Central Asian Journal of Medicine

Volume 2021 | Issue 3 Article 2

10-4-2021

G308A POLYMORPHISM OF TNFα GENE AND ITS INFLUENCE ON THE SYNTHESIS OF THE ALPHA TUMOR NECROSIS FACTOR IN RECURRENT BRONCHITIS IN CHILDREN

Sh.S. Bakhronov
Samarkand State Medical Institute, Samarkand, 140100, Uzbekistan

O.A. Sharipova
Samarkand State Medical Institute, Samarkand, 140100, Uzbekistan

T.A. Bobomuratov
Tashkent Medical Academy, Tashkent, 100109, Uzbekistan

D.Kh. Mamatkulova
Samarkand State Medical Institute, Samarkand, 140100, Uzbekistan

Central Asian Journal of Medicine

G308A POLYMORPHISM OF TNFα GENE AND ITS INFLUENCE ON THE SYNTHESIS OF THE ALPHA TUMOR NECROSIS FACTOR IN RECURRENT BRONCHITIS IN CHILDREN

Sh.S. Bakhronov[1], O.A. Sharipova[1], T.A. Bobomuratov[2], D.Kh. Mamatkulova[1]

1 Samarkand State Medical Institute, Samarkand, Uzbekistan
2 Tashkent Medical Academy, Tashkent, Uzbekistan

ABSTRACT

In the formation and outcome of recurrent bronchitis, the participation of the genetic characteristics of the organism, in particular, the polymorphism of the genes of some cytokines, is not excluded. One of which is tumor necrosis factor alpha. 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 groupcorresponded 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.

INTRODUCTION

Despite the use of modern diagnostic methods of research, the proportion of recurrent bronchitis 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 tract damage in children, genes for 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 cytokine gene polymorphism, 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 the respiratory system. In this regard, it is relevant to study the association of cytokine gene polymorphism in children of RB 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 (LGD) in the Uzbek population.

Research materials and methods: The survey included 119 children aged 2 to 7 years with RB (main group): 77 (64.7%) boys and 42 (35.3%) girls. 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 average age of children was 4.1 ± 0.82 years. In 12 (35%) patients with RB on the background of LGD, chest X-ray 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 CTEE <0.37), grade 2 in 17 (37.7%; ≤ 037 CTTI <0.42) and grade 3 in 12 (26.6%; ≥ 0.42 CTEE) in children with LGD. The control group consisted of 110 apparently healthy children of the same age. RB was diagnosed in accordance with the ICD criteria. The diagnosis of LGD was made on the basis of clinical and laboratory studies. The extent of thymomegaly was determined by chest x-ray. The patients were examined in the dynamics of the disease twice: in the acute period of bronchitis and in the follow-up, 1 month after the last episode of bronchitis. All patients were hospitalized in 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 from simple bronchitis. Whereas 42 (73.7%) children of the second subgroup suffered from obstructive bronchitis and 15 (26.3%) children from simple bronchitis.

The concentration of cytokines TNF-α in blood serum was carried out by the method of enzyme-linked immunosorbent assay using the ELISA test system "ELISA-TNF-α" ("Vector-Best", Russia, 2009).

In all RB patients with LGD, as well as conditionally healthy children of Uzbek nationality, who made up the control group, PCR genotyping of the TNFα gene G308A polymorphism was carried out in the laboratory of molecular genetics of the Research Institute of Hematology and Blood Transfusion. Blood sampling was carried out on an empty stomach from the cubital vein of the examined children under sterile conditions.

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

Table 1.

TNFα content in children with RB and RB on the background of LGD (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 on the background of LGD II subgroup		32,68±1,97	30,72±1,97	<0.0001

Note: *P is the significance of the difference in comparison with the data of the control group.*

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

When studying the level of TNFα in RB children against the background of LGD, we revealed an increase in the concentration of TNFα 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 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 patients with RB against the background of LGD, the process of acute immune inflammation lasts longer and can be transformed into a chronic one.

The gene encoding TNF-α is located on the short arm of chromosome 6 6 (6p21.1 - 6p21.3). In the regulatory area, it has several single nucleotide polymorphisms [4,5,6]. To date, the most significant variant is the replacement of guanine for adenine in position 308 (G / A) (rs1800629). The allelic 308A variant of this gene affects the level of mRNA transcription and the biosynthesis of this cytokine in the body [2,8].

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

Table 2.

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

Alleles and genotypes	The 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 group of the general sample and control was statistically insignificant and amounted to 90% and 92.7%. The unfavorable allele rs1800629 A was rare and was found in 7.3% in the control group and 10% in the main group of patients. Statistical processing, despite minor differences, revealed a high ratio of detecting an unfavorable allele A in RB patients in the general sample (OR = 1.4; 95% CI: 0.701-2.655). Moreover, in subgroup I (n = 62), the frequency of the unfavorable allele was noted 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 ($\chi2$ = 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 3; 4.

It is known that the association of allele A 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 with 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 of the disease was not revealed.

Table 3.

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

Alleles and genotypes	Subgroup I n= 62		Control group n=110		$\chi2$	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 G308A of gene TNFα II 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	-	-	-	-	-	-

The analysis of the distribution of G / G genotypes 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% were recorded. 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. An increase in the number of homozygous G / G genotype in children of the control group indicates a possible protective effect of this genotype in relation to 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 the I subgroup and 21% in the II subgroup of patients) in the control group there were 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: 0.7-283). The study of the frequency of occurrence of the heterozygous G / A genotype between subgroups, revealed the highest frequency of occurrence of this genotype in patients with subgroup II (17.7% in subgroup I and 21% in subgroup II) compared to conditionally healthy children. At the same time, the chance of RB developing 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 alleles and genotypes of the G-308A polymorphism of the TNF-α gene is characterized by some differences in frequencies between ethnic groups [10]. According to these data, the frequency of occurrence of the unfavorable genotype A / A for Mongoloids 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 obtained 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 RB patients against the background of LGD. An unreliable decrease in this cytokine in the remission phase shows that in patients with RB on the background of LGD, the acute immune inflammatory process persists longer and can be transformed into the chronic one.

Conclusion: The results obtained indicate that the highest frequency of the G allele and genotype G / G of the promoter of the tumor necrosis factor alpha gene rs1800629 in the studied groups reduces the likelihood of developing the disease, and indicates a possible protective effect of this allele and genotype in relation to the formation of RB and RB against the background of LGD. At the same time, 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 is accompanied by an increase in TNFα production in RB patients against the background of LGD, regardless of the phase of the disease.

REFERENCES

1. Guliev N.D., Ragimova. N. D. Polymorphism of cytokine genes in newborns with intrauterine infections. \\ Russian Bulletin of Perinatology and Pediatrics, 2015 No. 6. p.42-47.

2. Zykov M.V., Makeeva O.A., Golubenko M.V. et al. Study of the rs1800629 (G308A) polymorphism of the TNFα gene in patients with ST-segment elevation myocardial infarction. Russian Journal of Cardiology 2014. No. 10. S.-13-18.

3. Mirmanova N.A. Association of genetic polymorphism of the TNFα gene (G-308) with the formation of severe and complicated forms of influenza in children. Chita State Medical Academy Chita. Volume 5, No. 4 2013 p.30-34.

4. Khotko E.A., Taganovich A.D. Polymorphism of receptor genes and their ligands in chronic obstructive pulmonary disease. Medical journal No. 3. P. 36-42. Belarus 2016.

5. Dawid S. [et al.] The signifcace of IL1β +3953C>T, IL-6 -174G>C and -596G>A, TNF-α -308G>A gene polymorphisms and 86 bp variable number tandem repeat polymorphism of IL-1RN in bronchopulmonary dysplasia in infants born before 32 weeks of gestation/ Cent Eur J Immunol. 2017: 42(3): pp.287-293

6. Hong S.J. [et al.] TNF-alpha (-308G/A) and CD14 (-159T/C) polymorphisms in the bronchial responsiveness of Korean children with asthma // J. Allergy Clin Immunol. 2007. No. 173. pp. 970-976.

7. HuGENavigator (2013). Available at: http://www.Hugenavigator.net/ HuGENavigator/ gene Prospector (accessed June 2013).

8. Hajeer A.H, Hutchinson I.V. Influence of TNF alpha gene polymorphisms on TNF alpha production and disease. Hum Immunol. 2001 Nov; 62(11):1191-9;

9. Patel J.A., Reuben Matalon Polymorphisms of immunity genes and susceptibility to otitis media in children. Published: April 9.2014. https:// doi.org/10.1371/journal.pone.0093930

10. Shevchenko A.V, Golovanova O.V, Konenkov V.I, Features of polymorphism of promoter regions of genes cytokines IL1, IL4, IL5, IL6, IL10 and TNFα at the European population of Western Siberia. Immunology 2010; 4; 176-181.