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POLYMORPHISMS OF THE CYTOKINE GENES TNF-A (–308 G>A) AND IL23R (G/A) AND THEIR ASSOCIATION WITH CLINICAL CHARACTERISTICS OF APLASTIC ANEMIA IN THE UZBEK POPULATION

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ABSTRACT

The study revealed no statistically significant differences in the distribution of allele and genotype frequencies of the TNF-α (–308 G>A) and IL23R (G/A) polymorphisms between patients with AA and the control group (P > 0.05). No association was established between these genetic variants and disease severity. These results suggest that these polymorphisms are not the primary genetic determinants of predisposition to AA in the studied population.

Keywords: *aplastic anemia, TNF-α (rs1800629), IL23R (rs11209026), genetic polymorphism, cytokines, Uzbek population, disease severity, pancytopenia.*

RESUME

Purpose. To assess the association of TNF-α (–308 G>A) and IL23R (G/A) gene polymorphisms with clinical characteristics of aplastic anemia (AA) in the Uzbek population.

Methods. This case-control study was conducted to evaluate the distribution of TNF-α (–308 G>A, rs1800629) and IL23R (G/A, rs11209026) gene polymorphisms among patients with aplastic anemia (AA) and healthy controls in the Uzbek population. The study included 86 patients with acquired AA who were recruited from the Republican Specialized Scientific and Practical Medical Center of Hematology, Ministry of Health of the Republic of Uzbekistan (RSSPMC, Tashkent) between 2019 and 2023.

The diagnosis and severity classification of AA were established based on the Camitta criteria, dividing patients into three categories:

• Mild AA (n=16)

• Severe AA (n=46)
• Supersevere AA (n=24)

The control group consisted of 98 healthy individuals, matched for age and gender, without hematological or autoimmune diseases, recruited from voluntary blood donors and outpatients. Sample collection and DNA extraction

For molecular genetic analysis, 4.0 ml of venous blood was collected in sterile vacutainers containing EDTA. Blood samples were stored at –80°C until analysis. Genomic DNA was extracted using AmpliPrime RIBO-prep reagent kits (Russia) according to the manufacturer's protocol. DNA purity and concentration were measured using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, USA) with an optical density ratio of A260/A280 = 1.7–1.8.

Genotyping of TNF-α (–308 G>A) and IL23R (G/A) polymorphisms

Genotyping for both polymorphisms was performed using real-time polymerase chain reaction (RT-PCR) with specific primers and LITEX test systems (Russia). PCR amplification was performed on the following platforms:

• Applied Biosystems 2720 Thermal Cycler (USA)
• RotorGeneQ (QIAGEN, Germany)
• Corbett Research CG1-96 (QIAGEN, Germany)

PCR amplification conditions

The amplification conditions were as follows:

• Initial denaturation at 93°C for 1 minute
• 35 cycles:
-Denaturation at 93°C for 10 seconds
- Bonding of primers at 64°C for 10 seconds
- Extension at 72°C for 20 seconds
- Final extension at 72°C for 1 minute

PCR products were separated by electrophoresis in a 3% agarose gel prepared with TAE buffer and stained with ethidium bromide (1% solution, 5 µl/50 ml gel). Amplified DNA fragments were visualized under UV illumination at 310 nm.

INTRODUCTION

Aplastic anemia (AA) is a severe hematological disease that is caused by immune-mediated destruction of blood stem cells. Proinflammatory cytokines play a key role in suppressing hematopoiesis [1,2,3]. Among them, special attention is paid to TNF-α and the interleukin-23 system, which can modulate the activity of T-lymphocytes. The patient's genetic background can determine the expression level of these molecules [7,8,11,12]. Genetic polymorphisms in the promoter region of the TNF-α gene, especially at position –308 (G>A, rs1800629), are associated with

altered TNF-α expression and predisposition to various autoimmune and hematological diseases [5,9]. Another immunoregulatory factor is the interleukin-23 receptor (IL23R), which plays an important role in T-helper 17 (Th17)-mediated immune responses. Although IL23R gene polymorphisms, such as the G>A variant (rs11209026), have been associated with autoimmune diseases, including inflammatory bowel disease and psoriasis [4,6,10], their role in AA remains unclear. However, data on the role of TNF-α and IL23R gene polymorphisms in AA are contradictory and largely depend on the ethnicity of the patients. The study of these markers in the Uzbek population is relevant for understanding the regional characteristics of AA pathogenesis.

Results. Analyzing the structure of genetic polymorphism TNFα (G-308A) in the main group of patients with AA(n=86) No significant differences were found compared to the control group. Thus, the dominant variants for the TNFα gene (G-308A) in the patient group were also the major allele G (95.0%) and the genotype G/G (89.5%), while the mutant allele A (5.0%) and heterozygote G/A (10.5%) occupied a recessive position in frequency (see Table 1).

In patients with a mild form of AA (n=16), compared to the control and main groups, the major allele G (96.9%) and genotype G/G (93.8%) were recorded with a slightly higher frequency for the studied gene, according to which the mutant allele A (3.1%) and heterozygote G/A (6.2%) had lower frequencies (see Table 1).

Table 1

Structural analysis of the TNFα gene polymorphism (G-308A) in healthy controls and patients with AA

No.	Group	Alleles (n/%)				Genotypes (n/%)					
		G		A		G/G		G/A		A/A	
		n	%	N	%	N	%	N	%	N	%
1	Primary with AA, n=86	163	95.0	9	5.0	77	89.5	9	10.5	0	0.0
2	Mild AA, n=16	31	96.9	1	3.1	15	93.8	1	6.2	0	0.0
3	Severe AA, n=46	87	94.6	5	5.4	41	89.1	5	10.9	0	0.0
4	Super heavy, n=24	45	93.8	3	6.2	21	87.5	3	12.5	0	0.0
5	Comparison control, n=98	185	94.4	11	5.6	87	88.8	11	11.2	0	0.0

Among patients with a severe form (n=46), the major allele G (94.6%) and genotype G/G (89.1%) for the TNFα gene (G-308A) also had higher frequencies than the mutant allele A (5.4%) and heterozygote G/A (10.9%), which had lower frequencies, respectively. At the same time, in the super-severe form of AA (n=24), compared with the control group and the other groups of patients with AA described above, the major allele G (93.8%) and genotype G/G (87.5%) were recorded

relatively less frequently, and the frequencies of unfavorable variants of the allele A (6.2%) and genotype G/A (12.5%), according to the distribution pattern, had higher proportions (see Table 1).

Analyzing the significance of differences in the frequencies of alleles and genotypes of a polymorphic geneTNFα (G-308A) in a group of patientswith mild AA compared with severe and super-severe forms of AA, there were no significant differences in carriage of the mutant allele A(3.1% vs. 5.4%; $\chi2=0.3$; P=0.6; RR=1.0; CI:0.47 - 2.03;OR=0.6; CI:0.06-4.86 and3.1% vs. 6.3%; $\chi2=0.4$; P=0.6; RR=1.0; CI:0.3-3.11;OR=0.5; CI:0.05-4.66), the main genotype G/G (93.8% vs. 89.1%; $\chi2=0.3$; P=0.6; RR=1.1; CI:0.03-38.79;OR=1.8; CI:0.2-16.49 and93.8% vs. 87.5%; $\chi2=0.4$; P=0.6; RR=1.1; CI:0.04-32.48;OR=2.1; CI:0.21-21.68) and heterozygote G/A (6.3% vs. 10.9%; $\chi2=0.3$; P=0.6; RR=0.6; CI:0.02- 21.2;OR=0.5; CI:0.06-4.93 and6.3% vs. 12.5%; $\chi2=0.4$; P=0.6; RR=0.5; CI:0.02-15.16;OR=0.5; CI:0.05-4.72) was not found.

Along with this, comparing the degree of differences in the frequencies of alleles and genotypes of a polymorphic geneTNFα (G-308A) betweensevere and super-severe forms of AA have no significant differences in carriage of the mutant allele A(5.4% vs. 6.3%; $\chi2<3.84$; P=0.9; RR=1.0; CI:0.16-6.08;OR=0.9; CI:0.2-3.77), the main genotype G/G (89.1% vs. 87.5%; $\chi2<3.84$; P=0.9; RR=1.0; CI:0.34-3.09;OR=1.2; CI:0.26-5.38) and heterozygote G/A (10.9% vs. 12.5%; $\chi2<3.84$; P=0.9; RR=0.9; CI:0.29- 2.63;OR=0.9; CI:0.19-3.92) was also not established.

The results of the SNP distribution of the polymorphic gene IL23R (G/A) allowed us to conduct an analysis of the compliance of the observed (Ho) and expected (He) genotype frequencies with their canonical distribution. The statistically insignificant differences between the actually observed and theoretically expected genotype frequencies in the main ($\chi2=0.05$; P=0.79; df=1) and in a healthy ($\chi2=0.01$; P=0.876; df=1) groups showed that their distribution corresponded to the Hardy-Weinberg law (HWL).

WITHpolymorphic gene structureIL23R (G/A) in the control groupwas characterized by carriage of the major G allele in 99.0% of cases and only the mutant variant A in 1.0%. Among the genotypic variants, the major genotype G/G was determined in 98.0%, and the heterozygous G/A in 2.0% of healthy individuals. Whereas the homozygous mutant variant A/A was not observed in this group (see Table 2).

In the main group of patients with AA, the frequency of the major allele G was 97.7%, and the attenuated A variant was 2.3%. At the same time, the frequencies of

the major G/G and heterozygous G/A genotypes were 95.3% and 4.7%, respectively, also in the absence of the mutant variant A/A (0.0%) (see Table 2).

The lowest frequency of the main allele G (96.9%) and the genotype G/G (93.8%) were determined in the group of patients with a mild course of the disease, while in this group the maximum frequencies of the unfavorable variants of the allele A (3.1%) and the genotype G/A (6.2%) were simultaneously recorded (see Table 2).

Table 2

Structural analysis of IL23R gene polymorphism (G/A) in healthy controls and patients with AA

No.	Group	Alleles (n/%)				Genotypes (n/%)					
		G		A		G/G		G/A		A/A	
		n	%	n	%	N	%	N	%	N	%
1	Primary with AA, n=86	168	97.7	4	2.3	82	95.3	4	4.7	0	0.0
2	Mild AA, n=16	31	96.9	1	3.1	15	93.8	1	6.2	0	0.0
3	Severe AA, n=46	90	97.8	2	2.2	44	95.7	2	4.3	0	0.0
4	Super heavy, n=24	47	98.0	1	2.0	23	95.8	1	4.2	0	0.0
5	Comparison control, n=98	194	99.0	2	1.0	96	98.0	2	2.0	0	0.0

At the same time, in severe and extremely severe forms of AA, the main allele G and the genotype G/G were determined with a frequency of 96.9% and 97.8%, as well as 93.8% and 95.7%, while their unfavorable variants A and the heterozygote G/A were recorded with a frequency of 2.2% and 2.0%, as well as 4.3% and 4.2%, respectively (see Table 2).

Thus, in the structure of the IL23R (G/A) genetic polymorphism, the dominant role belonged to the major allele (G) and genotype (G/G) in all studied groups, while the low frequencies of the A and G/A variants allowed them to be classified as recessive. No significant differences were visually observed between the allele and genotype frequencies of the IL23R (G/A) polymorphism, which likely indicates the absence of their independent contribution to the risk of AA and its severity.

Thus, despite a noticeable increase in the frequencies of the unfavorable allele A and genotype G/A for the polymorphic geneIL23R (G/A) in mild, severe, and ultra-severe forms of AA compared to similar ones in the healthy group, the differences between these allele and genotype variants did not reach statistically significant values. In turn, based on the obtained results, the independently polymorphic IL23R (G/A) gene does not participate in the mechanisms that determine the severity of AA. Another supporting result of this conclusion was the absence of reliable differences in

the studied IL23R (G/A) gene between the groups with mild AA compared to severe (allele A – 3.1% versus 2.2%; $\chi2$=0.1; P=0.8; RR=1.0; DI:0.21-4.91;OR=1.5; CI:0.13-16.35; genotypeG/A – 6.3% versus 4.3%; $\chi2$=0.1; P=0.8; RR=1.4; CI:0.06-37.11;OR=1.5; CI:0.13-17.13) and super-severe forms (allele A – 3.1% versus 2.1%; $\chi2$=0.1; P=0.8; RR=1.0; DI:0.07-15.64;OR=1.5; CI:0.09-16.24.69; genotypeG/A – 6.3% versus 4.2%; $\chi2$=0.1; P=0.8; RR=1.5; CI:0.09-25.25;OR=1.5; CI:0.09-25.95), as well as between severe and extremely severe forms (allele A in severe AA – 2.2% versus 2.1%; $\chi2<3.84$; P=0.98; RR=1.0; CI:0.04-23.8;OR=1.0; CI:0.09 - 11.82; genotypeG/A – 4.3% versus 4.2%; $\chi2<3.84$; P=0.98; RR=1.0; CI:0.21 - 5.19;OR=1.0; CI:0.09 - 12.15).

CONCLUSION

The analysis demonstrates the absence of a direct association between TNF-α (–308 G>A) and IL23R (G/A) polymorphisms and the risk of developing and clinically prone to aplastic anemia in Uzbekistan. The fact that the obtained data diverges from the results of several studies in European or East Asian populations underscores the unique genetic profile of the Uzbek ethnic group.

The absence of association (negative result) is of great scientific importance, as it allows us to exclude these loci from the list of potential prognostic markers for this population and indicates the need to search for other genetic factors, as well as to study epigenetic mechanisms and environmental factors influencing the development of pancytopenia in the Central Asian region.

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