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CONTRIBUTION OF IL-17F (rs763780) AND IL23R (rs11209026) GENE POLYMORPHISMS IN THE DEVELOPMENT OF APLASTIC ANEMIA

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Abstract

Purpose of the study. To study the contribution of IL-17F (rs763780) and IL23R (rs11209026) gene polymorphisms to the development of aplastic anemia.

Methods. The material for clinical and laboratory studies in the work were patients with AA (n=86) who sought diagnostic help and subsequent inpatient examination at the republican specialized Scientific and Practical Medical Center of Hematology (RSSPMCH, Tashkent) from 2019 to 2023. Patients with AA ranged in age from 18 to 79 years, while the median age was 40.8±1.8 years. The diagnosis was made taking into account clinical and laboratory data.

A four milliliter peripheral blood sample was taken from all participants to study single nucleotide polymorphisms IL-23R (rs11209026; G1142A) and IL-17F (rs763780; A7488G) using polymerase chain reaction of restriction fragment length polymorphism (PCR-PDRF). The samples were stored at a temperature of -80 °C until DNA was isolated. The results were statistically processed using the PC application package "OpenEpi 2009, Version 2.3".

Conclusions. The results obtained by analyzing differences in polymorphic loci of the IL17F (His161Arg) gene in the main group of patients with AA compared with the control showed a statistically significant association between His/Arg heterozygote and the risk of AA. Moreover, the detected tendency to increase the frequency of the weakened Arg allele by 2.1 times and His/Arg heterozygote by 2.2 times in the group with severe AA, as well as to increase the frequencies of the weakened Arg allele by 2.4 and His/Arg heterozygote by 2.6 times in the group of patients with superheavy AA allows them to be considered as genetic predictors of AA weighting. The results of the study of IL23R (G/A) genetic polymorphism indicate the absence of independent associative links with an increased risk of AA development and severity.

Keywords:IL-17F (rs763780), IL23R (rs11209026), polymorphism, allele, genotype, relationship, risk of development, aplastic anemia.

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Introduction

Single nucleotide polymorphisms (SNPs) are the most common type of genetic diversity in the human genome, usually associated with a harmful phenotype [3,10]. This can lead to the formation of an altered protein, which can cause its functional impairment or affect its expression, especially if they are located in coding regions or regulatory regions of genes [12].

It is reported that IL-17F and IL-23 play an important role in maintaining the vital activity of Th17 cells. The gene encoding IL-17F is located on chromosome 6 (6p12). A functional polymorphism (IL-17F rs763780) was found in IL-17 genes, leading to a change in the expression of mRNA and protein [11,4,9].

The IL-23 receptor (IL-23R) contains two subunits: the IL-23R subunit, which is expressed by the IL-23R gene, and the IL-12Rb1 subunit, which is expressed by the IL12Rb1 gene. The single nucleotide polymorphism (SNP) rs11209026 is associated with the replacement of Arg by Gln at position 381 in the IL-23R (IL-23R381Gln) gene [6,1]. The data indicate that variants of the IL-17F and IL23R genes affect the concentration of IL-17 cytokine in blood serum and the associated metabolic pathway. In addition, there is evidence that these SNPs are associated with some autoimmune diseases [2, 5,7,8].

Purpose of the study. To study the contribution of IL-17F (rs763780) and IL23R (rs11209026) gene polymorphisms to the development of aplastic anemia.

Methods. The material for clinical and laboratory studies in the work were patients with AA (n=86) who sought diagnostic help and subsequent inpatient examination at the Republican Specialized Scientific and Practical Medical Center of Hematology (RSSPMCH, Tashkent) from 2019 to 2023. Patients with AA ranged in age from 18 to 79 years, with the median age being 40.8±1.8 years. The diagnosis was made taking into account clinical and laboratory data. Patients with AA (n=86), who made up the main group, were divided into three groups depending on the severity of the disease: mild, severe and superheavy.

To carry out molecular genetic analyses of immune modulator genes, 4.0 ml venous blood samples were taken from AA and healthy patients into test tubes containing EDTA IL17F (rs763780) and IL23R (rs11209026), which were stored in freezers at a temperature of -80 °C until the biomaterial was completely collected.

After blood collection, DNA of patients and healthy people was isolated using Ampli Prime RIBOT-prep reagents (Russia) on a Nano Drop 2000 spectrophotometer (Nano Drop Technologies, USA) with a wavelength equal to A260/280 nm with a determination of DNA concentration by a ratio of 1.7/1.8.

Polymorphic genes IL17F (rs763780) and IL23R (rs11209026) were detected by RT PCR using a thermal cycler (Applied Biosystems 2720, USA; RotorGeneQ, QUAGEN Germany and Corbett Research - CG1-96, QUAGEN Germany).

The amplification procedure was carried out by pre-denaturation of DNA at a temperature of 94 ° C for 3 minutes; 30 denaturation cycles at a temperature of 94 ° C for 20 seconds; annealing of primers at a temperature of 58 ° C for 20 seconds; elongation process at a temperature of 61 ° C for 30 seconds; DNA synthesis at a temperature of 61 °C for 7 minutes; electrophoresis on 2% agarose gel with final analysis of the results using a UV transilluminator with an integrated imaging camera.

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LITECH test systems (Russia) were used to detect the studied genetic markers. The SNPs were tested for deviations from the Hardy-Weinberg equilibrium using the Chi-square test. The relative risk associated with alleles and genotypes was calculated as the odds ratio (OR) with a 95% confidence interval (CI), which were performed using the PC program "OpenEpi 2009, Version 2.3".

Results

Evidence of the involvement of unfavorable loci of the polymorphic IL17F gene (His161Arg) in increasing the risk of AA was the observed tendency to increase the frequency of the weakened Arg allele by 2.1 times (11.0% vs. 5.6%; $\chi 2=3.6$; P=0.1; RR=1.1; DI: 0.41-2.72; OR=2.1; CI: 0.98-4.46) and a statistically significant increase in the frequency of His/Arg heterozygote by 2.2 times (22.1% vs. 11.2%; $\chi 2=4.0$; P=0.05; RR=2.0; CI: 1.04-3.73; OR=2.2; CI: 1.01-4.97) in the main group of patients with AA compared with the control (see Table 1).

Table 1 Structural analysis of IL 17F (His161Arg) gene polymorphism in healthy control groups and patients with AA

№	Group	Alleles, (n/%)				Genotypes, (n/%)					
		His		Arg		His/His		His/Arg		Arg/Arg	
		n	%	N	%	n	%	n	%	n	%
1	Basic with AA, n=86	153	89.0	19	11.0	67	78.0	19	22.0	0	0.0
2	Non-severe AA, n=16	29	90.6	3	9.4	13	81.2	3	18.8	0	0.0
3	Severe AA, n=46	82	89.1	10	10.9	36	78.3	10	21.7	0	0.0
4	Very severe AA, n=24	42	87.5	6	12.5	18	75.0	6	25.0	0	0.0
5	The control being compared, n=98	185	94.4	11	5.6	87	88.8	11	11.2	0	0.0

In addition, a tendency to decrease the frequency of the protective main His allele was found in the patient group (89.0% vs. 94.4%; $\chi 2=3.6$; P=0.1; CI: 0.22-1.02) and a significant decrease in the frequency of the main His/His genotype (77.9% vs. 88.8%; -2=4.0; P=0.05; CI: 0.2 - 0.99).

Meanwhile, differences in the distribution of polymorphic loci of the IL17F (His161Arg) gene between groups with mild AA and controls did not reach a statistically significant level, which indicated the absence of the role of SNP loci of the studied gene in the formation of mild AA, among the group of patients the frequency of the weakened Arg allele was 1.7 times (9.4% vs. 5.6%; $\chi 2=0.7$; P=0.5; RR=1.0; CI: 0.6 - 1.8; OR=1.7; CI: 0.46-6.52), and the frequency of His/Arg heterozygote is 1.8 times (18.8% vs. 11.2%; $\chi 2=0.7$; P=0.4; RR=1.7; CI: 0.18-15.11; OR=1.8; CI: 0.46 - 7.3) were higher than in the control group.

However, according to the results of the analysis of differences in the distribution of polymorphic loci of the IL17F (His161Arg) gene between groups with severe AA and healthy, a tendency was found to increase the frequency of the weakened Arg allele by 2.1 times (10.9% vs. 5.6%; $\chi 2=2.6$; P=0.2; RR=1.1; CI: 0.47-2.39; OR=2.1; CI: 0.85-4.94) and His/Arg heterozygotes by 2.2 times (21.7% vs. 11.2%; $\chi 2=2.8$; P=0.1; RR=1.9; CI: 0.69-5.43; OR=2.2; DI: 0.87-5.54) in the sample of patients.In turn, these results serve as the basis for the assertion that unfavorable loci of the IL 17F (His161Arg) gene may contribute to the formation of severe AA.

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The possible participation of unfavorable loci of the IL17F polymorphic gene (His161Arg) in increasing the risk of worsening the course of AA is confirmed by the observed trend in increasing the frequencies of the weakened Arg allele by 2.4 times (12.5% vs. 5.6%; χ2=2.8; P=0.1; RR=1.1; CI: 0.54-2.17; OR=2.4; CI: 0.86-6.68) and His/Arg heterozygotes by 2.6 times (25.0% vs. 11.2%; χ2=3.1; P=0.1; RR=2.2; CI: 0.49-10.0; OR=2.6; CI: 0.89-7.82) in the group of patients with superheavy form of AA in comparison with the control.

Nevertheless, comparing the differences in the distribution of polymorphic loci of the IL17F (His161Arg) gene in the group with mild AA compared with severe and superheavy forms of the disease, differences of a non-significant nature were found (Arg allele - 9.4% vs. 10.9%; χ2=0.1; P=0.9; OR=0.8; DI: 0.22 - 3.29 and 9.4% vs. 12.5; χ2=0.1; P=0.7; OR=0.7; DI: 0.17 - 3.12; His/Arg heterozygote - 18.8% vs. 21.7%; χ2=0.1; P=0.9; OR=0.8; DI: 0.2-3.49 and 18.8% vs. 25.0%; χ2=0.2; P=0.7; OR=0.7; DI: 0.15-3.28) .

Similarly, no statistically significant differences were found between groups with severe and superheavy forms of AA in the studied SNP loci of the IL17F gene (His161Arg) (Arg allele – 10.9% vs. 12.5; χ2=0.1; P=0.8; OR=0.9; DI: 0.29 – 2.51 and His/Arg heterozygote – 21.7% vs. 25.0%; χ2=0.1; P=0.8; OR=0.7; DI: 0.26-2.65).

In the distribution of polymorphic loci of the IL23R (G/A) gene in the main group, compared with healthy ones, an increase in the frequencies of the mutant A allele was found (2.3% vs. 1.0%; χ2=1.0; P=0.4; RR=1.0; DI: 0.11-9.38; OR=2.3; DI: 0.44-12.2) and heterozygotes G/A by 2.3 times (4.7% vs. 2.0%; χ2=1.0; P=0.4; RR=2.3; DI: 0.72-7.21; OR=2.3; DI: 0.44-12.5).However, the established differences between these polymorphic loci did not reach a statistically significant level. Moreover, the absence of significantly significant differences was also observed in the frequencies of the main G allele (97.7% vs. 99.0%; χ2=1.0; P=0.4; RR=1.0; DI: 0.32-3.05; OR=0.4; CI: 0.08-2.28) and G/G genotype (95.3% vs. 98.0%; χ2=1.0; P=0.4; RR=0.1; DI: 0.31-3.08; OR=0.4; DI: 0.08-2.28) (see Table 2).

Table 2 Structural analysis of IL23R (G/A) gene polymorphism in healthy control groups and patients with AA

№	Группа	Аллели, (n/%)				Генотипы, (n/%)					
		G		A		G/G		G/A		A/A	
		n	%	N	%	n	%	N	%	n	%
1	Basic with AA, n=86	168	97.7	4	2.3	82	95.3	4	4.7	0	0.0
2	Non-severe 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	Very severe AA, n=24	47	98.0	1	2.0	23	95.8	1	4.2	0	0.0
5	The control being compared, n=98	194	99.0	2	1.0	96	98.0	2	2.0	0	0.0

The absence of statistically significant differences in the distribution of polymorphic loci of the IL23R (G/A) gene in the main group compared with healthy ones proves the absence of their independent association with the risk of AA.

Next, we evaluated the association of polymorphic loci of the IL23R (G/A) gene with the risk of AA severity. Comparing the differences in alleles and genotypes for the IL23R (G/A) gene found in the group with a mild form of AA compared with healthy ones, it was found that they did not

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reach a statistically significant level as between allelic variants (G allele – 96.9% vs. 99.0%; χ2=0.9; P=0.4; RR=1.0; DI: 0.04-24.0; OR=0.3; DI: 0.03-3.21 and allele A - 3.1% vs. 1.0%; χ2=0.9; P=0.4; RR=1.0; DI: 0.21-4.92; OR=3.1; CI: 0.31-31.5), and genotypic variants (genotype G/G - 93.8% vs. 98.0%; χ2=1.0; P=0.4; RR=1.0; DI: 0.04-25.1; OR=0.3; CI: 0.03-3.24 and genotype G/A - 6.3% vs. 2.0%; χ2=1.0; P=0.4; RR=3.1; CI: 0.12-80.5; OR=3.2; DI: 0.3-33.14).

At the same time, in the group of patients with severe AA, in a comparative aspect with healthy ones, differences in the distribution of polymorphic loci of the IL23R (G/A) gene also did not differ in their significance between allelic variants (G allele – 97.8% vs. 99.0%; χ2=0.6; P=0.5; RR=1.0; DI: 0.14-6.94; OR=0.5; DI: 0.07 - 3.2 and allele A – 2.2% vs. 1.0%; χ2=0.6; P=0.5; RR=1.0; DI: 0.15-6.95; OR=2.2; DI: 0.31-14.8) and genotypic variants (genotype G/G – 95.7% vs. 98.0%; χ2=0.6; P=0.5; RR=1.0; DI: 0.13-7.07; OR=0.5; DI: 0.07-3.21 and genotype G/A - 4.3% vs. 2.0%; χ2=0.6; P=0.5; RR=2.1; DI: 0.29-5.42; OR=2.2; DI: 0.31-15.3).

In the group of patients with superheavy AA, in relation to the control, the differences in the distribution of polymorphic loci of the IL23R (G/A) gene were not significant (the G allele was 97.9% vs. 99.0%; χ2=0.4; P=0.6; RR=1.0; DI: 0.04-23.7; OR=0.5; DI: 0.05-5.19 and allele A – 2.1% vs. 1.0%; χ2=0.4; P=0.6; RR=1.0; DI: 0.21-4.87; OR=2.1; DI: 0.19-22.1), and genotypic variants (genotype G/G – 95.8% vs. 98.0%; χ2=0.4; P=0.6; RR=1.1; DI: 0.04-24.4; OR=0.5; DI: 0.04-5.24 and genotype G/A – 4.2% vs. 2.0%; χ2=0.4; P=0.6; RR=2.0; DI: 0.08-51.0; OR=2.1; DI: 0.19-22.8).

Conclusion

Thus, we studied the structural features of the polymorphic IL 17F (His161Arg) gene among healthy and AA patients. The results obtained by analyzing differences in polymorphic gene loci in the main group of patients with AA compared with the control showed a tendency to increase the risk of AA when carrying the mutant Arg allele 2.1 times (χ2=3.6; P=0.1) and a statistically significant association between the risk of the disease and the His/Arg heterozygote, which is associated with a 2.2-fold increase in the risk of AA (χ2=4.0; P=0.05).Moreover, there was a tendency to increase the frequency of the attenuated Arg allele by 2.1 time (χ2=2.6; P=0.2) and His/Arg heterozygotes by 2.2 times (χ2=2.8; P=0.1) in the group with severe AA, as well as in an increase in the frequencies of the weakened Arg allele by 2.4 times (χ2=2.8; P=0.1) and His/Arg heterozygotes by 2.6 times (χ2=3.1; P=0.1) in the group of patients with the superheavy form of AA allows them to be considered as genetic predictors of AA weighting.

Despite a noticeable increase in the frequencies of unfavorable allele A and genotype G/A for the polymorphic IL23R (G/A) gene in non-severe, severe and superheavy forms of AA compared with those in the healthy group, the differences between these loci did not reach statistically significant values. In turn, based on the results obtained independently, polymorphic loci of the IL23R (G/A) gene do not participate in the mechanisms implementing the severity of AA. Another proving result of this conclusion was the absence of significant differences in the studied loci of the IL23R (G/A) gene between groups with mild AA compared with severe AA (allele A – 3.1% vs. 2.2%; χ2=0.1; P=0.8; RR=1.0; DI: 0.21-4.91; OR=1.5; DI: 0.13-16.35; genotype G/A – 6.3% vs. 4.3%; χ2=0.1; P=0.8; RR=1.4; DI: 0.06-37.11; OR=1.5; DI: 0.13-17.13) and superheavy forms (allele A – 3.1% vs. 2.1%; χ2=0.1; P=0.8; RR=1.0; DI: 0.07-15.64; OR=1.5; DI: 0.09-16.24.69; genotype G/A – 6.3% vs. 4.2%; χ2=0.1; P=0.8; RR=1.5; DI: 0.09-25.25; OR=1.5; DI: 0.09-25.95), as well as between severe and superheavy forms (allele A in severe AA – 2.2% vs. 2.1%; χ2<3.84; P=0.98;

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RR=1.0; DI: 0.04-23.8; OR=1.0; CI: 0.09 - 11.82; genotype G/A – 4.3% vs. 4.2%; $\chi 2<3.84$; P=0.98; RR=1.0; CI: 0.21 - 5.19; OR=1.0; DI: 0.09 - 12.15).

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