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DETECTION OF RS9939609 POLYMORPHISM OF FTO GENE AND RS324011 POLYMORPHISM OF STAT6 GENE AND SEVERITY DEGREE OF BRONCHIAL ASTHMA ASSOCIATED WITH OBESITY

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Abstract

The aim of this research was to analyze the distribution of FTO and STAT6 genes polymorphism in patients with bronchial asthma (BA), associated with obesity (Ob) depending on the main disease severity degree.

Materials and methods. The study included 117 patients 18–48 years old, divided in 3 groups. The main group (bronchial asthma, associated with obesity) included 57 patients, two groups of comparison – 30 patients with the diagnosis BA and a normal body weight, and 30 patients with obesity, but without the pathology of the bronchopulmonary system.

The general genomic DNA was extracted from blood according to the standard protocol. The genetic typing was realized by the method of allele-specific amplification with the detection of results in the real time regime using TaqMan-probes, complementary to polymorphic parts of DNA. The detection of deletions in FTO and STAT 6 genes was realized by the method of polymerase chain reaction (PCR) using specific primers.

Results. In the main group, among patients with BA and Ob, carriers of T/T genotype were 36,84 %, T/A – 45,61 %, A/A – 17,55 % against 40 %, 60 % and 0 % respectively in PHP group by FTO gene. Carriers of C/C genotype in the main group were 38,6 %, C/T – 35,09 %, T/T – 26,31 % against 40 %, 55 % and 5 % respectively in PHP group by STAT6 gene. In the main group the light persisting BA was diagnosed in 20,0 % of cases, middle severity – in 60,0 % and severe – in 20,0 % of patients. In the group of comparison this disease severity was observed in 17,7 %, 66,5 % and 15,8 % of observations, respectively.

Conclusions. So, among patients with BA, associated with Ob with the middle and severe course of asthma the percent of heterozygous (T/A) and mutant carriers (A/A) rs9939609 polymorphism of FTO gene is higher than at the light course. The analogous situation is observed at the study of rs324011 polymorphism of STAT6 (C2892T) gene among this category of patients. So, the determination of FTO and STAT6 genes polymorphism in patients with BA, associated with Ob, can be considered as a marker of the more severe course of asthma.

Keywords: bronchial asthma, obesity, genes polymorphism.

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1. Introduction

Bronchial asthma (BA) it is a classic example of a multifactor pathology, realized at the interaction of numerous factors of environment and hereditary predisposition [1, 2]. Hereditary nature of the disease had been observed already in the last centuries and proved in XX century by hereditary forms of the disease and BA cases in twins. The genetic polymorphism is a base of the phenotypic difference between persons and may cause the hereditary predisposition to different nosologies [3, 4]. Today there is revealed a series of genes, involved in the asthma pathogenesis, that exist in different allele variants (single nucleotide polymorphisms) and favor BA appearance. The detection of "predisposition genes" may favor the purposeful primary prophylaxis in receptive persons [5]. It was established, that at the combination of BA and obesity can be observed the heavier asthma course, worsening of disease control and inadequate response of patients to the treatment [6]. In patients with BA and obesity is formed the difficultly controlled phenotype with manifestations of a dose-dependence or resistance to inhalation CS (ICS) [7]. In the cross research

by L. Barclay was demonstrated, that the risk of hospitalization in patients with BA and obesity is 5 times higher than in ones with a normal body mass [8]. The feature of BA course at the modern stage is the growth of specific weight of severe forms, including among youth that the high invalidism and lethality are connected with [9, 10].

So, mechanisms of the main questions of both BA and obesity are interconnected and need a deeper study at both molecular and genetic level. The most spread method of studying BA genetic mechanisms is the search for disease associations with candidate genes polymorphism. Thus, there are found genes, responsible at the same time for asthma and obesity formation, and also for the synthesis of anti-inflammatory cytokines [11, 12]. It was established, that at obesity the risk of BA development is 1,4–2,2 times higher than at a normal body mass [13, 14]. Alongside with it the testing of genes-candidates demonstrated that none of them has a preferential value, because the genetic base of the disease has the multifactor nature [15].

2. Aim of research

To analyze distributions of rs9939609 polymorphism of FTO gene and rs324011 polymorphism of STAT6 gene in patients with bronchial asthma, associated with obesity, depending on the main disease severity degree.

3. Materials and methods

The study included 117 patients 18–48 years old, divided in 3 clinical groups. The main group (bronchial asthma, associated with obesity – II group) included 57 patients with mean age 38 years old. Among them were 30 women (52 %) and 27 men (48 %). At the same time two groups of comparison were formed: the first one (1 group) included 30 patients with the diagnosis BA and normal body mass, the third (III group) – 30 patients with Ob and without the pathology of the bronchopulmonary system (BMI >25,0 kg/m^2). The control group consisted of 20 practically healthy persons (PHP). The mean duration of bronchial asthma was 8 years (2–15 years). The mean duration of obesity was 5 years. For setting the diagnosis, clinical course severity degree were used general clinical, laboratory and instrumental research methods based on GINA recommendations (2011, 2014) [16], order of MHP of Ukraine No. 128 "About approval of clinical protocols of medical care for the specialty "Pulmonology" and "Unified clinical protocol of the primary, secondary (specialized) medical care "Bronchial asthma" No. 868 of 08.10.2013.

Genetic studies were realized on the base of the medical-genetic laboratory "Hermedtech", Odessa city (license of MHP of Ukraine No. 196563 of 03.01.2013). The general genomic DNA was extracted from blood according to the standard protocol using the set for DNA extraction from clinical samples "AmpliPrime DNA-sorb-B" (made by AmpliSense, CSRI of epidemiology of MH RF, Russia). The genetic typing was realized by the method of allele-specific amplification with the detection of results in the real time regime using TaqMan-probes, complementary to polymorphic parts of DNA. The detection of deletions in FTO and STAT 6 genes was realized by the method of multiplex polymerase chain reaction (PCR) using specific primers. For FTO (T/A) gene the size of the amplicon for external primers – 321 pb, T-allele: 178 pb, A-allele: 201 pb. For STAT6 (C2892T) gene the size of the amplicon – 275 pb.

The obtained results of the study were analyzed using computer packages "STATISTICA" StatSoft Inc. and Excell XP Windows on a personal computer using parametric and non-parametric calculation methods. The distribution of genotypes in the studied sample was verified for the correspondence to Hardy-Weinberg equilibrium using the program Hardy-Weinberg Equilibrium Calculator for 2 Alleles.

4. Results of researches

In the process of the research were complexly examined 117 patients, 18–48 years old, mean age was 33±5,2 y. (**Table 1**).

Among patients wasn't observed any essential difference by gender. Thus, the male share was 49,2 %, female – 50,8 %.

Table 1

Clinical characteristics of patients

	I group (BA), n=30		II group (BA+Ob), n=57		III group (Ob), n=30		Control group (PHP) n=20	
Sex, n	M	F	M	F	M	F	M	F
	19	11	27	30	11	19	9	11
Mean age, years	28,5±4,63		38,52±3,25		29,52±7,77		30,33±5,33	

The expression of T/A polymorphism of FTO gene (rs9939609) and C2892T polymorphism of STAT6 (rs324011) gene was determined in 57 patients with bronchial asthma, combined with obesity, 30 patients with BA, 30 patients with Ob and 20 practically healthy persons. In the main group, carriers of T/T genotype were 36,84 %, T/A – 45,61 %, A/A – 17,55 % against 40 %, 60 % and 0 % respectively in PHP group by FTO gene. Carriers of C/C genotype in the main group were 38,6 %, C/T – 35,09 %, T/T – 26,31 % against 40 %, 55 % and 5 % respectively in PHP group by STAT6 gene.

Distributions of polymorphisms of selected genes in the main group (in patients with bronchial asthma, combined with obesity) are presented in **Table 2**.

Table 2

Distributions of polymorphisms of FTO and STAT6 genes in the main group

Genes	Absolute number, n %		II group (BA+Ob), n=57		χ^2	p	p1
FTO	Alleles	T	68	59,65			
		A	46	40,35			
	Genotypes	T/T	21	36,84	0,16	0,6922	>0,05
		T/A	26	45,61			
		A/A	10	17,55			
STAT6	Alleles	C	64	56,14			
		T	50	43,86			
	Genotypes	C/C	22	38,60	4,71	0,03	<0,05
		C/T	20	35,09			
		T/T	15	26,31			

Note: p – reliability of differences of frequencies between genotypes by χ^2 criterion; p1 – reliability of differences of parameters relative to Hardy-Weinberg scale of genotypes equilibrium

At the same time patients were distributed depending on BA severity degree and detection of FTO and STAT6 genes polymorphism (**Table 3**).

Table 3

Clinical-phenotypic characteristic of patients with bronchial asthma, combined with obesity, depending on FTO and STAT6 genes polymorphism and asthma severity degree

Genes			Patients with BA, combined with Ob (n=57)		
			Persisting light BA, n (%)	Persisting middle-severe BA, n (%)	Persisting severe BA, n (%)
FTO	Genotypes	T/T	8(14,0)	9(15,8)	4(7,0)
		T/A	5(8,8)	14(24,6)	7(12,3)
		A/A	1(1,7)	6(10,5)	3(5,3)
STAT6	Genotypes	C/C	4(7,0)	12(21,1)	6(10,5)
		C/T	4(7,0)	10(17,5)	6(10,5)
		T/T	2(3,5)	8(14,0)	5(8,8)

Note: n – number of observations

In the main group the light persisting bronchial asthma was detected in 21,0 % of cases, middle severity – in 52,6 % and heavy – in 27,2 % of patients.

5. Discussion of research results

Last time more and more studies are devoted to the genetic predisposition to certain diseases. The last years' studies, including the full genomic analysis demonstrated the essential connection between obesity and rs9939609 single nucleotide polymorphism in the first intron of FTO fat gene, fat mass and obesity associated [17, 18]. But mechanisms of this gene influence still insufficiently studied. Alongside with it among genes – candidates of BA and atopy the researchers' attention is mostly fixed on the interleukin gene – 4 (IL-4). IL-4 is often called the "critical cytokine of inflammation". It is used to think, that STAT-6 is activated by IL-4 and takes part in the induction of switching the synthesis of immunoglobulins to the synthesis of IgE and differentiation of Th2-cells [19]. There was also demonstrated the dependence for BA in 53 % of cases and for obesity in 77 %, that indicates the genetic interconnection between these diseases. In parallel with it, at the association of BA and obesity the effect of "mutual complication" is fully realized that favors the heavier clinical course of asthma, worse control of the disease and bad patients' response to the treatment [20, 21].

Considering general clinical characteristics of examined patients (sex, age, disease duration), it must be noted, that groups didn't essentially differ by age structure. At the same time among patients wasn't observed any essential difference by gender. Thus the male share was 49,2 %, female – 50,8 %.

Analyzing the obtained data, it can be seen, that among patients with bronchial asthma of middle severity, combined with obesity, the percent of heterozygous (*T*/*A*) and mutant carriers of (*A*/*A*) of rs9939609 polymorphism of FTO gene is 3 times higher than at the light course and 1,7 higher at the severe one. The analogous situation is observed at studying rs324011 polymorphism of STAT6 (C2892T) gene among this category of patients. The percent of heterozygous (*C*/*T*) and mutant (*T*/*T*) carriers of rs324011 polymorphism of STAT6 among patients with bronchial asthma of middle severity, associated with obesity is 3 times higher than at the light course and 1,8 times higher than at the severe one.

It was revealed 45,61 % of heterozygous (*T*/*A*) and 17,55 % of mutant (*A*/*A*) carriers of rs9939609 polymorphism of FTO gene and 35,09 % of heterozygous (*C*/*T*) and 26,31 % of mu-

tant (*T*/*T*) carriers of rs324011 polymorphism of STAT6 (C2892T) gene among patients with bronchial asthma and obesity.

So, the determination of FTO and STAT6 genes polymorphism in patients with BA, associated with Ob may be considered as a marker of the more severe asthma course, probably, with a worse response to the treatment.

The prospect of further researches is the study of interconnections of FTO and STAT6 genes polymorphism in patients with bronchial asthma in the combination with obesity, with the effectiveness of the basic treatment and asthma controllability.

6. Conclusions

1. Among patients with bronchial asthma of middle severity and severe one, combined with obesity, the percent of heterozygous (*T*/*A*) and mutant (*A*/*A*) carriers of rs9939609 polymorphism of FTO gene and heterozygous (*C*/*T*) and mutant (*T*/*T*) carriers of rs324011 polymorphism of STAT6 is higher than at the light course.

2. The determination of FTO and STAT6 genes polymorphism in patients with BA, associated with Ob may be considered as a marker of the more severe asthma course, probably, with a worse response to the treatment.

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