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ASSOCIATION OF POLYMORPHIC VARIANTS OF HHIP, ADRB2 AND IL-33 GENES WITH CLINICAL MANIFESTATIONS OF BRONCHIAL ASTHMA IN CHILDREN

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АССОЦИАЦИЯ ПОЛИМОРФНЫХ ВАРИАНТОВ ГЕНОВ HHIP, ADRB2 И IL-33 С КЛИНИЧЕСКИМИ ПРОЯВЛЕНИЯМИ БРОНХИАЛЬНОЙ АСТМЫ У ДЕТЕЙ

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Objective. To study the association of polymorphic variants HHIP, ADRB2 and IL-33 genes with phenotypes of clinical course of bronchial asthma in children and effective treatment.
Materials and methods. 90 patients aged from 5 to 17 with the diagnosis of bronchial asthma were included in the investigation. Diagnostic procedures were carried out in all the patients. They included the study of genetic polymorphism of HHIP, ADRB2 and IL-33 genes to establish the association with the clinical phenotypes, findings of laboratory and instrumental study determining the course of bronchial asthma and the degree of its control.
Results. The study of polymorphism of HHIP, ADRB2 and IL-33 genes in children with bronchial asthma with different phenotypes of the disease revealed the association of genetic polymorphism with the severity of course of the disease as well as concomitant diseases. It was determined that allele T of genetic variant rs12504628 (T>C) of HHIP gene reduces the risk of a severe course of BA. Its protective role in the development of drug allergy was also proved. Genotype AA of ADRB2 gene is associated with reduced risks of the development of congenital defects of the tracheobronchial tree in BA. Polymorphic variants in the 4th and 6th exon of IL-33 gene are more frequently associated with moderate and severe course of asthma and base substitution in the 4th and 6th exon are associated with the severe course.
Conclusions. Associations of polymorphic variants of HHIP, ADRB2 and IL-33 genes with clinical manifestations of BA in children are determined in this study. They can be considered in a personalized monitoring of the patients and can help to control the disease totally.
Keywords. Genetic polymorphism, bronchial asthma, children, severity of asthma, level of control.

Цель. Изучение ассоциации полиморфных вариантов генов HHIP, ADRB2 и IL-33 с фенотипами клинического течения бронхиальной астмы (БА) у детей и эффективностью терапии заболевания.
Материалы и методы. В исследование включены 90 пациентов в возрасте от 5 до 17 лет с установленным диагнозом бронхиальной астмы. Всем пациентам были проведены диагностические процедуры, включающие исследование генетического полиморфизма генов HHIP, ADRB2 и IL-33 для установления связи с клиническими фенотипами, показателями лабораторных, инструментальных исследований, определяющими течение бронхиальной астмы и степень контроля заболевания.
Результаты. Проведенное исследование полиморфизмов генов HHIP, ADRB2 и IL-33 у детей, страдающих БА, с разными фенотипами заболевания выявило ассоциацию между полиморфизмами генов и тяжестью заболевания, а также с сопутствующими заболеваниями. Установлено, что аллель Т генетического варианта rs12504628 (Т > С) гена HHIP снижает риск реализации тяжелой БА, а также доказана его протективная роль в отношении реализации лекарственной аллергии. Генотипа АА гена ADRB2 ассоциирован со снижением риска реализации врожденных пороков развития трахеобронхиального дерева на фоне БА. Полиморфные варианты в 4-м и 6-м экзонах гена IL-33 чаще сочетаются со среднетяжелой и тяжелой астмой, а замены нуклеотидов в экзонах 4 и 6 ассоциированы с тяжелым течением БА.

Выводы. В данном исследовании установлены ассоциации полиморфных вариантов генов HHIP, ADRB2 и IL-33 с клиническими проявлениями бронхиальной астмы у детей, которые могут учитываться при персонализированном наблюдении за этими пациентами, и помочь в достижении полного контроля над заболеванием.
Ключевые слова. Полиморфизм генов, бронхиальная астма, дети степень тяжести астмы, уровень контроля.

INTRODUCTION

Bronchial asthma (BA) is a crucial problem of modern theoretical and practical medicine. The Global Asthma Network reported that approximately 348 million people currently suffer from BA, and at least 14 % of them are children[1]. In 2019, there were estimated 262 million people with BA and 461,000 deaths from the disease [1].

BA is a heterogeneous disease involving chronic inflammation of the airways and characterized by reversible broncho-obstructive syndrome, repeated episodes of wheezing, dyspnea, chest congestion, and cough. Its symptoms vary in time and intensity [2; 3]. BA treatment is mainly aimed at achieving and maintaining optimal control of the disease and preventing exacerbations [4].

Despite the wide availability of inhaled glucocorticosteroids (IGC) and standardized recommendations for asthma treatment, disease control remains suboptimal in most children. More than 50 % of pediatric patients with BA experience at least one exacerbation each year, including those with mild asthma. In Russia, the problem of asthma control is critical, because only 23 % of patients achieve complete control of the disease. The causes of insufficient control of BA include low adherence to therapy (43 %), lack of elimination of triggers (29 %), presence of concomitant diseases (15 %), smoking (15 %), and others [5].

Single-nucleotide substitutions in the genome can determine the influence of genetic polymorphism on the disease phenotype and predict differences in clinical manifestations of the disease, including symptom control.

A characteristic aspect of molecular medicine as a science based on data on the molecular structure of the human genome is its focus on correcting the pathological process in every individual, considering his unique genetic characteristics. Another feature is a preventive focus, when information about the genome obtained long before the obvious manifestations of the disease can prevent its development. Genetic predisposition can manifest through interaction with environmental factors, which forms a pathological phenotype. A common method for studying the contribution of genetic mechanisms to the development of BA is the search for associations of the disease and its phenotypes with polymorphic markers of candidate genes.

Over the past decade, genetic studies have identified several candidate genes de-

[1] Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2020, available at: https://ginasthma.org

termining susceptibility to BA. However, the results are often contradictory, confirming the need for further study of BA associations with polymorphic markers of candidate genes.

Some polymorphic candidate gene markers may lead to airway obstruction due to loss of lung elasticity, whereas others contribute to the formation of chronic inflammation resulting in airway obstruction or poor response to drugs such as β_2-agonists or IGC [6].

Genetic aspects of BA control in pediatric patients continue to be studied. In particular, polymorphism of the β_2-adrenergic receptor gene (*ADRB2* gene) is associated with the therapeutic response of patients to bronchodilators, β_2-agonists. Stimulation of β_2-adrenergic receptors leads to bronchodilation and improvement of bronchial conductance, affects the functioning of T cells and eosinophilic inflammation, and causes a decrease in the secretion of proinflammatory mediators from mast cells [7].

The product of the Hedgehog-interacting protein (*HHIP*) gene is an evolutionarily conserved signaling protein that is significant in various processes. There is evidence of an association of the rs1828591 single-nucleotide polymorphism of the *HHIP* gene with a predisposition to the development of bronchial obstruction [8]. The association of single-nucleotide polymorphism rs1512288 of the *HHIP* gene with bronchial obstruction reversibility is indicated, whereas such association has not been revealed with bronchial hyperreactivity [9].

Among the BA candidate genes, the cytokine alarmins are highlighted, which play a key role at all stages of allergic reactions. As regards molecular genetic methods, the gene for the cytokine alarmin interleukin (IL)-33 is promising for research. IL-33 is one of the central signaling molecules of immune responses in BA [10]. Its role has been confirmed in the pathogenesis of BA in children [11; 12]. IL-33 levels have been found to be elevated in sputum and bronchial biopsies of patients with asthma [13]. IL-33 is a tissue-derived cytokine that induces and enhances eosinophilic inflammation and has become a new target for the treatment of asthma and allergic diseases [14].

The binding of IL-33 to its receptor (ST2) enhances the expression of several proinflammatory mediators (IL-5, IL-4, and IL-13) and affects Th2 cell-mediated eosinophilic airway inflammation. Increased IL-33 was associated with the presence of the rs1342326 allele of the *IL-33* gene. The *IL-33* gene polymorphism rs1342326 was associated with a lower risk of asthma in children in Tunisia and higher cytokine IL-33 expression [15]. Single-nucleotide polymorphism rs992969 of the *IL-33* gene was found to be associated with blood eosinophil levels, asthma, and eosinophilic asthma. The rs4008366 polymorphism of the *IL-33* gene showed a weak association with eosinophilic asthma [16].

Using whole-genome sequencing, a rare variant of the *IL-33* gene (NM_001199640: exon7: c.487-1G > C (rs146597587-C), allele frequency = 0.65 %) was discovered in an Ice-

landic population, which disrupts the canonical acceptor splice site before the last coding exon of the gene [17]. This variant occurs at low frequency in European populations and is associated with lower eosinophil counts and reduced risk of asthma in Europeans (*OR* = 0.47, 95 %). In heterozygotes, the overall IL-33 mRNA expression is approximately 40 % lower than in noncarriers. This polymorphism results in a shortened form of the IL-33 protein. The shortened version does not form the IL-33R/ST2 complex and does not activate ST2-expressing cells. These data demonstrate that rs146597587-C is a loss-of-function cytokine mutation [17].

Despite several presented studies of polymorphisms of the *HHIP, ADRB2*, and *IL-33* genes in BA, the significance of the association of polymorphisms of the above-mentioned genes with the clinical course of BA in pediatric patients remains unclear. Hence, there is a need to improve a comprehensive assessment of the degree of BA control and determine the influence of clinical, laboratory, functional, and genetic characteristics on it.

This study aimed to analyze the association between the polymorphic variants of the *HHIP, ADRB2*, and *IL-33* genes and clinical phenotypes of BA in children and efficiency of the disease therapy.

MATERIALS AND METHODS

A single-center cohort study was conducted on 90 patients aged 5–17 years diagnosed with bronchial asthma of varying degrees of severity and control between November 2019 and March 2021. The diagnosis of BA was made based on current clinical guidelines[2]. The study was conducted at the Regional Children's Clinical Hospital of Perm and polyclinics of the Perm region within a scientific grant from the Russian Center for Scientific Information, formerly the Russian Foundation for Basic Research.

Inclusion criteria were children aged 5–17 years with an established BA diagnosis and signed informed consent.

Exclusion criteria were any acute respiratory infections during the examination period and age <5 years (owing to the impossibility of spirography in this age group).

The clinical condition and external respiratory function of all participants were examined. Subsequently, a set of diagnostic procedures was implemented, including the study of genetic polymorphism of the *HHIP, ADRB2*, and *IL-33* genes to establish an association between clinical phenotypes, indicators of laboratory and instrumental studies that determine the course of bronchial asthma, and the degree of disease control. The medical history of all patients was obtained, including allergic history, general blood test, rhinocytogram, the level of general and specific IgE, and immunogram as indicated. Spirography, pulse oximetry, chest X-ray, and peak expiratory flow rate (PEFR) test were performed. Based on clinical data and indicators of external respiratory function, exacerbation severity was determined according to clinical recommen-

[2] Bronchial asthma: clinical guidelines. 2021, available at: https://cr.minzdrav.gov.ru/recommend/359_2.

dations. The severity of BA exacerbations was determined according to the clinical criteria of clinical symptoms, PEFR, respiratory rate, pulse rate, frequency of use of emergency medications, and night awakenings. The BA control level was determined according to the Asthma Control Test, C-ACT, and Composite Asthma Severity Index.

The material for the molecular genetic study was DNA isolated from dried capillary blood spots in 90 children. A study was conducted on the frequencies of alleles and genotypes of polymorphic gene loci rs12551256-A and rs146597587-G of the *IL-33* gene in 70 children and rs12504628 of the *HHIP* gene and ARG16GLY rs1042713 of the *ADRB2* gene in 90 patients with BA, considering the severity and control of the disease. To identify mutant gene alleles, the polymerase chain reaction method was used. In pediatric patients with severe BA, and in children with poorly controlled/uncontrolled asthma ($n = 26$), the entire coding sequence of the *IL-33* gene, located on the ninth chromosome in the 9p24.1 region, was additionally sequenced (search for mutations in nine exons).

Cases were selected using the continuous sampling method, and the sample size was determined using a specialized equation for an unknown general population size:

$$n = t^2 \cdot p \cdot q / \Delta^2,$$

where n is the sample size; t, a coefficient depending on the confidence level chosen by the researcher; p, the proportion of respondents with the presence of the studied characteristic; $q = 1–p$, the proportion of respondents who do not have the studied characteristic; and Δ, the maximum sampling error.

Statistical processing of the results was performed using statistical software packages Microsoft Excel 2010.

The hypothesis about the normal distribution of the studied indicators was tested using the Shapiro–Wilk test. To extend the conclusions to general populations (95 % confidence), some indicators were presented as $M \pm 2m$ ($\% \pm 2m$).

When comparing dependent and independent groups of signs characterizing the level of control and/or severity of the disease (depending on the type of distribution of the analyzed indicators), a two-sample Student's t-test or the Mann – Whitney U test was used. In analysis of contingency tables of characteristics, Pearson's χ^2 criterion was used. The relationship between variables was studied using the correlation analysis. The significance level for the hypotheses being tested was set to 0.05.

The expected distribution of genotypes was estimated using the Hardy – Weinberg equation:

$$(q + p)^2 = q^2 + 2pq + p^2,$$

where q is the incidence of the recessive gene; p, the incidence of the dominant gene; q^2, the incidence of the aa genotype; p^2, the incidence of the AA genotype; and $2pq$, the incidence of Aa genotype.

To assess the correspondence of the observed distribution of genotypes to the ex-

pected one based on the Hardy – Weinberg equilibrium, the χ^2 criterion was used.

For statistically significant parameters, the relative risk (RR) was calculated using the equation:

$$\text{OP} = \frac{A \cdot (C + D)}{C \cdot (A + B)}.$$

The confidence (95 %) interval for the RR was calculated using the equations:

upper limit $e^{\ln(\text{OP})+1{,}96\cdot\sqrt{\frac{B}{A\cdot(A+B)}+\frac{D}{C\cdot(C+D)}}}$,

lower limit $e^{\ln(\text{OP})-1{,}96\cdot\sqrt{\frac{B}{A\cdot(A+B)}+\frac{D}{C\cdot(C+D)}}}$,

where A, B, C, and D in the equations were the number of cases in the cells of the four-field contingency table.

This study was conducted in accordance with the principles of the Declaration of Helsinki of the World Medical Association. Before including a pediatric patient in the study, written informed consent was obtained from his legal representative in accordance with local laws and regulations. Conclusion of the local ethics committee at Perm State Medical University no. 5/20 dated August 4, 2020, was obtained.

RESULTS AND DISCUSSION

All children in the study group were under regular medical check-up by a pulmonologist owing to a diagnosis of bronchial asthma. The median age of the patients was 13 years [Q_1–Q_3: 9; 15] years. Overall, 100 children participated in the study, including 72 boys (72 %) and 28 girls (28 %). Sixty-two patients had mild asthma, 27 had moderate BA, and 11 had severe asthma. The largest number of patients in the study cohort (67 patients) had incomplete control of bronchial asthma; lack of control was detected in 7 children, and 26 had complete control.

When collecting anamnestic data from 87 % of children, factors for violating the hypoallergenic regime were identified (i.e., the presence of animals in the house, carpets, flowering indoor plants, mold, passive smoking, pollution of the place of residence with exhaust fumes from cars or nearby industrial enterprises).

Outpatient medical records of children undergoing dispensary follow-up by a local pediatrician were analyzed to assess the comorbid background. Evaluation of the concomitant diseases of the examined children revealed that the largest percentage was accounted for by allergic rhinitis (80.0 %), atopic dermatitis (32.0 %), and pollinosis (40.4 %) (Table 1). Most children had two or more concomitant diseases.

The structure of complaints of the examined children was dominated by dyspnea during physical activity (70.0 ± 9.0), dyspnea on the street in the spring–summer period (34.0 ± 9.3) and with a respiratory infection (42.0 ± 9.7), difficulty breathing with wheezing (45.0 ± 9.8), and dry paroxysmal cough (48.0 ± 9.8).

Examination of the external respiratory function using the spirography method identified obstructive-type disorders in 29 children, and peak flowmetry control data showed significant deterioration in indicators

Table 1

Comorbidities in BA pediatric patients

Concomitant disease	Number of pediatric patients with an established diagnosis, *n*
Allergic rhinitis	80
Atopic dermatitis	32
Pollinosis	40
Food allergy	20
Diseases of the gastrointestinal tract (chronic gastroduodenitis/biliary tract dysfunction/chronic constipation)	17
Overweight/obesity	15
Congenital malformations of the tracheobronchial tree: accessory bronchus/ bronchial transposition	16
Urticaria fever	14

in 18 % of the patients and indicated their shift to the "red" zone, which confirms the hypothesis that the disease is not controlled in BA patients in the course of baseline therapy.

Characteristics of the studied genotypes. The distribution of genotypes in BA patients corresponded to the Hardy – Weinberg equilibrium, with the exception of the genetic variant rs146597587 of the *IL-33* gene (G > C), with only carriers of one genotype GG (Table 2).

Search for associations of genetic markers of the *HHIP, ADRB2*, and *IL-33* genes with the clinical course of bronchial asthma. Comparison of genetic markers in patients with severe BA (sBA) and mild/moderate BA revealed a tendency to reduce the risk of severe disease among individuals carrying the TT genotype (*OR* = 0.221 (95 % *CI*: 0.059–0.828; χ^2 = 5.759; p = 0.056)) and T allele (*OR* = 0.491 (95 % *CI*: 0.190–1.269; χ^2 = 4.270; p = 0.039)) of the studied genetic variant rs12504628 (T > C) of the *HHIP* gene; the frequency of the CC genotype in severe BA was 64 % versus 28 % for non-severe BA and that of C allele was 77 % versus 52 % (Table 3).

Table 2

Frequency distribution of genotypes of the studied polymorphisms (rs12504628 (T > C), rs1042713 (G > A), rs12551256, rs146597587 (G > C)) in a group of patients with bronchial asthma

Gene/ polymorphism	Genotype	N.O.	N.E.	χ^2 df = 1	Allele frequency	$h_{obs+\pm}$SE $h_{exp\pm}$SE	*D*
HHIP gene rs12504628 (T > C)	TT	21	18.68	0.254 p = 0.614	T = 0.455 C = 0.544	h_{obs} = 0.444 ± 0.052 h_{exp} = 0.101 ± 0.012	–0.586
	TC	40	44.64				
	CC	29	26.68				
	T	82	45.56				
	C	98	54.44				
ADRB2 gene rs1042713 (G > A)	GG	40	39.34	0.004 p = 0.952	G = 0.661 A = 0.339	h_{obs} = 0.433 ± 0.052 h_{exp} = 0.685 ± 0.049	–0.368
	GA	39	40.33				
	AA	11	10.34				
	G	119	66.11				
	A	61	33.89				

End of the Table 2

Gene/ polymorphism	Genotype	N.O.	N.E.	χ^2 $df = 1$	Allele frequency	$h_{obs+\pm}$SE $h_{exp\pm}$SE	*D*
IL-33 gene rs12551256 (A > G)	GG	24	22.56	0.095 $p = 0.758$	G = 0.593 A = 0.407	$h_{obs} = 0.438 \pm 0.062$ $h_{exp} = 0.825 \pm 0.047$	−0.470
	GA	28	30.88				
	AA	12	10.56				
	G	76	59.38				
	A	52	40.63				
IL-33 gene rs146597587 (G > C)	GG	40	100		G = 1.0 C = 0	$h_{obs} = 0$ $h_{exp} = 0$	0
	CG	0	0				
	CC	0	0				
	G	80	100				
	C	0	0				

Note: N.O., observed number of genotypes; N.E., expected number of genotypes. The χ^2 criterion was used to assess the correspondence of the observed distribution of genotypes to the expected one based on the Hardy – Weinberg equilibrium. *df,* the number of degrees of freedom; $h_{obs} \pm$ s.e. and $h_{exp} \pm$s.e., observed and expected heterozygosity, respectively, with error; *D,* the relative deviation of the observed heterozygosity from the expected one.

Table 3

Case-control analysis of the studied genetic variants for severe and non-severe BA

Gene/ polymorphism	Genotypes/ alleles	severe BA		non-severe BA		χ^2	*p*	*OR*
		N	%	*N*	%			
HHIP gene rs12504628 (T > C) 1-T/C, 2-T/T, 3-C/C	**TT**	1	9	20	**25**	5.759	0.056	**0.221 (0.059 < *OR* < 0.828)**
	TC	3	27	37	47			
	CC	7	64	22	28			
	T	5	***23***	77	***48***	**4.270**	**0.039**	**0.309 (0.109 < *OR* < 0.880)**
	C	17	*77*	81	*52*			
ADRB2 gene rs1042713 (G > A) 1-G/A, 2-G/G, 3-A/A	GG	5	45	35	44	1.898	0.387	1.048 (0.295 < *OR* < 3.720)
	GA	6	55	33	42			
	AA	0	0	11	14			
	G	16	73	103	65	0.211	0.646	1.424 (0.527 < *OR* < 3.847)
	A	6	27	55	35			
IL-33 gene rs12551256 (A > G) 1-A/G, 2-A/A, 3-G/G	GG	3	11	21	14	4.345	0.114	0.470 (0.190 < *OR* < 1.166)
	GA	c	45	28	40			
	AA	c	44	12	46			
	G	6	*33*	70	*34*	0.027	0.870	0.950 (0.646 < *OR* < 1.396)
	A	0	*67*	52	*66*			
IL-33 gene rs146597587 (G > C) 1-G/C, 2-G/G, 3-C/C	GG	8	11	22	14	4.345	0.114	0.470 (0.190 < *OR* < 1.166)
	GC	0	45	0	40			
	CC	0	44	0	46			
	G	16	*33*	44	*34*	0.027	0.870	0.950 (0.646 < *OR* < 1.396)
	C	0	*67*	0	*66*			

Note: *N*, the absolute number of observed genotypes; *p*, given for the χ^2 test; *c,* sequencing.

Comparison of genetic markers in patients with a combination of atopic dermatitis (AD) and bronchial asthma (BA + AD) and BA without AD (BA without AD) revealed an increased risk of a combination of asthma and dermatitis among individuals carrying the TT genotype [*OR* = 2.875 (95 % *CI*: 1.130–7.316; $\chi^2 = 5.751$; $p = 0.056$)] of genetic variant rs12504628 (T > C) of the *HHIP* gene, but without a significant difference.

Analysis of genetic markers in patients with a combination of congenital malformations of the tracheobronchial tree with asthma (BA + CM) and bronchial asthma without malformations (BA without CM) revealed associations with the combination of asthma and CM with the AA genotype [*OR* = 0.182 (95 % *CI*: 0.051–0.646; $\chi^2 = 8.567$; $p = 0.014$)] of the genetic variant rs1042713 (G > A) of the *ADRB2* gene, which has a protective effect against CM; it was found that carriers of the AA genotype have CM of the bronchial tree in 40 % of cases, versus 11 % of carriers of genotype AA without CM.

Analysis of genetic markers in BA patients and an aggravated allergic history among first-degree relatives (BA + hereditary history) and bronchial asthma without an aggravated hereditary allergic history (BA without a hereditary history) showed that carriers of the AA genotype [*OR* = 0.112 (95 % *CI*: 0.013 –0.932; $\chi^2 = 5.554$; $p = 0.062$)] and A allele (OR = 0.453 (95 % *CI*: 0.213–0.964; $\chi^2 = 3.537$; $p = 0.059$) of the genetic variant rs1042713 (G > A) of the *ADRB2* gene tend to have a lower incidence of an aggravated allergic anamnesis among first-degree relatives (4 % vs 26 % for the AA genotype and 26 % vs 44 % for the A allele). However, further study of genetic markers in a large cohort of patients is required to confirm the above statements.

Analysis of genetic markers in patients with BA and drug allergies (BA + DA) and bronchial asthma without drug allergies (BA without DA) revealed that carriers of the CC+TC genotype tend to have combined asthma and drug allergies (*OR* = 2.917 (95 % *CI*: 1.009–8.427; $\chi^2 = 4.984$; $p = 0.083$) of the genetic variant rs12504628 (T > C) of the *HHIP* gene; the T allele was shown to have a protective role against drug allergies (31 % vs. 52 %) (*OR* = 0.416 (95 %) *CI*: 0.190–0.909; $\chi^2 = 4.204$; $p = 0.040$).

Analysis of genetic markers in patients with uncontrolled BA and bronchial asthma with partial and complete control of the disease symptoms did not reveal associations with the studied genetic variants.

Carriage of the AA genotype of the *ADRB2* gene is associated with a reduced risk of aggravated allergic anamnesis among first-degree relatives and of congenital malformations of the tracheobronchial tree against BA (Table 4).

Sequencing and exome analysis of the *IL-33* gene showed a significant positive relationship between the frequency of damage in exons 4 ($r = 0.417$; $p = 0.034$) and 6 ($r = 0.593$; $p = 0.001$) and severity of BA. Nucleotide substitutions in these exons are more often associated with severe bronchial asthma.

Table 4

Associations of bronchial asthma with studied genetic variants

Gene/ polymorphism	Genotype/ allele comparison	*OR* (95 % *CI*)	Clinical associations
HHIP gene rs12504628 (T > C)	C vs T	0.309 (0.109–0.880)	Reduced risk of severe BA for carriers of the T allele
	TT vs CC+TC	2.875 (1.130–7.316)	Increased risk of a combination of BA and atopic dermatitis for carriers of the TT genotype
	C vs T	0.416 (0.190–0.909)	Reduced risk of drug allergies associated with BA for carriers of the T allele
ADRB2 gene rs1042713 (G > A)	AA vs GA+GG	0.182 (0.051–0.646)	Reduced risk of bronchial tree CM associated with BA for carriers of the AA genotype
	AA vs GA+GG	0.112 (0.013–0.932)	Reduced incidence of aggravated allergic anamnesis associated with BA for carriers of the AA genotype
	A vs G	0.453 (0.213–0.964)	Reduced incidence of aggravated allergic anamnesis associated with BA for carriers of the A allele

A study showed that the Gly16Gly genotype of the *ADRB2* gene is associated with an increased risk of a more severe form of bronchial asthma, as well as its nocturnal form, compared to the ADRB2 Arg16Arg genotype [13]. Analysis of 28 studies on the association of beta-2 adrenergic receptor gene polymorphisms with BA phenotypes confirmed the link between Gly16 polymorphism and nocturnal asthma; however, it did not reveal an association between the Arg16Gly variant and bronchial hyperresponsiveness [18].

Notably, in our sample, children with the indicated genotype AA of the *ADRB2* gene did not receive long-acting b_2-agonists as monotherapy as baseline therapy and did not use monotherapy with short-acting b_2-agonists as emergency medications over the past 6 months. Therefore, it was not possible to assess the risk of exacerbation of the disease in carriers of the Arg16 genotype who resort to the use of b_2-agonists.

Currently, it has been established that the *HHIP* gene affects the condition of both small and large airways [19]. The presence of A allele of the rs13118928 polymorphism of the *HHIP* gene may be associated with the emphysema–hyperinflation phenotype in patients with chronic obstructive pulmonary disease [20]. The state of external respiration function is the most crucial criterion for the severity of bronchial asthma. The presence of CC + CT genotypes increases the risk of drug allergies associated with BA by 2.9 times.

We did not identify an association between genetic variants of the rs12551256 polymorphism of the *IL-33* gene and features of the clinical course of BA. When analyzing the genotypes of the rs146597587 (G > C) polymorphism of the *IL-33* gene, all children were carriers of the same genotype. However, nucleotide substitutions in exons 4 and 6 of the *IL-33* gene were found to be associated with severe bronchial asthma. This indicates the need for further

studies with larger sample sizes on the exon polymorphism of the *IL-33* gene and its associations with the clinical course of bronchial asthma in pediatric patients.

Study limitations. The association of polymorphisms of the *HHIP*, *ADRB2*, and *IL-33* genes in pediatric patients cannot be concluded to the entire population of Russian children owing to the small study sample size. The distributions of genotypes and alleles of these genes will possibly differ from those given in this article with an increase in sample size. We have not conducted a multivariate analysis adjusted for the detected gene associations, considering the carriage of polymorphic variants of other genes and environmental factors, which may affect the results of assessing the effect of the genes under study.

Conclusions

A study of polymorphisms of the *HHIP*, *ADRB2*, and *IL-33* genes in BA pediatric patients with different phenotypes of the disease revealed an association between gene polymorphisms and the disease severity, as well as with concomitant diseases.

It was observed that the TT genotype of the genetic variant rs12504628 (T > C) of the *HHIP* gene reduces the risk of severe BA; however, it increases the risk of atopic dermatitis combined with BA by 2.8 times. The CC + CT genotype of the *HHIP* gene increases the risk of drug allergies associated with BA by 2.9 times.

The AA genotype of the *ADRB2* gene is associated with the absence of an aggravated allergy history among first-degree relatives and a reduced risk of congenital malformations of the tracheobronchial tree in the presence of BA. Nucleotide substitutions in exons 4 and 6 of the *IL-33* gene are associated with severe BA. Thus, attention should be paid to exons 4 and 6 to predict the course of the disease and for timely correction of baseline therapy.

Thus, this study established associations of polymorphic variants of the *HHIP, ADRB2*, and *IL-33* genes with the clinical manifestations of bronchial asthma in children, which can be considered in personalized monitoring of these patients and help in achieving complete control of the disease.

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