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TNF α POLYMORPHISM (- 308 G> A) IN CHILDREN WITH CHRONIC BRONCHITIS

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Abstract. In order to determine the role of polymorphic variants of the tumor necrosis factor α gene (TNFα -308G> A) in the development and course of chronic respiratory diseases, an analysis was made of the frequency of alleles and genotypes in patients with bronchial asthma and chronic bronchitis and healthy individuals of the Uzbek population. An analysis of the frequency distribution of genotypes and alleles of the studied gene showed that risk allele A and genotype A/A and A/G are markers of an increased risk of developing a disease of the bronchopulmonary system both in the group of patients with bronchial asthma and in children with chronic bronchitis.

Keywords: chronic bronchitis, bronchial asthma, gene polymorphism TNFα -308 G> A.

Introduction. Over the past decade, in the state of health of children and adolescents, there has been increasing in the overall incidence in almost all classes of diseases, a significant increase in the number of diseases with recurrent and chronic course [4,10]. Particularly noteworthy are diseases in children of the bronchopulmonary tract, such as acute and chronic bronchitis. The causes of diseases of the bronchopulmonary tract are viral, bacterial and fungal infections, various environmental factors (hypothermia, environmental instability, lack of vitamins and minerals in the diet), the presence of background diseases and comorbid conditions. However, studies of recent years show that the negative impact of environmental factors, as a rule, is realized against the background of an individual genetic predisposition for almost any pathology known today, including diseases of the bronchopulmonary system.

Despite the fact that the problem of lung pathology in children, it would seem, is well covered in the literature and national programs for their treatment and prevention have been developed, the genetic basis of diseases of the bronchopulmonary system remains little studied.

One of the most promising approaches in assessing the genetic predisposition to many recurrent diseases, in particular to respiratory diseases, is to identify their association with certain candidate genes. Based on current data on the pathogenesis of respiratory tract damage, the genes of pro- and anti-inflammatory cytokines are one of these candidate genes. Of greatest interest is tumor necrosis factor (TNFα), which belongs to cytokines that have many biological functions, such as cytotoxicity, immunoregulation, induction of inflammation, proliferation and apoptosis. TNFα, a potent pro-inflammatory cytokine produced primarily by macrophages and monocytes, is found in high concentrations in the

lungs of patients suffering from cystic fibrosis and probably plays an important role in leukocyte damage of the lungs during inflammation [1,5,9].

The variant A2 allele (adenine at position -308 of the TNFα gene promoter region) is associated with a high level of TNFα production as a result of the direct influence of the -308 G>A polymorphism on gene transcriptor activity [2,11]. TNFα is directly involved in the development of clinical signs of inflammation such as pain, fever, loss of muscle and bone mass, and also stimulates the proliferation of fibroblasts [2,7].

The ongoing research in this direction can be used in the development of prognostic markers of acute pathology in children and the optimization of treatment tactics and preventive measures with an individual approach for each patient.

The aim of the work was to study the association of TNFα –308G> A gene polymorphism with the development of chronic bronchitis in children of the Uzbek population.

Material and methods. A total of 116 children aged 4 to 14 years were examined. Based on the results of the examination, groups of patients with chronic kidney disease (n = 42), asthma (n = 28), and a control group (n = 46) were formed. Clinical examination and diagnosis were carried out on the basis of the pulmonology department of the RSSPMC of Pediatrics of the Ministry of Health of the Republic of Uzbekistan. Verification of the diagnosis was carried out according to the international classification of WHO (ICD-10). Examination of patients included general clinical methods (interrogation, study of objective status, clinical tests - general analysis of blood and urine, general analysis of sputum, electrocardiography, chest x-ray. External respiration function, which included determination of peak expiratory flow rate (EFR), forced expiratory volume in the first second (FSV^1), conducting bronchomotor tests.

Conditions for inclusion in the control group: the absence of a cough history, the absence of acute respiratory diseases during the previous three months, normal indices of the function of external respiration according to spirometry data.

For typing the TNFα candidate gene (- 308G> A), pyrosequencing methods (PyroMark Q24, PyroMark Gold Q24 Reagents, Qiagen, Germany), qPCR method (DT-Prime, Russia) and microarray PCR detection method (MCE 202 MultiNA, Zhimadzu, Japan).

Thermostable DNA polymerase Taq from DNA technology (Moscow, RF) was used in the work. Single nucleotide primers TGGAAGTTAGAAGGAAACAGAC and ACACAAGCATCAAGGATACC were used. DNA concentration was measured on a NanoDrop ™ Lite spectrophotometer (Thermo Fisher Scientific, USA) and all DNA samples were 50-100 ng / pl. Optimization of qPCR genotyping of TNFα (- 308G> A) was carried out with the following program parameters: preliminary heating of 95 ° C for 5 minutes, then three-stage PCR 35 cycles of denaturation 94 ° C for 20 seconds, annealing of primers 66 ° C for 25 seconds, elongation 72 ° C - 2 minutes and finally holding 5 minutes at 72 ° C for the final elongation of amplicons. Composition of the PCR reaction: PCR buffer (UtM Tris-HCI pH 8.3, 50 tM KCI, Tween-20 1%), dNTP's mixture 0.25 tM each, 2.5 tM MgCI, primers 0.4 cM each, Taq polymerase 0.05i / q1, genomic DNA 50-100ng.

The statistically significant differences ($p < 0.05$) of the frequencies of the G308 / 308A alleles of the TNF-α gene were calculated using the non-parametric Fisher method, $\chi 2$ (ksi-square), OR (odds-ration - odds ratios), 95% confidence interval (95% CI)

Results and discussion.

The frequency distribution of the genotypes of the studied TNFa genes in the group of patients with BA and healthy, consistent with the expected Hardy-Weyenberg equilibrium. When comparing the sample of patients with BA and the control group, significant

differences were revealed in the frequencies of the genotypes of the polymorphic locus -308G> A of the TNF α gene (Fig. 1).

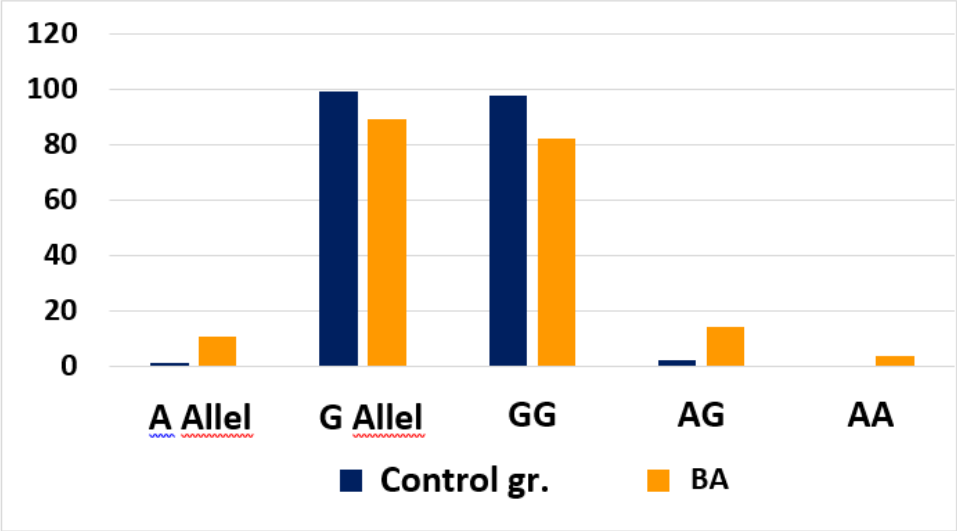

Fig. 1. The frequency of the genotype and allele of TNF-α variants (G-308 A) in patients with BA and the control group

As can be seen from the data in Fig. 1, the distribution of A and G alleles was almost the same in patients and the control group. The AA genotype was present in patients with BA in 3.6%, while in the control group it was completely absent.

Among the group of patients, 10 samples had an unfavorable genotype: in 5 individuals in the group with bronchial asthma, we identified the risk allele A, which was OR = 10.92 at 95% Cl 1.28-93.28 (p-0.007, x2_7.16). In addition to the significant risk allele A, risk genotypes A/A and A/G were also determined (p-0.05, x2-5.89) (Table 1.).

Table 1

The frequency of mutations in the TNF α gene (-308G> A) among children with bronchial asthma compared with the control.

Allels	Case	Control	X^2	P	*OR*	
	n = 28	n = 46			mean.	95% Cl
Allel *A*	0.107	0.011	7.16	0.007	10.92	1.28-93.28
Allel *G*	0.893	0.989			0.09	0.01-0.78

Genotype	Case	Control	x^2	P	*OR*	
	n = 28	n = 46			mean.	95% Cl
Genotype *A/A*	0.036	0.000	5.89	0.05	5.07	0.20-128.91
Genotype *A/G*	0.143	0.022			7.50	0.79-70.92
Genotype *G/G*	0.821	0.978			0.10	0.01-0.93

* A vs G *Pvalue* =0.001, χ^2=10.28; **AA vs AG, GG *Pvalue* =0.04, χ^2=6.54

A comparative analysis of the total sample of patients with CB and the control group showed statistically significant differences in the studied genotypes (Fig. 2). Allele A in the control group was found with a frequency of 1.1%, and in sick children with chronic

bronchitis - with a frequency of 7.1%. Allele G was found in patients and in the control group at approximately the same frequency.

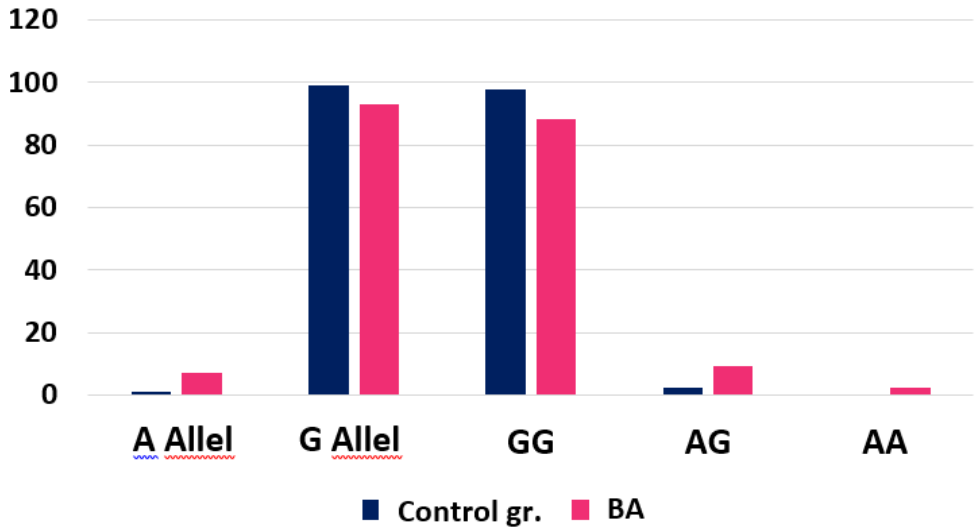

Fig. 2. The frequency of the genotype and allele of TNF-α variants (G-308 A) in patients with CB and the control group

The AA genotype was present in patients with chronic kidney disease in 2.4%, while in the control group it was completely absent. The AG genotype in patients with chronic kidney disease was found with a frequency of 9.5%, and among individuals who made up the control group with a frequency of 2.2%.

When studying the frequency of the polymorphic TNF α variant (- 308G> A) in the group of children diagnosed with chronic bronchitis, the risk allele A was also detected, which was OR = 7.00, with 95% Cl 0.82-59.41 (X2 = 4.22; P = 0.04) (table 2).

Table 2

The frequency of mutations in the TNF α gene (-308G> A) among children with chronic bronchitis compared with the control.

Alleles	Case	Control	X^2	P	*OR*	
	n = 42	n = 46			mean.	95% Cl
Allele A	0.071	0.011	4.22	0.04	7.00	0.82-59.41
Allele G	0.929	0.989			0.14	0.02-1.21

Genotype	Case	Control	X^2	P	*OR*	
	n = 46	n = 46			mean.	95% CI
Genotype *A/A*	0.024	0.000	3.41	0.18	3.36	0.13-84.80
Genotype *A/G*	0.095	0.022			4.74	0.51-44.21
Genotype *G/G*	0.881	0.978			0.16	0.02-1.47

* A vs G *Pvalue* =0.001, χ^2=10.28; **AA vs AG, GG *Pvalue* =0.04, χ^2=6.54

Thus, the results of molecular genetic studies have shown that carriers of the –308 * A polymorphic allele of the TNFα gene have a high risk of developing chronic bronchopulmonary pathology. This probably contributes to the development of a cell-

mediated adaptive immune response [3,6,8]. Under physiological conditions, TNFα is produced in the body in extremely small quantities, locally showing its effects. In pathological processes, its production is activated, and, getting into the blood, the tumor necrosis factor has a stimulating effect on neutrophils, epithelial and endothelial cells. The results of this study indicate that the lack of point substitution in the rs1800629 region of the TNFα gene affects the concentration of the cytokine of the same name, causing a decrease in the concentration of the molecule in carriers of the A/A and A/G variants, which, in turn, may not provide sufficient protection mechanisms. Thus, the obtained results allow us to conclude that the allele A and the genotype A/A and A/G of the TNFα rs1800629 gene promoter predispose to the development of chronic bronchopulmonary disease.

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