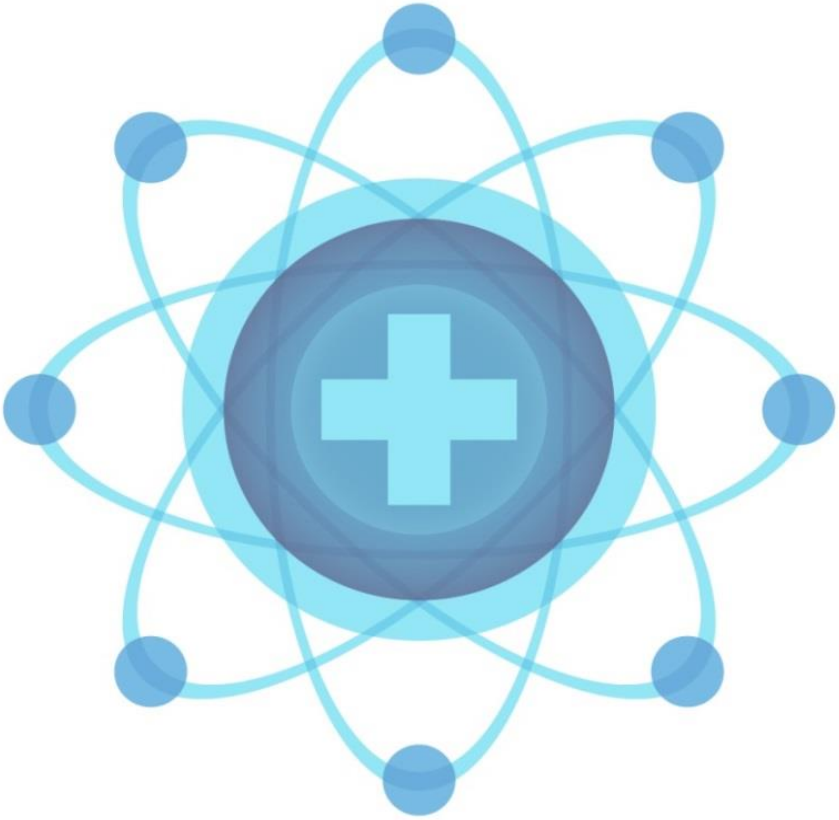

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STUDY OF THE FEATURES OF SINGLE-NUCLEOTIDE GENETIC POLYMORPHISM IL6 (C174G) IN PURULENT DISEASES OF THE MIDDLE EAR

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Abstract: The features of the uninucleid genetic polymorphism IL6 (C174G) in purulent-inflammatory diseases of the middle ear in Uzbekistan were studied. Polymorphisms of the IL6 gene (C174G) were investigated by analyzing DNA samples by PCR in real time. The results of the study made it possible to establish among carriers of the heterozygous C/ G genotype an unexpressed tendency to increase the risk of purulent-inflammatory diseases of the middle ear of the lung course by 1.5 times (χ 2=1.1; P = 0.3) compared with the control and 2.4 times (χ2 = 3.8; P = 0.1) compared with the moderate and severe course of the disease, as well as among carriers of the mutant genotype G / G tend to increase the likely risk of the formation of purulent-inflammatory diseases of the middle ear with moderate and severe course compared with the control by 2.2 times (χ2 =1.5; P=0.3). The findings indicate a possible contribution of the polymorphic IL6 (C174G) gene to the mechanisms of increased risk of formation of purulent-inflammatory diseases of the middle ear in Uzbekistan.

Keywords: purulent-inflammatory diseases of the middle ear, polymorphism of the IL6 (C174G) gene, allele, frequency, genotype, share of carriage.

Reevance. Purulent-inflammatory diseases of the middle ear are a common medical problem worldwide. Characterized by frequent recurrence, leading to damage to the eardrum, they are one of the most common causes of hearing impairment, the development of severe complications (brain abscess, meningitis) and high mortality [3,10]. According to the World Health Organization (WHO), more than 65,000,000 people suffer from purulent-inflammatory diseases of the ear, 50% of which have various forms of hearing impairment up to its loss [1,2,4].

Complex disorders resulting from exposure to various agents of the external and internal environment initiate the onset of purulent-inflammatory diseases of the middle ear [5,6]. Along with the lack of full disclosure of the pathogenetic mechanisms of their formation [8], there are reports of an important contribution to their implementation of genetic components, in particular a number of polymorphic genes of pro-inflammatory cytokines that play a key role in the processes of immunoregulation of the body [11]. In this regard, many functions belong to the

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cytokine IL6, which is involved in the complex processes of maturation of megakaryocytes and B-cells, differentiation of monocytes into macrophage cells, as well as in the processes of activation of T-cells [9,12].

The variety of genetic studies on the involvement of various polymorphic variants of the IL-6 gene in the development of inflammatory processes in the middle ear also do not differ in the presence of convincing results [7,12], which served as the basis for this study.

Material and methods. The study was conducted with the participation of 87 patients with purulent-inflammatory diseases of the middle ear (I general group, in the age range from 15 to 67 years) who were treated in a specialized hospital of the Urgench branch of the Tashkent Medical Academy and in a private hospital KHRAZM ENT SHIFO in the period from 2016 to 2022 Depending on the severity of the course of the disease, patients (n = 87) are divided into two groups: II (n = 36) group of patients with mild course and III (n = 51) group of patients with moderate and severe course of purulent-inflammatory diseases of the middle ear. Control IV group consisted of 71 conditionally healthy persons without inflammatory diseases, comparable in age and sex to the general group of patients.

Molecular genetic studies were conducted in the laboratory of molecular genetics, cytogenetics and FISH of the Republican Specialized Scientific and Practical Medical Center of Hematology (Republic of Uzbekistan, Tashkent). In accordance with the generally accepted technique, DNA was isolated from blood leukocytes. At the same time, an analysis was carried out using the Applied Biosystems 2720 system (USA) (SNP-PCR Real time).) polymorphic gene IL6 (C174G) using test systems "Liteh" (Russia). Mathematical analysis of the results was carried out using the program "OpenEpi 2009, Version 9.3".

Results and discussion. In the distribution of frequencies of genotypic variants of the studied single-nucleotide polymorphic il6 (C174G) gene among patients with purulent-inflammatory diseases of the middle ear and healthy individuals, discrepancies from the canonical distribution for PCB ($\chi 2<0.84$; $p>0.05$) were not detected.

The results of a study to assess the incidence of single-nucleotide polymorphism of the IL6 gene (C174G) among patients of the general group of patients with purulent-inflammatory diseases of the middle ear (n = 87) and healthy individuals (n = 71) were characterized by the carriage of the main C allele in 71.8% (n = 125) and 74.6% (n = 106) cases, despite the fact that cases of bearer of minor G alleles were naturally smaller percentages - 28.2% (n = 49) and 25.4% (n = 36) respectively to the studied groups (Table 1).

Table-1

Distribution of allelic and genotypic variants of single-nucleotide polymorphism IL6 (C174G) among patients with purulent-inflammatory diseases of the middle ear and healthy individuals

№	Group	Frequency of allelic	Frequency of genotypic

		variants				variants					
		C		G		C/ C		C/G		G /G	
		n	%	n	%	n	%	n	%	n	%
I	Core group, (n= 87)	125	71.8	49	28.2	46	52.9	33	37.9	8	9.2
II	Group with a slight current, (n = 36)	50	69.4	22	30.6	16	44.4	18	50.0	2	5.6
III	Group with moderate course, (n = 51)	75	73.5	27	26.5	30	58.8	15	29.4	6	11.8
IV	Control group, (n = 71)	106	74.6	36	25.4	39	54.9	28	39.4	4	5.6

As with regard to allele frequencies between the general group of patients and healthy, a slightly different frequency was found for the main wild genotype C / C (52.9% vs. 54.9%) and the heterozygous genotype/ C / G (37.9% vs. 39.4%). from 5.6% in the group of healthy increased to 9.2% in the group of patients, which may indicate its participation in the processes of initiation of purulent-inflammatory diseases of the middle ear.

In addition to analyzing the features of the occurrence of single-nucleotide polymorphism of the IL6 (C174G) gene between the above groups, in order to detail the features of the changes found in the general group, we conducted studies among patients depending on the severity of the course of the main pathological process.

Dwelling in detail on the results of the study in the group of patients with a mild course of purulent-inflammatory diseases of the middle ear examined (n = 36), the fact of a decrease in the proportion of the main C allele to 69.4% was visualized due to an increase in the proportion of the minor G allele to 30.6%. Similarly, these data, compared with the control, there was a noticeable decrease in the frequency of the wild C genotype among this group of patients to 44.4% against 54.9%. At the same time, the heterozygous C/G genotype increased to 50.0% versus 39.4% at the same frequency of the mutant G/G genotype (5.6% vs. 5.6%).

At the same time, in the group of patients with moderate and severe course of purulent-inflammatory diseases of the middle ear (n = 51) compared with control, an insignificant decrease in the carriage of the main C allele (73.5% vs. 74.6%) and an increase in the proportion of the minor G allele (26.5% vs. 25.4%) was revealed. mutant G/G genotype among patients (11.8% vs. 5.6%).

Thus, analyzing the results of a study to study the occurrence of alleles and genotypes of the single nucleotide polymorphism of the IL6 gene (C174G) among the surveyed groups of patients compared with those among healthy ones, an increase in the proportion of carriage of the mutant G / G genotype in the general group of patients (group I) (9.2% versus 5.6%) was revealed due to an increase in the share of carriage of the mutant G / G genotype in the general group of patients (group I) (9.2% versus 5.6%) due to an increase in the share of carriage of the mutant genotype G /

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G I have its frequency among patients with moderate and severe purulent-inflammatory diseases of the middle ear (11.8% versus 5.6%). Moreover, among patients with mild purulent-inflammatory diseases of the middle ear, compared with control, an increase in the frequencies of the minor G allele (30.6% vs. 25.4%) and the heterozygous genotype C / G (50.0% vs. 39.4%) was found. Consequently, an increase in the frequencies of functionally unfavorable allele (G) and genotypes (C / G and G / G) polymorphism of the IL6 (C174G) gene among patients may be associated with their participation in the mechanisms of formation of purulent-inflammatory diseases of the middle ear.

In this regard, it is possible to reliably assess their contribution to the processes of initiation of purulent-inflammatory diseases of the middle ear only on the basis of a statistical comparative analysis between groups of patients and healthy, the results of which are described below.

Thus, withthe equal results of differences in the carriage of alleles and genotypes for the polymorphic IL6 gene (C174G) between the main group of patients with purulent-inflammatory diseases of the middle ear and healthy ones, it was found that the minor G allele was 1.2 times (28.2% versus 25.4%; $\chi2=0.3$; P=0.6; OR=1.2; 95%CI: 0.7-1.91) was more common among patients than among healthy ones, but as statistical analysis shows, this difference was not significant.

Moreover, in the studied groups, differences between the frequencies of the basic and heterozygous C/C genotypes (52.9% vs. 54.9%; $\chi2=0.1$; P=0.8; AboutR=0.9; 95%CI: 0.49-1.73) and G/C (37.9% vs 39.4%; $\chi2<3.84$; P=0.9; OR=0.9; 95%CI: 0.49-1.79) not reaching one were also not statistically significant. Meanwhile, while the percentage frequency of the mutant G/G genotype was significantly higher among patients by 1.7 times (9.2% vs. 5.6%; $\chi2=0.7$; P=0.5; AboutR=1.7; 95%CI: 0.49-5.81) the differences identified did not reach a statistically significant result (Table 2).

Table 2

Differences in the distribution of frequencies of allelic and genotypic variants of single-nucleotide polymorphism IL6 (C174G) among patients with purulent-inflammatory diseases of the middle ear and healthy individuals

Alleles and genotypes	Number of alleles and genotypes examined				χ^2	R	RR	95%CI	OR	95%CI
	I group		IV group							
	n	%	n	%						
C	125	71.8	106	74.6	0.3	0.6	1.0	0.63-1.47	0.9	0.52 - 1.43
G	49	28.2	36	25.4	0.3	0.6	1.0	0.59-1.82	1.2	0.7 - 1.91
C/ C	46	52.9	39	54.9	0.1	0.8	1.0	0.55-1.67	0.9	0.49 - 1.73
C/ G	33	37.9	28	39.4	0.0	0.9	1.0	0.54-1.7	0.9	0.49 - 1.79
G /G	8	9.2	4	5.6	0.7	0.5	1.6	0.71-3.77	1.7	0.49 - 5.81

Thus, despite the presence of the predominance of carriage of unfavorable alleles G and G / G for the polymorphic IL6 gene (C174G) among patients of the general group with purulent-inflammatory diseases of the middle ear in 1.2 ($\chi2 = 0.3$; P = 0.6) and 1.7 times ($\chi2 =0.7$; P = 0.5), respectively, in comparison with similar among healthy people, the lack of their statistically significant significance proves the absence of their role in the mechanisms of initiation of purulent-inflammatory diseases of the middle ear as a whole.

In the distribution of allele frequencies and genotypes by the polymorphic IL6 (C174G) gene in the group of patients with a mild course of purulent-inflammatory diseases of the middle ear compared with those among healthy ones, the fact of a statistically unreliable increase in the occurrence of the minor G allele by 1.3 times (30.6% vs. 25.4% was established; $\chi2=0.7$; P=0.5; About R=1.3; 95%CI: 0.69-2.43). Moreover, the distribution of wild C/C frequencies was also characterized by the absence of statistically significant differences between the groups (44.4% versus 54.9%; $\chi2=1.1$; P=0.4; AboutR=0.7; 95%CI: 0.29-1.47) and mutant G/G (5.6% vs 5.6%; $\chi2<3.84$; P=0.99; AboutR=1.0; 95%CI: 0.17-5.65) genotypes. However, it is important to note that in the frequency of the heterozygous C/G genotype, although not strongly expressed, but still traced a tendency to its increase of 1.5 (50.0% vs. 39.4%; $\chi2=1.1$; P=0.3; AboutR=1.5; 95%CI: 0.69-3.44) times among patients compared to similar in the healthy group.

Consequently, the results of statistical analysis indicate the absence of significance of the minor alleles G ($\chi2 = 0.7$; P = 0.5) and genotype G / G ($\chi2<3.84$; P = 0.99) for the polymorphic gene IL6 (C174G) in the processes of formation of a mild course of purulent-inflammatory diseases of the middle ear. But at the same time, an increase in the proportion of heterozygous genotype C / G is accompanied by a tendency to increase the risk of mild course of purulent-inflammatory diseases of the middle ear by 1.5 times. Perhaps, with a larger coverage of patients, the identified difference could be statistically significant.

The results of a comparative analysis of allele frequencies and genotypes for the polymorphic IL6 (C174G) gene in the group of patients with moderate and severe purulent-inflammatory diseases of the middle ear compared with the healthy group did not differ in the presence of statistically significant differences. For example, in the proportion of the carrier of the minor G allele between the groups, the insignificant difference was equal to 1.1 (26.5% vs. 25.4%; $\chi2<3.84$; P=0.9; OR=1.1; 95%CI: 0.59-1.89) with negligible differences of 1.2 in the frequency of genotype C/C (58.8% vs. 54.9%; $\chi2=0.2$; P=0.7; About R=1.2; 95%CI: 0.57-2.43) and not reaching one in the frequency of the heterozygous C/G genotype (29.4% vs. 39.4%; $\chi2=1.3$; P=0.3; About R=0.6; 95%CI: 0.3-1.38). Differences in their frequencies did not reach a significant level due to their close values in the studied groups.

Meanwhile, the apparent high frequency of the mutant G/G genotype among patients showed a tendency to increase the likely risk of formation of purulent-

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inflammatory diseases of the middle ear with a medium and severe course by 2.2 times (11.8% vs. 5.6%; $\chi2$=1.5; P=0.3; AboutR=2.2; 95%CI: 0.61-8.14).

In the distribution of allele frequencies and genotypes according to the polymorphic IL6 (C174G) gene in the II group of patients with lung and in the III group of patients with moderate and severe purulent-inflammatory diseases of the middle ear, an insignificant increase in the occurrence of the minor G allele was found by 1.2 times (30.6% versus 26.5%; $\chi2$=0.3; P=0.6; OR=1.2; 95%CI: 0.63-2.38) among patients of group II.

Also, between the groups, the absence of statistically significant differences was recorded in the distribution of frequencies of wild C / C (44.4% versus 58.8%; $\chi2$=1.8; P=0.2; AboutR=0.6; 95%CI: 0.24-1.32) and mutant G/G (5.6% vs 11.8%; $\chi2$=1.0; P=0.4; AboutR=0.4; 95%CI: 0.09-2.24) genotypes. At the same time, in the frequency of the heterozygous C/G genotype, a pronounced tendency to increase it by 2.4 times was traced (50.0% versus 29.4%; $\chi2$=3.8; P=0.1; About R=2.4; 95%CI: 1.0-5.79) among patients of group II compared with a similar one in group III, which proves the fact of an increased risk of development in relation to the mild course of purulent-inflammatory diseases of the middle ear among carriers of this genotype for the polymorphic il6 gene (C174G).

Conclusion. Thus, analyzing the results, on the study of the features of the distribution of frequencies of occurrence of alleles and genotypes for the polymorphic gene IL6 (C174G) among the studied groups of patients with purulent-inflammatory diseases of the middle ear and healthy among carriers of the heterozygous genotype C / G, a tendency to increase the risk of purulence was foundinflammatory diseases of the middle ear of mild course by 1.5 times ($\chi2$ = 1.1; P = 0.3) compared with the control and by 2.4 times ($\chi2$ = 3.8; P = 0.1) compared with the medium and severe course of the disease, as well as among carriers of the mutant genotype G / G, a tendency was revealed to increase the likely risk of formation of purulent-inflammatory diseases of the middle ear with a middle ear average2.2 times the current and severe in comparison with the control ($\chi2$ = 1.5; P = 0.3). Perhaps, with greater coverage of patients, these differences could reach statistically significant significance. In this regard, according to the polymorphic il6 (C174G) gene, the C/ G and G / G genotypes can be considered as genetic predictors of an increased risk of formation of purulent-inflammatory diseases of the middle ear in Uzbekistan.

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