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G308a Tnf-A Gene Polymorphism in Children's Recurrent Bronchitis and its Effect on Tnf-A Interleukin Synthesis

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Resume

The aim of this study was to study the frequency of polymorphic alleles and genotypes G-308A of the TNF-α gene, as well as their influence on the synthesis of TNF-α in patients with RB. The study was carried out in 119 children aged 2 to 7 years with RB (main group). All patients of the main group were divided into 2 subgroups: subgroup I of 62 children with recurrent bronchitis, subgroup II consisted of 57 patients with RB on the background of LGD. The control group consisted of 110 conventio healthy children of the same age. The distribution of alleles and genotypes of the TNF-α gene in the studied groups of patients with RB and RB on the background of LGD and the control group corresponded to the Hardy-Weinberg equilibrium. In the active phase of RB, the level of TNFα was 6.7 times higher than in the control group. In children of RB against the background of LGD, a 4.9-fold increase in the TNFα concentration was revealed. In patients of subgroup II in the remission phase, there was a tendency towards a decrease in TNFα ($P > 0.49$), while in patients of subgroup I in the remission phase, the level of TNFα was significantly reduced ($P < 0.0001$). The data obtained indicate that in RB patients against the background of LGD, the process of acute immune inflammation lasts longer. The highest frequency of the G allele and the G/G genotype was noted in the studied groups. At the same time, the indices of the G allele and the homozygous G/G genotype in patients of the II subgroup tended to decrease in comparison with the control group, i.e. this genotype has a protective effect. The minor allele A and the heterozygous G / A genotype were greatest in subgroup II (17.7% in subgroup I and 21% in subgroup II) compared to conditionally healthy children. At the same time, the chance of developing of RB carriers of the heterozygous genotype G / A in subgroup II is 1.6. Not a single case of carriage of mutant genotypes A / A was revealed among patients with RB and healthy people. The presence of the A allele was accompanied by an increase in TNFα production in RB patients against the background of LGD, regardless of the phase of the disease.

Key words: recurrent bronchitis, lymphotic-hypoplastic diathesis, gene polymorphism, cytokines, rs1800629, tumor necrosis factor alpha.

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Despite the use of modern diagnostic research methods, the proportion of bronchitis of a recurrent nature is increasing. Thus, the prevalence of RB in children is currently 2.5 per 1000 children. Despite the fact that the problem of treatment and prevention of bronchitis in children is well covered in the literature, the genetic basis remains poorly understood. In this regard, it is relevant to identify and study genetic markers in children with RB. Based on modern data on the pathogenesis of respiratory damage in children, the genes of pro- and anti-inflammatory cytokines are candidate genes and are closely related to the development and clinical course of these diseases [1,9]. The study of polymorphism of cytokine genes, regulation of the functional activity of cells of the immune system and genetic control of the immune response makes it possible to develop criteria for susceptibility to diseases, including those of the respiratory system. In this regard, it is relevant to study the association of cytokine gene polymorphism in children of the Republic of Belarus in the Uzbek population.

Purpose of the study: to study the information content and frequency of distribution of alleles and genotypes of the G308A polymorphism of the TNFα gene in children with recurrent bronchitis against the background of lymphatic-hypoplastic diathesis (LHD) in the Uzbek population.

Materials and research methods: The survey included 119 children aged 2 to 7 years with RD (main group): 77 (64.7%) boys and 42 (35.3%) girls. All patients of the main group were divided into 2 subgroups: subgroup I consisted of 62 children with recurrent bronchitis, subgroup II consisted of 57 patients with RB on the background of LHD. The average age of the children was 4.1±0.82 years. In 12 (35%) patients with RB on the background of LHD, chest radiography did not reveal an enlargement of the thymus gland. The degree of thymomegaly was assessed according to J. Gewolb (1979). At the same time, grade 1 thymomegaly was found in 22 (48.8%; ≤ 0.33 CTTI < 0.37), grade 2 in 17 (37.7%; ≤ 037 CTTI < 0.42) and grade 3 in 12 (26.6%; ≥ 0.42 CTTI) children with LHD. The control group consisted of 110 conditionally healthy children of the same age. The diagnosis of RB was established in accordance with the ICD criteria. The diagnosis of LGD was made on the basis of clinical and laboratory studies. The degree of thymomegaly was determined using chest radiography. Patients were examined in the dynamics of the disease twice: in the acute period of bronchitis and in follow-up, 1 month after the last episode of bronchitis. All patients were hospitalized during the acute period of recurrent bronchitis. At the same time, 19 (30.6%) children of the first subgroup suffered from obstructive bronchitis and 43 (69.4%) children suffered from simple bronchitis. While 42 (73.7%) children of the second subgroup suffered from obstructive bronchitis and 15 (26.3%) children suffered from simple bronchitis.

The concentration of TNF-α cytokines in blood serum was carried out by enzyme-linked immunosorbent assay using the ELISA test system "ELISA-TNF-α" (Vector-Best, Russia, 2009). All patients with RB against the background of LHD, as well as conditionally healthy children of Uzbek nationality who formed the control group, underwent PCR genotyping of the G308A polymorphism of the TNFα gene in the laboratory of molecular genetics of the Research Institute of Hematology and Blood Transfusion. Blood samples were taken on an empty stomach from the antecubital vein of the examined children under sterile conditions.

Research results: The results of studying the concentration of TNFα in blood serum are presented in Table 1.

Table 1. TNFα content in children with RB and RB against the background of LHD (M±m)

	Control	Active phase	Remission	P
Recurrent bronchitis I subgroup	7,6±0,81	51,1±4,14	18,9 ±1,86	<0.0001
Recurrent bronchitis due to LHD II subgroup		32,68±1,97	30,72±1,97	<0.0001

Note: P-significance of difference in comparison with data from the control group.

As can be seen from Table 1, in children with RB in the active phase, an increase in the level of TNFα was detected, which was 6.7 times higher in the active phase and 2.5 times higher in the remission phase compared to the control group (P < 0.0001).

When studying the level of TNFα in children with RB against the background of LHD, we found an increase in the concentration of TNFα by 4.9 times in the active phase of the disease and 4.3 times in the remission phase compared to the control (P<0.0001). In patients of subgroup II in the remission phase, there was a tendency to decrease TNFα (P>0.49), while in patients of subgroup I in the remission phase, the level of TNFα was significantly reduced (P<0.0001). The data obtained indicate that in patients with RB against the background of LHD, the process of acute immune inflammation persists longer and can transform into chronic inflammation. The gene encoding TNF-α is located on the short arm of chromosome 6 (6p21.1 – 6p21.3). It has several single nucleotide polymorphisms in the regulatory region [4,5,6]. To date, the most significant variant is the substitution of guanine for adenine at position 308(G/A) (rs1800629). The 308A allelic variant of this gene affects the level of mRNA transcription and the biosynthesis of this cytokine in the body [2,8].

A study of the frequency distribution of alleles and genotypes of the G308A polymorphism of the TNFα gene is presented in table. 2

Table 2. Frequency of distribution of alleles and genotypes of the G-308A insertion-deletion polymorphism of the TNF-α gene in observation groups (case-control)

Alleles and genotypes	Main group n=119		Control group n=110		χ^2	P	RR	95% CI	OR	95% CI
	n	%	n	%						
G	215	90.3	204	92.7	0.8	0.4	1.3	0.721; 2.448	1.4	0.701; 2.655
A	23	9.7	16	7.3						
G/G	96	80.7	94	85.5	0.9	0.3	0.9	0.84; 1.061	0.7	0.353; 1.429
G/A	23	19.3	16	14.5	0.9	0.3	1.3	0.742; 2.38	1.4	0.712; 2.830
A/A	0	0	0	0	-	-	-	-	-	-

As can be seen from Table 2. The frequency of occurrence of the wild G allele of the TNF-α gene in the general sample and control group was statistically insignificant and amounted to 90% and 92.7%. The unfavorable allele rs1800629 A was rare and occurred in 7.3% of the control group and 10% of the main group of patients. When carrying out statistical processing, despite minor differences, a high odds ratio for detecting an unfavorable allele A in patients with RB in the general sample was revealed (OR=1.4; 95% CI: 0.701-2.655). Moreover, in subgroup I (n=62) the frequency of the unfavorable allele was observed in 8.9%, and in subgroup II (n=57) in 10.5% of cases. Carriage of allele A in subgroup II was 1.4 times higher than in the control group (χ2=1.03; P=0.3; OR=1.5; 95% CI 0.684; 3.29) and 1.2 times higher than in subgroup I (OR=0.8; 95% CI 0.35; 1.957) table 2. It is known that the association of the A allele with a higher level of TNF-α production indicates the possible significance of this allele as a risk factor for complications [3,5,7]. In our studies, children in subgroup I showed a slight increase in the

frequency of the minor allele A compared to the control group (8.9% versus 7.3%) and a month after the active phase there was a significant decrease in the level of TNF-α (P <0.0001) and a complicated course no diseases were detected.

Table 3. Differences in the frequency of occurrence of alleles and genotypes of the G308A gene of the TNFα I subgroup of patients and the control sample

Alleles and genotypes	Subgroup I n= 62		Control group n=110		χ2	P	RR	95% CI	OR	95% CI
	n	%	n	%						
G	113	91.1	204	92.7	0.3	0.6	1.2	0.585; 2.545	1.2	0.557; 2.766
A	11	8.9	16	7.3						
G/G	51	82.3	94	85.5	0.3	0.6	1.0	0.838; 1.106	0.8	0.341; 1.828
G/A	11	17.7	16	14.5	0.3	0.6	1.2	0.605; 2.461	1.3	0.547; 2.935
A/A	0	0	0	0	-	-	-	-	-	-

Table 4. Differences in the frequency of occurrence of alleles and genotypes of the G308A gene of TNFα II in the subgroup of patients and the control sample

Alleles and genotypes	Subgroup II n=57		Control group n=110		χ^2	P	RR	95% CI	OR	95% CI
	n	%	n	%						
G	102	89.5	204	92.7	1.03	0.3	1.4	0.709; 2.954	1.5	0.684; 3.29
A	12	10.5	16	7.3						
G/G	45	79	94	85.4	1.14	0.3	0.9	0.791; 1.078	0.6	0.279; 1.462
G/A	12	21	16	14.5	1.14	0.3	1.4	0.736; 2.847	1.6	0.684; 3.588
A/A	0	0	0	0	-	-	-	-	-	-

Analysis of the distribution of genotypes G/G in the main group of patients was 80.7% (82.3% in subgroup I and 79% in subgroup II of patients), in the control group 85.4% was recorded. The highest frequency of the G allele and G/G genotype was noted in the studied groups. At the same time, the indicators of the G allele and homozygous genotype G/G in patients of subgroup II tended to decrease compared to the control group, i.e. this genotype has a protective effect. An increase in the number of homozygous G/G genotype in children of the control group indicates a possible protective effect of this genotype regarding the formation of RB against the background of LGD. The frequency of heterozygous carriage of the G/A genotype in the main group of patients was 19.3% (17.7% in subgroup I and 21% in subgroup II of patients); in the control group it was 14.5%. Indicators of heterozygous carriage of the G/A genotype in the main group of patients tended to increase. At the same time, the chance of development in relation to RB was 1.4 (95% CI: 0.7-2.83). Studying the frequency of occurrence of the heterozygous G/A genotype between subgroups, the highest frequency of occurrence of this genotype was revealed in patients in subgroup II (17.7% in subgroup I and 21% in subgroup II) compared to conditionally healthy children. At the same time, the chance of developing RB in carriers of the heterozygous genotype G/A in subgroup II is 1.6 [CI95%: 0.684; 3.588] table 4. It is known from the literature that the polymorphic variant for the alleles and genotypes of the G-308A polymorphism of the TNF-α gene is characterized by some frequency differences between ethnic groups [10]. According to these data, for Mongoloids, the frequency of occurrence of the unfavorable A/A genotype is –0-2% [NCBI (http:www.ncbi.nlm.nlm.nih.gov/snp) and Allele Frequencies in Worldwide populations].

This is confirmed by our study, which showed that in all studied groups there were no cases of carriage of A/A genotypes of the TNF-α gene. Thus, the results of the study indicate that the G-308A polymorphism

of the TNF-α gene affects the level of tumor necrosis factor alpha in the blood of patients with RB on the background of LHD. An unreliable decrease in this cytokine in the remission phase shows that in patients with RB against the background of LHD, the acute immune inflammatory process persists longer and can transform into a chronic one.

Conclusion: The results obtained indicate that the highest frequency of the G allele and the G/G genotype of the promoter of the tumor necrosis factor alpha gene rs1800629 in the studied groups reduce the likelihood of developing the disease, and indicate a possible protective effect of this allele and genotype regarding the formation of RB and RB against the background of LGD . At the same time, not a single case of carriage of mutant A/A genotypes was detected among patients with RB and healthy people. The presence of the A allele is accompanied by an increase in TNFα production in patients with RB against the background of LHD, regardless of the phase of the disease.

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