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ANALYSIS OF MOLECULAR GENETIC STUDIES IN CHILDREN WITH CHRONIC BRONCHITIS

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ABSTRACT

Background: 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, and analysis the frequency and alleles in patients with bronchial asthma and chronic bronchitis and healthy individuals of the Uzbek population was carried out. **Objective:** 116 children aged 4 to 14 were examined. According to the results of the survey, groups of patients with chronic bronchitis (CB) (n=42), BA (n=28) and a control group (n=46) were formed. **Methods:** For typing the candidate gene TNFa (-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). **Results:** Analysis of the frequency distribution of genotypes and alleles of the studied gene showed that A allele and the A/G genotype of TNFα rs1800629 gene promoter predispose to the development of chronic bronchopulmonary pathology.

KEYWORDS: Chronic bronchitis, Bronchial asthma, TNF α -308 G/A gene polymorphism.

INTRODUCTION

Over the past decade, in the state of health of children and adolescents, there has been an increase in almost all classes of diseases, a significant increase in the number of diseases with recurrent and chronic course.[1-7] Diseases of the bronchopulmonary tract, such as acute and chronic bronchitis, deserve special attention. The causes leading to diseases of the bronchopulmonary tract are viral, bacterial and fungal infections, various environmental factors (hypothermia, environmental instability, lack of vitamins and trace elements in the diet), the presence of background diseases and comorbid conditions.[12] However, recent studies show that the negative impact of environmental factors, is realized against the background of an individual genetic predisposition in almost any currently known pathology, including diseases of the bronchopulmonary system.[8,13]

Despite the fact that the problem of lung pathology in children seems to be well covered in the literature and national programs for their treatment and prevention, the genetic basis of diseases of the bronchopulmonary system remains poorly understood.

One of the most promising approaches to 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 lesions, genes for pro- and anti-inflammatory cytokines are one of such candidate genes. Of greatest interest is tumor necrosis factor (TNFa), which belongs to cytokines with multiple biological functions such as cytotoxicity, immunoregulation, inflammation induction, proliferation, and apoptosis. TNFa, a potent pro-inflammatory cytokine produced predominantly by macrophages and monocytes, is found at high concentrations in the lungs of patients with cystic fibrosis and appears to play an important role in leukocyte lung injury during inflammation.[9,10,14]

The variant A2 allele (Adenine at position -308 of the promoter region of the TNFA gene) is associated with a high level of TNFα production as a result of the direct effect of the -308 G-A polymorphism on the transcriptional activity of the gene. TNFα is directly involved in the development of such clinical signs of inflammation as pain, fever, loss of muscle and bone mass, and also stimulates the proliferation of fibroblasts.[7,9]

The ongoing research in this direction can be used in the development of prognostic markers for acute pathology in children and optimization of treatment tactics and

preventive measures with an individual approach for each patient.

The aim of the study was to study the association of TNF-308G/A gene polymorphism with the development of chronic bronchitis in children of the Uzbek population.

MATERIAL AND METHODS

116 children aged 4 to 14 were examined. According to the results of the survey, groups of patients with chronic bronchitis (CB) (n=42), BA (n=28) and a control group (n=46) were formed. Clinical examination and diagnostics were carried out on the basis of the pulmonology department of the Republican Scientific and Practical Center for Pediatrics of the Ministry of Health of the Republic of Uzbekistan. Verification of the diagnosis was carried out according to the international WHO classification (ICD-10). Examination of patients included general clinical methods (survey, examination of the objective status, clinical tests - general blood and urine analysis, general sputum analysis, ECG, chest x-ray. External respiration function, which included the determination of peak expiratory flow rate (PSV), forced expiratory volume in the first second (FEV^1), bronchomotor tests.

Conditions for inclusion in the control group: no cough history, absence of acute respiratory diseases during the previous three months, normal indicators of respiratory function according to spirometry.

For typing the candidate gene TNFa (-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).

The thermostable Taq DNA polymerase from DNA technology (Moscow, RF) was used in the work. Single nucleotide primers were used - TGGAAGTTAGAAGGAAACAGAC and ACACAAGCATCAAGGATACC. 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 TNFa (-308G>A) was performed with the following program parameters: preheating 95°C - 5 minutes followed by three-stage PCR 35 cycles denaturation 94°C - 20 seconds, primer annealing 66°C - 25 seconds, elongation 72 °C - 2 minutes and finally hold for 5 minutes at 72°C for the final elongation of the amplicons. Composition of the PCR reaction: PCR buffer (JutM Tris-HCI pH8.3, 50mM KCI, Tween-20 1%), dNTP's mixture 0.25mM each, 2.5mM MgCI, primers 0.4mM each, Taq polymerase 0.05u /c1, genomic DNA 50-100ng.

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

RESULTS AND IT'S DISCUSSION

The distribution of genotype frequencies of the studied TNFa genes in the group of BA patients and healthy controls corresponded to that expected from the Hardy-Weinberg equilibrium. When comparing the sample of patients with BA and the control group, significant differences in the frequencies of the genotypes of the -308G>A polymorphic locus of the TNFa gene were revealed (Fig. 1).As can be seen from the data in Fig. 1, the distribution of alleles A and G was almost the same in patients and the control group. The AA genotype was present in 3.6% of patients with BA, while it was completely absent in the control group.

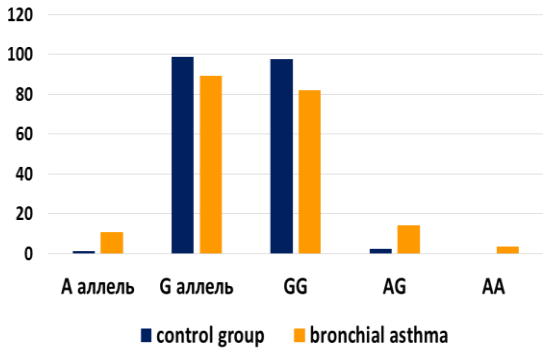

Fig. 1: Frequency of the genotype and allele of TNF-α variants (G-308 A) in patients with BA and the control group.

Among the group of patients with bronchial asthma, 10 samples had an unfavorable genotype: 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 risk significant allele A, risk genotypes A/A and A/G were also identified (p-0.05, x2-5.89) (Table 1.).

Table 1: The frequency of occurrence of mutations in the TNFA gene (rs361525) among children with bronchial asthma in comparison with the control.

Alleles	Cases	Controls	X^2	P	*OR*	
	n = 28	n = 46			values	95% Cl
Allele *A*	0.107	**0.011**	7.16	0.007	10.92	1.28-93.28
Allele *G*	0.893	0.989			0.09	0.01-0.78

Genotypes	Cases	Controls	x^2	P	*OR*	
	n = 28	n = 46			values	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 CB patients and the control group showed statistically significant differences in the studied genotypes (Fig. 2). Allele A in the control group met with a frequency of 1.1%, and in children with chronic bronchitis - with a frequency of 4.1%. The allele occurred in patients and in the control group with approximately the same frequency.

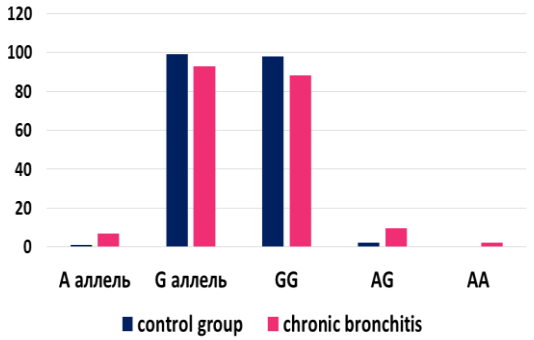

Fig. 2: Frequency of the genotype and allele of TNF-α variants (G-308 A) in patients with chronic bronchitis and the control group.

The AA genotype was present in 3.6% of patients with BA, while it was completely absent in the control group. The AG genotype in patients with CB was found with a frequency of 9.5%, and among those who made up the control group with a frequency of 2.2%.

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

Table 2: The frequency of occurrence of mutations in the TNF-α gene (rs361525) among children with chronic bronchitis in comparison with the control.

Alleles	Cases	Controls	X^2	P	*OR*	
	n = 42	n = 46			values	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

Genotypes	Cases	Controls	X^2	P	*OR*	
	n = 46	n = 46			values	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 the molecular genetic study showed that carriers of the -308*A polymorphic allele are at high risk of developing chronic bronchopulmonary pathology. This probably contributes to the development of a cell-mediated adaptive immune response.[7] Under physiological conditions, TNF-α is produced in the body in an extremely small amount, locally manifesting its effects. In pathological processes, its production is activated and getting into the blood, tumor necrosis

factor has a stimulating effect on neutrophils, epithelial and endothelial cells.

The results of this study indicate that the absence of a point substitution in the rs1800629 region of the TNF-α gene affects the concentration of the same cytokine, causing a decrease in the concentration of the molecule in carriers of the A/G variant, which, in turn, may not provide sufficient implementation of sanogenic defense mechanisms. Thus, the results obtained allow us to conclude that the A allele and the A/G genotype of the TNFα rs1800629 gene promoter predispose to the development of chronic bronchopulmonary pathology.

The carriage of the G allele and the A/G genotype of the promoter of the tumor necrosis factor-α rs1800629 gene reduce the likelihood of developing a chronic process.

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