic and genotypic variants of ST polymorphism in MMP9 C1562T (rs3918242) gene in the group of RA patients with FR and control group

Alleles and genotypes	Number of alleles and genotypes examined				χ2	p	RR	95%CI	OR	95%CI
	2 group - RA patients with RF		Control group							
	n	%	n	%						
C	45	66,2	47	78,3	2,3	0,20	0,8	0,45 - 1,59	0,5	0,25 - 1,19
T	23	33,8	13	21,7	2,3	0,20	1,2	0,46 - 3,02	1,9	0,84 - 4,06
C/C	17	50,0	17	56,7	0,3	0,60	0,9	0,36 - 2,17	0,8	0,29 - 2,05
C/T	11	32,4	13	43,3	0,8	0,40	0,8	0,27 - 2,03	0,6	0,23 - 1,73

A statistically significant increase in the occurrence of the T allele was revealed in group 2 (Table 5) compared to the control group (33.8% vs. 21.7%; p=0.20, OR=1.9, 95%CI= 0.84 - 4.06). The predominance

of heterozygous ST genotype was observed in control group individuals compared to group 3 patients, however, without statistical significance (43.3% vs. 32.4%; p=0.40, OR=0.6, 95%CI= 0.23 - 1.73).

Table 6: Differences in the frequency of allelic and genotypic variants of ST polymorphism in MMP9 C1562T (rs3918242) gene in the group of RA patients with CVDP and control group RA patients with CVD and control group

Alleles and genotypes	Number of alleles and genotypes examined				$\chi 2$	p	RR	95%CI	OR	95%CI
	3 group - RA patients with CVD		Control group							
	n	%	n	%						
C	35	62,5	47	78,3	3,5	0,10	0,8	0,39 - 1,63	0,5	0,2 - 1,04
T	21	37,5	13	21,7	3,5	0,10	1,3	0,5 - 3,13	2,2	0,96 - 4,88
C/C	12	42,9	17	56,7	1,1	0,30	0,8	0,26 - 2,19	0,6	0,2 - 1,62
C/T	11	39,3	13	43,3	0,1	0,80	0,9	0,31 - 2,66	0,9	0,3 - 2,41

The analysis of MMP-9 gene polymorphism showed statistically significant prevalence of homozygous variant CC (Table 6) in the control group (56.7% vs. 42.9%; OR=0.6; 95%CI=0.2-1.62), as well as increased occurrence of T allele in group 3 patients compared to healthy controls (37.5% vs. 21.7%; OR=2.2; 95%CI=0.96-4.88). The occurrence of homozygous variant of the MMP9-1562 TT gene was observed in groups 2 and 3 relative to healthy controls.

The mean age of RA patients in heterozygotes with MMP9 C1562T genotype (47±4.6) was lower than in carriers of MMP9-1562 CC genotype (51±3.94), and in carriers of homozygous MMP9-1562 TT genotype this index amounted to (41±5.3) years, this indicates that the T allele variant is associated with an increased risk of earlier onset of the disease (p = 0.024).

The study revealed statistically significant correlation of high disease activity according to DAS28 indexes and VAS scale with mutant homozygous TT genotype of MMP9-1562 gene, where the indices amounted to 4.8±2.1 and 6±3, respectively.

Carriers of the mutant homozygous MMP9-1562 TT genotype had higher disease activity than carriers of the MMP9-1562 CC genotype: mean COE was 34.13±11.16 vs. 25.42±12.14; mean CRP was 21.13±16.5 vs. 13.24±12.42; blood RF concentration was 50.76±26.43 vs. 39.35±22.75; ADCC concentration was 116.2±26.35 and 93.09±43.42, respectively.

Conclusions.

Thus, some immunogenetic features of VEGF and MMP 9 gene in Uzbek population in Samarkand region were demonstrated, where heterozygosity for VEGF gene was revealed in 63% of cases. At the same time, there is an increase in the occurrence of allele T and genotype TT in groups 2 and 3 compared to the control group, which indicates the aggressiveness of this allele and genotype in RA patients. The molecular genetic markers, allele T and genotype TT of VEGF 936 (rs3025039) and MMP9 1562 (rs3918242) genes detected in RA patients are associated with increased cardiovascular risk in rheumatoid arthritis.

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