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ASSOCIATION ANALYSIS OF AMINOXIDASE 1 AOC1 AND HISTAMINE-N-METHYLTRANSFERASE HNMT GENE POLYMORPHISMS WITH THE DEVELOPMENT OF ASTHMA IN CHILDREN

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Asthma is a common multifactorial disease characterized by chronic inflammation of the respiratory tract, and respiratory symptoms such as wheezing, shortness of breath (dyspnea), coughing, that vary over time and intensity. The main goal of asthma management is to achieve and maintain clinical control of the disease over a long period of time, considering the safety of therapy and potential adverse reactions. At present, total asthma control is achieved in less than half of patients, and 10-20% of patients show signs of therapeutic resistance to certain groups of drugs. Several studies have revealed that heredity has a significant effect on the individual sensitivity to asthma therapy. In this regard, it is actual to study the genes involved in the metabolism of major groups of drugs used to asthma treatment. Antihistamines are frequently prescribed in the treatment of allergic diseases, it blocks the binding of histamine to its receptors by the mechanism of competitive inhibition, it has antipruritic, antiedema, antispasmodic and local anesthetic effect.

The aim of this work was to analyze associations of polymorphic variants of amine oxidase, copper-containing 1 *AOC1* and histamine N-methyltransferase *HNMT* genes involved in the histamine metabolism with the development of asthma in children living in the Republic of Bashkortostan. **Material and methods.** DNA samples of 430 unrelated individuals aged 2-17 years living in the Republic of Bashkortostan were used as the study material. Genotyping of polymorphic variants is carried out by PCR-RFLP method. **Results.** The associations of rs1049793**CC* genotype and rs1049793**C* allele of the *AOC1* gene with asthma development, with significant decreases in spirometry measures in Russians were revealed. The associations of rs1801105**CT* genotype and rs1801105**T* allele of the *HNMT* gene with significant decrease of MEF25 in asthma patients of Tatar ethnicity were established. **Conclusion.** The results of the study suggest that *AOC1* and *HNMT* polymorphic variants are involved in asthma development.

Keywords: bronchial asthma, polymorphic variant, association, aminoxidase 1 *AOC1* gene, histamine N-methyltransferase *HNMT* gene.

Introduction. Asthma is a severe heterogeneous disease characterized by chronic airway inflammation. The global prevalence of asthma is 1-18% [4]. Despite the rapid development of modern medicine in 10-20% of Russian patient's severe course of asthma with the signs of therapeutic resistance to various groups of drugs are diagnosed [2]. Genetic variability has a significant influence on the patient's sensitivity to the prescribed therapy [5]. Histamine is a biogenic amine that plays an important role in the development of inflammatory processes. The activation of histamine receptors in the lungs leads to bronchospasm and airway obstruction. Histamine is metabolized by two main enzymes, which are histamine-N-methyltransferase (HNMT) and aminoxidase (DAO, AOC1) [7]. Several studies have shown that polymorphic loci in the genes encoding histamine metabolizing enzymes are associated with allergic diseases [1, 7]. **The aim** of this work was to assess the significance of amiloride-sensitive amine oxidase *AOC1* and histamine-N-methyltransferase *HNMT* genetic polymorphisms involved in the histamine metabolism in the prediction of asthma developing in children of different ethnicities living in the Republic of Bashkortostan (RB).

Materials and methods. DNA samples of 430 unrelated individuals aged 2-17 years old from the RB were used in this work. The group of patients included

Table1

Distribution of allele and genotype frequencies of *AOC1* rs1049793 and *HNMT* rs1801105 gene polymorphisms in asthma patients and controls

Polymorphic variant / study group		Genotypes			Alleles		N
		n (%)	n (%)	n (%)	n (%)	n (%)	
AOC1 (rs1049793)		CC	CG	GG	C	G	
Patients	Russians	46 (56.1) p=0.009 OR=2.36 (1.24-4.5)	29 (35.37)	7 (8.54)	121 (73.78) p=0.01 OR=1.86 (1.16-3.01)	43 (26.22) p=0.01 OR=0.54 (0.33-0.87)	82
	Tatars	48 (44.86)	45 (42.06)	14 (13.08)	141 (65.89)	73 (34.11)	107
	Bashkirs	11 (25.0)	24 (54.55)	9 (20.45)	46 (52.27)	42 (47.73)	44
Controls	Russians	26 (35.14)	37 (50.0)	11 (14.86)	89 (60.14)	59 (39.86)	74
	Tatars	35 (43.21)	41 (50.62)	5 (6.17)	111 (68.52)	51 (31.48)	81
	Bashkirs	15 (41.67)	15 (41.67)	6 (16.67)	45 (62.50)	27 (37.5)	36
HNMT (rs1801105)		CC	CT	TT	C	T	
Patients	Russians	66 (78.57)	15 (17.86)	3 (3.57)	147 (87.5)	21 (21.15)	84
	Tatars	76 (72.38)	29 (27.62)	-	181 (86.19)	29 (13.81)	105
	Bashkirs	31 (70.45)	12 (27.27)	1 (2.27)	74 (84.09)	14 (15.91)	44
Controls	Russians	58 (78.38)	13 (17.57)	3 (4.05)	129 (87.16)	19 (12.84)	74
	Tatars	67 (81.71)	15 (18.29)	-	149 (90.85)	15 (9.15)	82
	Bashkirs	30 (83.33)	6 (16.67)	-	66 (91.67)	6 (8.33)	36

Note to tables 1-2. N is the number of individuals; n is the number of groups; allele and genotype frequencies are given in brackets, %; p is the p-value and is shown in the case of statistical significance only (p<0,05); OR is the odds ratio and 95% confidence interval (in brackets).

236 asthma patients (70 girls, 166 boys) of different ethnicities (Russians - 84, Tatars - 108, Bashkirs - 44). All investigated individuals were the patients of the children's division of the Clinic at Bashkir State Medical University of the Russian Ministry of Health (Ufa, Russia) and the Allergology Department of the Republican Children's Clinical Hospital (Ufa, Russia). The evaluation of the external respiration parameters was carried out on a computer spirograph "Erich Jaeger" (Germany). The control group comprised 194 practically healthy individuals (119 girls, 75 boys) of the corresponding ethnicity (Russians - 75, Tatars - 83, Bashkirs - 36) without bronchopulmonary and allergic diseases, with a low level of total immunoglobulin E (0-60 ME/ml). Children from 15 years old and parents of children under 15 years old gave informed consent to participate in the study. The study protocol was approved by the local bioethics committee of the Institute of Biochemistry and Genetics - Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences (Protocol no 7 dated February 10, 2011).

Genomic DNA was isolated from the peripheral blood lymphocytes by phenol-chloroform extraction. The analysis of rs1049793 (p.1990C>G, p.His664Asp) polymorphism of the *AOC1* gene and

Table2

Distribution of allele and genotype frequencies of AOC1 rs1049793 and HNMT rs1801105 gene polymorphisms in asthma patients and control groups with different parameters of external respiration function

Polymorphic variant/ study group		Genotypes			Alleles		N
		n (%)	n (%)	n (%)	n (%)	n (%)	
AOC1 (rs1049793)		*CC*	*CG*	*GG*	*C*	*G*	
Asthma patients of Russian ethnicity	FEV1 > 78.1%	9(60)	5(33.33)	1 (6.67)	24(80)	6(20)	15
	FEV1 56.5-78.1%	3	-	4	6	8	7
	FEV1 < 56.5%	18(58.06) p=0.03, OR=2.56. (1.08-6.03)	11(35.48)	2(6.45)	47(75.81) p=0.03, OR=2.08. (1.07-4.05)	15(24.19)	31
	MEF25 > 71.7%	13(65)	6(30)	1(5)	32(80)	8(20)	20
	MEF25 37.7-71.7%	6(54.55)	4(36.36)	1(9.09)	16(72.73)	6(27.27)	11
	MEF25 < 37.7%	18(58.06) p=0.03, OR=2.56. (1.08-6.03)	11(35.48)	2(6.45)	47(75.81) p=0.03, OR=2.08. (1.07-4.05)	15(24.19)	31
Control group of Russian ethnicity		26 (35.14)	37 (50.0)	11 (14.86)	89 (60.14)	59 (39.86)	74
HNMT (rs1801105)		*CC*	*CT*	*TT*	*C*	*T*	
Asthma patients of Tatar ethnicity	MEF25 > 71.7	35(89.74)	4(10.26)	-	74(94.87)	4(5.13)	39
	MEF25 37.7-71.7	6	3	-	15	3	9
	MEF25 < 37.7%	14(51.85) p=0.002, OR=0.24. (0.09-0.62)	13(48.15) p=0.002. OR=4.15, (1.62-10.62)	-	41(75.93) p=0.004, OR=0.32. (0.14-0.72)	13(24.07) p=0.004. OR=3.15, (1.39-7.14)	27
Control group of Tatar ethnicity		67 (81.71)	15 (18.29)	-	149 (90.85)	15 (9.15)	82

rs1801105 (p.314C>T, p.Thr105Ile) polymorphism of the *HNMT* gene was performed by PCR-RFLP. The primer sequences, the amplifiable fragment sizes, and restriction enzyme's names are described earlier [3]. A pairwise comparison of genotype and allele frequencies in asthma patients and in controls was performed by using the χ2 criterion for 2x2 conjugency tables with Yates correction. In the case of statistically significant differences between the compared samples, the odds ratio (Odds Ratio, OR) and the boundaries of 95% confidence interval (CI 95%) were assessed.

Results and discussion. The study of the allele and genotype distributions of the amine oxidase, copper-containing 1 gene *AOC1* rs1049793 and of the histamine-N-methyltransferase gene *HNMT* rs1801105 polymorphic variants in asthma children and healthy individuals of different ethnicities living in RB was conducted (Table 1). The distribution of genotype frequencies in studied polymorphisms corresponded to the Hardy-Weinberg equilibrium (p>0,05).

The *AOC1* gene is located on chromosome 7q36.1 and contains 10 exons. We found statistically significant differences between asthma patients and controls in Russians, based on the analysis of allele frequencies and genotype distributions of rs1049793 polymorphism of the *AOC1* gene. The association of rs1049793**CC* genotype and rs1049793**C* allele of the *AOC1* gene with risk of asthma development in Russians was established (p=0,009, OR=2,36, 95%CI 1,24-4,5 and p=0,01, OR=1,86, 95%CI 1,16-3,01, respectively) (Table 1). A comparative analysis of allele and genotype frequencies of studied polymorphism in asthma patients with different spirometry measures showed that rs1049793**CC* genotype and rs1049793**C* allele of the *AOC1* gene are associated with a significant decrease in the volume of forced expiratory volume in 1 second (FEV1) (p=0,03, OR=2,56, 95%CI 1,08-6,03 and p=0,03, OR=2,08, 95%CI 1,07-4,05) and with a significant decrease in forced expiratory flow during the 25% of forced vital capacity (MEF25) (p=0,03, OR=2,56, 95%CI 1,08-6,03 and p=0,03, OR=2,08, 95%CI 1,07-4,05) (Table 2) in Russians. According to the literature, the association of the rs1049793**CC* genotype of the *AOC1* gene with higher values of maximal response over baseline (Emax) to histamine in asthma children was identified [5]. On the contrary, Szczepankiewicz et al. has not found any associations between polymorphic loci of the *AOC1* gene and asthma [8].

The histamine-N-methyltransferase gene *HNMT* is located at the chromosome region 2q22.1 and contains 9 exons. Analysis of allele and genotype frequencies distribution of the rs1801105 polymorphic variant of the *HNMT* gene did not reveal statistically significant differences between the asthma children and control group from RB (p>0,05) (Table 1). We found a higher frequency of rs1801105**CT* heterozygous genotype and rs1801105**T* allele of the *HNMT* gene in asthma patients of Tatar ethnicity with a significant decrease of FEV25 (48,15% and 24,07%), compared to the corresponding control group of children (18,29%, p=0,002, OR=4,15, 95%CI 1,62-10,62, and 9,15%, p=0,004, OR=3,15, and 95%CI 1,39-7,14) (Table 2). According to literature, an association of the rs1801105**T* allele with asthma in children [8] and with severe allergic rhinitis has been identified [6]. There is no association of the *HNMT* polymorphism rs1801105 with asthma development in Europeans [3].

Conclusion. Thus, an association of rs1049793**CC* genotype and rs1049793**C* allele of the *AOC1* gene with asthma, with a significant decrease in the values of FEV1 and MEF25 in the Russians was established. It was found the association of rs1801105**CT* genotype and rs1801105**T* allele of the *HNMT* gene with reduced MEF25 values in Tatars. The findings of this work demonstrate a certain aspects of asthma molecular pathogenesis, which may be needed to personalize the asthma treatment in the future.

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