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Характеристика полиморфизмов генов цитокинов у детей с различными фенотипами бронхиальной астмы

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АННОТАЦИЯ

Обоснование. Бронхиальная астма является хроническим воспалительным заболеванием дыхательных путей, в развитии которого особое значение имеют генетические предикторы, ассоциированные с дифференцировкой и функционированием Т-хелперов. Полиморфизмы генов цитокинов, участвующих в регуляции направления опосредованного Т-хелперами иммунного ответа, являются факторами риска развития болезни и реализации различных фенотипов бронхиальной астмы.

Цель — изучение структуры и встречаемости однонуклеотидных полиморфизмов генов цитокинов и оценка рисков реализации различных фенотипов бронхиальной астмы у детей.

Материалы и методы. В исследовании «случай-контроль» приняли участие 250 детей, из них 150 с верифицированным диагнозом бронхиальной астмы (в том числе 75 детей с вирусиндуцированным и 75 детей с аллергениндуцированным фенотипом болезни) и 100 сопоставимых по полу здоровых сверстников. Детям проведено комплексное общеклиническое и аллергологическое обследование, генотипирование, анализ структуры, частоты встречаемости полиморфизмов генов цитокинов и расчёт коэффициента отношения шансов риска развития различных фенотипов болезни. Материалом для молекулярно-генетического анализа служили образцы ДНК, выделенные из периферической венозной крови. Выбраны следующие точки мутаций: *IFN-γ (T-874A)*, *IL-4 (C-589T)*, *IL-6 (C-174G)*, *IL-17A (G-197A)*, *TNF-α (G-308A)*.

При обработке цифровых данных использовали методы описательной, параметрической и непараметрической статистики программы Statistica 10, сравнение несвязанных групп по качественным признакам, оценку соответствия распределений генотипов ожидаемым значениям при равновесии Харди–Вайнберга. Анализ распределений частот генотипов и аллелей в двух субпопуляциях проводили с использованием критерия Хи-квадрат (χ^2).

Результаты. Проведённый сравнительный анализ частоты аллелей и генотипов цитокинов различных Th-профилей с определением их особенностей при аллергениндуцированном и вирусиндуцированном фенотипах болезни выявил у детей с бронхиальной астмой преобладание гомозиготных генотипов *IFN-γ (A-874A)*, *IL-4 (T-589T)*, *IL-6 (G-174G)*, *IL-17A (A-197A)*, *TNF-α (A-308A)*, а у здоровых сверстников — превалирование *IFN-γ (T-874T)*, *IL-4 (C-589C)*, *IL-6 (C-174C)*, *IL-17A (G-197G)*, *TNF-α (G-308G)*. При бронхиальной астме у детей чаще, чем у здоровых сверстников, встречались гетерозиготные генотипы *IL-4 (C-589T)*, *IL-6 (G-174C)*, *IL-17A (G-197A)*, *TNF-α (G-308A)*, за исключением генотипа *IFN-γ (T-874A)*. У детей с вирусиндуцированным фенотипом болезни мутантный *T*-аллель *IL-4 (C-589T)* обнаружен в 30,67% случаев при коэффициенте отношения шансов 19,30 CI 95% (11,23–33,31). При носительстве мутантного *A*-генотипа *IFN-γ (T-874A)* коэффициент отношения шансов риска развития болезни отразил бóльшую степень вероятности реализации вирусиндуцированного фенотипа бронхиальной астмы (OR=5,11; CI 95% 3,18–8,23). Носительство гомозиготных генотипов *IL-6 (G-174G)* и *IL-17A (A-197A)* определяло увеличение риска развития аллергениндуцированной бронхиальной астмы (OR=2,71; CI 95% 1,73–4,18 и OR=0,51; CI 95% 0,32–0,71 соответственно). Среди детей с бронхиальной астмой отмечено статистически достоверное увеличение встречаемости функционально неблагоприятного генотипа *A308A* гена *TNF-α*, и уровень коэффициента отношения шансов отражает повышение риска развития вирусиндуцированного фенотипа болезни в 2,6 раза (χ^2=18,66; *p*=0,017; OR=2,60; CI 95% 1,67–4,01).

Заключение. В результате проведённого исследования в структуре и встречаемости полиморфизмов генов цитокинов у детей с аллерген- и вирусиндуцированной бронхиальной астмой определены значимые различия в зависимости от реализованного фенотипа болезни. Носительство мутантных аллелей *IFN-γ (A-874A)*, *IL-4 (T-589T)*, *IL-6 (G-174G)*, *IL-17A (A-197A)*, *TNF-α (A-308A)* можно охарактеризовать как генетические предикторы развития болезни: для реализации вирусиндуцированного фенотипа выше коэффициент отношения шансов при *IFN-γ (A-874A)*, *IL-4 (T-589T)*, *TNF-α (A-308A)*, для аллергениндуцированного фенотипа болезни — *IL-6 (G-174G)*, *IL-17A (A-197A)*.

Ключевые слова: полиморфизмы генов; цитокины; бронхиальная астма; вирусиндуцированный и аллергениндуцированный фенотипы; дети.

Как цитировать

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Characteristics of cytokine gene polymorphisms in children with different phenotypes of bronchial asthma

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ABSTRACT

BACKGROUND: Bronchial asthma is a chronic inflammatory disease of the airways, the development of which is based on genetic predictors associated with the differentiation and functioning of T-helper (Th) cells. Polymorphisms in the genes of cytokines involved in the regulation of the direction of the Th cell-mediated immune response are risk factors for the development of the disease and the realization of various phenotypes of bronchial asthma.

AIM: To study of the structure and frequency of occurrence of single nucleotide polymorphisms of cytokine genes with an assessment of the risk of various phenotypes of bronchial asthma in children.

MATERIALS AND METHODS: In this case–control study, 250 children were examined, including 150 children with a verified diagnosis of bronchial asthma (including 75 children with virus-induced and 75 children with allergen-induced disease phenotypes) and 100 sexually comparable healthy peers. The children underwent a comprehensive general clinical and allergological examination, genotyping, structure analysis, frequency of occurrence of cytokine gene polymorphisms, and calculation of the odds ratio of the risk of developing different bronchial asthma phenotypes. DNA samples isolated from peripheral venous blood were used as material for molecular genetic analysis. The following mutation points were selected: *IFN-γ (T-874A)*, *IL-4 (C-589T)*, *IL-6 (C-174G)*, *IL-17A (G-197A)*, and *TNF-α (G-308A)*.

When processing digital data, we used the methods of descriptive, parametric, and nonparametric statistics of the Statistica 10 program, comparison of unrelated groups by qualitative characteristics, and assessment of the correspondence of the distributions of genotypes to the expected values at the Hardy–Weinberg equilibrium. The frequency distributions of genotypes and alleles in two subpopulations were analyzed using the chi-square test ($\chi 2$).

RESULTS: A comparative analysis of the frequencies of alleles and genotypes of cytokines of various Th profiles with the definition of features in allergen-induced and virus-induced phenotypes of the disease revealed the predominance of homozygous genotypes *IFN-γ (A-874A)*, *IL-4 (T-589T)*, *IL-6 (G-174G)*, *IL-17A (A-197A)*, and *TNF-α (A-308A)* in children with bronchial asthma, and in healthy peers, *IFN-γ (T-874T)*, *IL-4 (C-589C)*, *IL-6 (C-174C)*, *IL-17A (G-197G)*, and *TNF-α G-308G* were prevalent. Heterozygous genotypes *IL-4 (C-589T)*, *IL-6 (G-174C)*, *IL-17A (G-197A)*, and *TNF-α (G-308A)* were found in children with bronchial asthma more often than in healthy peers, with the exception of the *IFN-γ* genotype *(T-874A)*. In children with the virus-induced bronchial asthma phenotype, the presence of the *IL-4 (C-589T)* mutant allele was found in 30.67% of cases with an odds ratio of 19.3 (95% CI, 11.23–33.31). When carrying the mutant *A*-genotype *IFN-γ (T-874A)*, the odds ratio of the risk of developing the disease reflected a high degree of probability of the virus-induced bronchial asthma phenotype (OR, 5.11; 95% CI, 3.18–8.23). Carriage of homozygous genotypes *IL-6 (G-174G)* and *IL-17A (A-197A)* determined an increased risk of developing allergen-induced bronchial asthma (OR, 2.71; 95% CI, 1.73–4.18, and OR, 0.51; 95% CI, 0.32–0.71, respectively). Among children with bronchial asthma, a statistically significant increase was noted in the incidence of the functionally unfavorable genotype *A308A* of the *TNF-α* gene, and the odds ratio reflects a 2.6-fold increase in the risk of developing a virus-induced bronchial asthma phenotype (χ^2=18.66; *p*=0.017; OR, 2.60; 95% CI, 1.67–4.01).

CONCLUSIONS: As a result of the study, significant differences were determined in the structure and frequency of occurrence of cytokine gene polymorphisms in children with allergen and virus-induced bronchial asthma, depending on the realized phenotype of the disease. Carriage of mutant alleles *IFN-γ (A-874A)*, *IL-4 (T-589T)*, *IL-6 (G-174G)*, *IL-17A (A-197A)*, and *TNF-α (A-308A)* can be characterized as genetic predictors of bronchial asthma, for the implementation of the virus-induced phenotype, and the odds ratio is higher in the presence of mutant alleles *IFN-γ (A-874A)*, *IL-4 (T-589T)*, and *TNF-α (A-308A)*, for the allergen-induced phenotype of the disease — *IL-6 (G-174G)* and *IL-17A (A-197A)*.

Keywords: gene polymorphism; cytokines; bronchial asthma; virus-induced and allergen-induced phenotype; children.

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BACKGROUND

Bronchial asthma is a chronic heterogeneous disease with numerous mechanisms involved in the development and regulation of inflammation, including genetic factors. Mutations in cytokine genes and cytokine regulation of immune response pathways, development, and activation of inflammation in the airways all play a role in the immunopathogenesis of bronchial asthma. The clinical polymorphism and heterogeneity of the condition are determined by a variety of etiological and pathogenetic factors. The implementation of the genetic predisposition for bronchial asthma development occurs in interaction with environmental factors and results in the formation of the disease pathological phenotype. The occurrence and structure of genotype polymorphisms can vary significantly depending on ethnicity and regional factors, influencing the levels of cytokines and immunoglobulin E in blood serum [1–5].

Several researchers have noted that genetic predictors are involved in the regulation of bronchial asthma phenotypic realization and that disease control is a gene-mediated process that is dependent on the allelic variant of the disease immunopathogenesis mediator genes. Modern publications focus primarily on the study of cytokine genes of the immune response Th2 profile in bronchial asthma, with only a few studies of the structure, occurrence, and significance of cytokine gene polymorphisms of Th1 and Th17 profiles [6–12].

Knowledge of genetic markers will allow for the prediction of phenotypic features of the bronchial asthma course [2, 3, 4, 9–12]. The study and analysis of bronchial asthma genetic predictors that determine pathogenetic disorders and realization of the biological phenotypes of the disease are relevant and allow to assess disease risk and personalize prevention and therapy programs, which defined the purpose and objectives of this study.

The aim was to characterize the occurrence of single nucleotide polymorphisms in cytokine genes and assess the risks of developing various phenotypes of bronchial asthma in children.

MATERIALS AND METHODS

Study design

An observational, single-center, prospective, randomized, parallel-group, controlled study was performed.

Eligibility criteria

The study included children with a confirmed diagnosis of virus-induced and allergen-induced bronchial asthma, with an isolated course or in combination with allergic rhinitis. The diagnosis and phenotype were verified in accordance with the national program "Bronchial asthma in children. Treatment strategy and prevention" and international consensus documents on bronchial asthma in children (Global Strategy for Asthma Management and Prevention; PRACTALL) [5].

Inclusion criteria include children aged 3 to 11 years, a confirmed diagnosis of bronchial asthma, and written parental consent.

Exclusion criteria include a history of severe somatic diseases; the presence of allergic skin disease, such as atopic dermatitis; as well as autoimmune and other chronic inflammatory diseases.

The control group included healthy peers with the first health group, no history of allergic disease or family history of allergy, and complaints at the time of examination; exclusion criteria were allergic diseases and evidence of a history of food/drug intolerance or realization of an allergic disease during the observation period from 2015 to 2020.

Experimental setting

Clinical and laboratory research was conducted at the clinical bases of Vladivostok Clinical Diagnostic Center (Vladivostok) and the laboratory of the Department of Clinical Laboratory Diagnostics, General and Clinical Immunology of the FSBEI HE "Pacific State Medical University" of the Ministry of Healthcare of the Russian Federation (Vladivostok).

Study duration

The study was conducted with annual ambulatory examinations from 2015 to 2020.

Description of medical intervention

In all children, blood was drawn from the cubital vein in accordance with the rules for conducting the preanalytical stage of laboratory studies.

Primary outcome of the study

All children underwent a thorough clinical and allergological examination, as well as genotyping, structural analysis, frequency of occurrence of cytokine gene polymorphisms, and estimation of the odds ratio of developing various disease phenotypes. In DNA samples isolated from peripheral venous blood, the following mutation points were determined: *IFN-γ (T-874A)*, *IL-4 (C-589T)*, *IL-6 (C-174G)*, *IL-17A (G-197A)*, and *TNF-α (G-308A)*.

Subgroup analysis

In the observation group of 150 children with a confirmed diagnosis of bronchial asthma, two subgroups were distinguished based on the disease phenotype, with 75 children having a diagnosed virus-induced phenotype and 75 children having an allergen-induced phenotype, matched by sex and age. The control group consisted of 100 healthy peers who were matched by sex.

Outcome registration methods

DNA samples were isolated using the organic solvent chloroform with Genomic DNA Purification Kits (Thermo Fisher Scientific, Waltham, USA). Typing of single nucleotide

genes polymorphisms of the studied cytokines was carried out by the polymerase chain reaction method with melting of reaction products in the presence of adjacent oligonucleotides with genotyping of polymorphisms. For amplification, we used detecting amplifier in thermal cycle (model Re Bis-M111 (000 Bis-N, Novosibirsk)) and standard sets of primers from Scientific and Production Company Litekh-SNP (Moscow). Electrophoresis in 3% agarose gel with ethidium bromide addition was used to visualize amplification products under ultraviolet light. Detection was conducted in agarose gel stained with ethidium bromide using horizontal electrophoresis. Photofixation was carried out using the VersaDoc Model 4000 gel documentation system (Bio-Rad, USA).

Ethical review

This study followed the ethical principles stated by the Declaration of Helsinki of the World Medical Association and the Rules of Clinical Practice in the Russian Federation; study design was approved by the Interdisciplinary Ethics Committee of the FSBEI HE Pacific State Medical University of the Ministry of Healthcare of the Russian Federation; informed voluntary consents were signed by parents. The decision to conduct the study was approved by the Interdisciplinary Ethics Committee of the FSBEI HE Pacific State Medical University of the Ministry of Healthcare of the Russian Federation, protocol No. 8 dated April 27, 2015.

Statistical analysis

When processing digital data, methods of comparing unrelated groups by qualitative traits and conformance evaluation of genotype frequency distribution to expected values under Hardy–Weinberg equilibrium were used, while the chi-square test (χ^2) was used to compare the distributions of genotype and allele frequencies in two subpopulations. Statistica 10, a statistical analysis system (StatSoft Inc., USA), was used to analyze the results.

The study employed standard statistical criteria: calculation of the odds ratio (OR), confidence interval (CI), and confidence factor of index and differences (p) with a critical significance level of p <0.05. The scope of the research performed made it possible to evaluate the results with a reliability of 95%–99%.

RESULTS

Objects (participants) of the study

The study included 250 children, 150 of whom had a confirmed diagnosis of bronchial asthma and 100 healthy peers who were matched by sex.

Primary outcome of the study

The study of the structure, comparison of the frequency distribution of the alleles, cytokine genotypes of various T-helper profiles in healthy children and in children with bronchial asthma was conducted, followed by an analysis of polymorphism features in the realization of allergen-induced and virus-induced disease phenotypes.

An assessment of the prevalence of cytokine genes polymorphisms in children with bronchial asthma revealed the predominance of homozygous genotypes: *IFN-γ (A-874A), IL-4 (T-589T), IL-6 (G-174G), IL-17A (A-197A),* and *TNF-α (A-308A)*. Homozygous genotypes *IFN-γ (T-874T), IL-4 (C-589C), IL-6 (C-174C), IL-17A (G-197G),* and *TNF-α (G-308G)* were more frequently detected in healthy peers. In terms of genotype structure, heterozygous variants of *IL-4 (C-589T), IL-6 (G-174C), IL-17A (G-197A),* and *TNF-α (G-308A)* were more common in children with bronchial asthma compared to healthy peers, and the *IFN-γ (T-874A)* genotype was found in a higher percentage of cases in the population of healthy children (Table 1).

In the distribution of *IFN-γ (T-874A)* genotypes among children with a virus-induced bronchial asthma phenotype, a statistically significant increase in the frequency of occurrence of the *A874A* allele (61.33 vs. 12.00%, respectively) was observed in comparison with healthy peers. When carrying a functionally unfavorable allele *A* of the *IFN-γ* gene *A874A* polymorphism, the OR value was 5.11, indicating a significant increase in the risk of developing a virus-induced disease phenotype. The heterozygous *T874A* genotype was found in 32.00% of children with virus-induced bronchial asthma and 56.00% of healthy peers (Table 2).

In children with the allergen-induced phenotype of bronchial asthma, the carriage of the *IFN-γ A874A* mutant allele was less common than in children with the virus-mediated phenotype of the disease (37.33 vs. 61.33% of cases, respectively). The presence of the single nucleotide polymorphism *IFN-γ A874A* increases the risk of developing allergen-induced bronchial asthma phenotype by 2.59 times (χ^2=18.66; p=0.013; OR=2.59; CI 95% 1.67–4) and virus-induced phenotype by 5.11 times (Table 2).

In a study of *IL-4 (C-589T)* polymorphism in the group with the virus-induced bronchial asthma phenotype, the frequency of homozygous allele *IL-4 T874T* occurrence was 30.67% versus 5.00% in healthy peers, while the frequency of heterozygous genotype occurrence was 53.33 and 20.00%, respectively. The presence of a functionally unfavorable *T* allele of the *T874T IL-4* gene polymorphism increased the risk of developing and manifesting a virus-induced disease phenotype (χ^2=56.97; p=0.001; OR=19.30; CI 95% 11.23–33.31). In children with the allergen-induced disease phenotype, the carriage of the mutant allele was less common than in children with the virus-mediated phenotype but significantly more frequent than in the group of healthy peers, increasing the risk of developing bronchial asthma (Table 3).

In children with virus-induced bronchial asthma phenotype, the occurrence of the *IL-6* gene *G174G* mutant genotype is higher than in healthy peers (24.00 vs. 7.00%, respectively) (OR=1.49 at χ^2=3.35; p=0.224 CI 95% 0.96–2.29).

Table 1. Structure and occurrence of cytokine gene polymorphisms in children with bronchial asthma and healthy peers

Cytokine	Polymorphism	Genotypes	Healthy children *n*=100 (%)	Children with bronchial asthma *n*=150 (%)
Interferon gamma (IFN-γ)	*874 T/A*	*TT*	32 (32.00)	13 (8.67)
		TA	56 (56.00)	63 (42.00)
		AA	12 (12.00)	74 (49.33)
	Allele frequency	*T*	120 (60.00)	89 (29.67)
		A	80 (40.00)	211 (70.33)
Interleukin-4 (IL-4)	*589 C/T*	*CC*	75 (75.00)	20 (13.33)
		CT	20 (20.00)	93 (62.00)
		TT	5 (5.00)	37 (24.67)
	Allele frequency	*C*	170 (85.00)	133 (44.33)
		T	30 (15.00)	167 (55.67)
Interleukin-6 (IL-6)	*174 G/C*	*GG*	7 (7.00)	39 (26.00)
		CG	55 (55.00)	84 (56.00)
		CC	38 (38.00)	27 (18.00)
	Allele frequency	*G*	69 (34.50)	162 (54.00)
		C	131 (65.50)	138 (46.00)
Interleukin-17A (IL-17A)	*197 G/A*	*GG*	34 (34.00)	15 (10.00)
		GA	56 (56.00)	85 (56.67)
		AA	10 (10.00)	50 (33.33)
	Allele frequency	*G*	124 (62.00)	115 (38.33)
		A	76 (38.00)	185 (61.67)
Tumor necrosis factor alpha (TNF-α)	*308 G/A*	*GG*	40 (40.00)	25 (16.67)
		GA	40 (40.00)	70 (46.67)
		AA	20 (20.00)	55 (36.66)
	Allele frequency	*G*	120 (60.00)	120 (40.00)
		A	80 (40.00)	180 (60.00)

Table 2. Frequency distributions of *IFN-γ* alleles and genotypes for *874T/A* polymorphism in children with virus-induced bronchial asthma phenotype

Polymorphism	Genotypes	Children with virus-induced bronchial asthma phenotype *n*=75 (%)	Healthy children (control) *n*=100 (%)
IFN-γ (T-874A)	*TT*	5 (6.67)	32 (32.00)
	TA	24 (32.00)	56 (56.00)
	AA	46 (61.33)	12 (12.00)
Allele frequency	*T*	34 (22.67)	120 (60.00)
	A	116 (77.33)	80 (40.00)
OR (CI 95%)		5.11 (3.18–8.23)	
χ^2 test		28.48; *p*=0.001	

Note: OR — odds ratio; CI — confidence interval.

The heterozygous *G174C* allele in this group of children was identified in 53.00% of cases, and in children with the allergen-induced disease phenotype, the carriage of this allele and the homozygous *G174G* was identified more frequently with a high OR (Table 4).

When analyzing the structure and occurrence of *IL-17A (G-197A)* single nucleotide polymorphisms in children with allergen-induced phenotype of bronchial asthma, a high proportion of the *A197A* genotype (40.00%) and a singular occurrence of the *G197G* allele at CI 95% 0.32–0.71 for the OR were identified (Table 5). In children with the virus-induced bronchial asthma phenotype, the presence of the mutant allele A was found in 26.70% of cases with an OR of 0.27 (CI 95% 0.19–0.37) and χ^2=12.02 (*p*=0, 39).

The prevalence of *TNF-α (G-308A)* polymorphism and the distribution of genotype frequency in children with various bronchial asthma phenotypes differed from that of healthy peers for the A allele — 63.30 versus 40.00% and 36.70 versus 60.00%. The sampling revealed a statistically significant increase in the incidence of the functionally unfavorable *A308A* genotype, as well as a 2.6-fold increase in the risk of developing a virus-induced phenotype of bronchial asthma (χ^2=18.66; *p*=0.017; OR=2.60; CI 95% 1.67–4.01).

The distribution of the heterozygous *A308G* genotype of the *TNF-α* gene in samples of children with various bronchial asthma phenotypes differed slightly, accounting for 46.60 and 46.70% of cases in the groups of children with allergen-induced and virus-induced phenotype, respectively, and 40.00% in the group of healthy peers (Table 6).

Thus, according to the findings, carriage of the mutant A genotype can be used to predict the relative risk of developing bronchial asthma in children.

Adverse events

No adverse events were reported during the course of the study.

Table 3. Frequency distribution of *IL-4* alleles and genotypes (*589C/T* polymorphism) in children with allergen-induced bronchial asthma phenotype

Polymorphism	Genotypes	Children with allergen-induced bronchial asthma phenotype *n*=75 (%)	Healthy children (control) *n*=100 (%)
IL-4 (C-589T)	*CC*	8 (10.67)	75 (75.00)
	CT	53 (70.67)	20 (20.00)
	TT	14 (18.66)	5 (5.00)
Allele frequency	*C*	69 (46.00)	170 (85.00)
	T	81 (54.00)	30 (15.00)
OR (CI 95%)		7.172 (4.31–11.92)	
χ^2 test		15.21; *p*=0.01	

Note: OR — odds ratio; CI — confidence interval.

Table 4. Carriage of *IL-6* gene polymorphism in children with allergen-induced bronchial asthma phenotype

Polymorphism	Genotypes	Children with allergen-induced bronchial asthma phenotype *n*=75 (%)	Control group *n*=100 (%)
IL-6 (C-174G)	*GG*	24 (32.00)	7 (7.00)
	CG	44 (58.67)	60 (60.00)
	CC	7 (9.33)	33 (33.00)
Allele frequency	*G*	92 (46.00)	74 (37.00)
	C	58 (54.00)	126 (63.00)
OR (CI 95%)		2.71 (1.73–4.18)	
χ^2 test		20.34; *p*=0.015	

Note: OR — odds ratio; CI — confidence interval.

Table 5. *197G/A* polymorphism of the *IL-17A* gene in children with allergen-induced bronchial asthma

Polymorphism	Genotypes	Children with allergen-induced bronchial asthma phenotype *n*=75 (%)	Control group *n*=100 (%)
IL-17A (G-197A)	*GG*	5 (6.70)	34 (34.00)
	GA	40 (53.30)	56 (56.00)
	AA	30 (40.00)	10 (10.00)
Allele frequency	*G*	50 (33.00)	124 (62.00)
	A	100 (67.00)	76 (38.00)
OR (CI 95%)		0.51 (0.32–0.71)	
χ^2 test		28.22; *p*=0.031	

Note: OR — odds ratio; CI — confidence interval.

Table 6. Frequency distribution of alleles and genotypes for the *308G/A* polymorphism of the *TNF-α* gene with the realization of the allergen-induced disease phenotype

Polymorphism	Genotypes	Children with allergen-induced bronchial asthma phenotype *n*=75 (%)	Control group *n*=100 (%)
TNF-α (G-308A)	*GG*	15 (20.00)	40 (40.00)
	GA	35 (46.60)	40 (40.00)
	AA	25 (33.40)	20 (20.00)
Allele frequency	*G*	65 (43.30)	120 (60.00)
	A	85 (56.70)	80 (40.00)
OR (CI 95%)		1.96 (1.27–3.01)	
χ^2 test		9.55; *p*=0.05	

Note: OR — odds ratio; CI — confidence interval.

DISCUSSION

Summary of the primary outcome of the study

Among the genetic risk factors for the development and realization of bronchial asthma phenotypic variants in children, cytokine genes, which are involved in both the regulation of the direction of immune response and the immunopathogenesis of the disease, play an important role. Variations in the level of expression and production of cytokines that regulate the differentiation of T-lymphocyte populations are determined by the structure of polymorphisms in the coding or promoter parts of genes [7–9, 11, 12].

Cytokines are important regulators of the development and progression of the chronic inflammatory process as well as immunopathogenesis of respiratory tract structural disorders in bronchial asthma [1, 3, 5–7]. Interferon gamma (IFN-γ) regulates immune response and differentiation of Th1 lymphocytes, whereas IL-4 is involved in the differentiation of Th2 lymphocytes and the development of allergic inflammation. The development of allergic inflammation is associated with the predominance of Th2 type immune response and aberrant production of the corresponding spectrum of cytokines. At the same time, data on the role of other subpopulations of T-helpers and their cytokines in the development of bronchial asthma are described in literature [1, 3, 4, 7, 10, 13]. Tumor necrosis factor (TNF), which is secreted by neutrophils and alveolar macrophages and initiates the secretion of IL-1, IL-6, and IL-8, plays a significant role in the implementation of the type of inflammatory process in the airways in bronchial asthma. Data on the causal significance of TNF-α in the chronicity of atopic inflammation, control of the infiltration degree of bronchial wall by neutrophils, and regulation of the expression of eosinophil adhesion molecules in the focus of inflammation have been published [6, 10].

The literature contains conflicting data about the role of cytokines and genetic factors in the pathogenesis of bronchial asthma [3, 8, 13–15]. Data on genetic predictors of the bronchial asthma phenotype realization in children are important for personifying prevention and therapy programs.

Discussion of the primary outcome of the study

In this case-control study single nucleotide substitutions in cytokines genes in various profiles of T-lymphocytes-helpers were studied. When assessing the prevalence of cytokine gene polymorphism in children with allergen-induced and virus-induced bronchial asthma phenotypes, a sample of 100 healthy Caucasian children living in the city of Vladivostok was used as a population control.

In children with bronchial asthma, there was a statistically significant increase in the frequency of occurrence of the *IFN-γ A874A* allele, an association of the *A* allele of *IFN-γ* gene with an increased risk of developing the disease, and the fact that the carriage of the functional unfavorable polymorphism *A874A* of the *IFN-γ* gene increases the risk of development and realization of virus-induced bronchial asthma phenotype by 5.11 times.

There was a statistically significant increase in the occurrence of the homozygous *T589T* allele in children with virus-induced phenotype of bronchial asthma, as well as an increased risk of developing this phenotype of the disease when carrying a functionally unfavorable allele *T* of the *T589T* polymorphism of the IL-4 gene. The results of the study of the frequency of the mutant *T* allele of *IL-4 (C-589T)* allowed to characterize the carriage of this homozygote as a genetic predictor of the risk of bronchial asthma with a higher degree of probability of the virus-induced phenotype realization.

In the scientific literature, conflicting data from studies of the relationship between *IL-4* gene polymorphism and the risk of developing asthma are presented. In a meta-analysis of the association of *IL-4 (C-589T) (rs224350)* polymorphism of *IL-4* gene with predisposition to bronchial asthma, A. Kousha et al. (2020) [8] found that this polymorphism increases the risk of asthma in all genetic models, including dominant (OR=1, 22), recessive (OR=1.17), allelic (OR=1.21), and TT *vs.in comparison with CC model* (OR=1.34*), with the exception of* CT vs. TT model (OR =1.13). Additional analysis of subgroups by age showed that *IL-4* gene polymorphism *(C-589T)* was significantly associated with the risk of asthma in children and adults, as well as a substantial association in subgroups by ethnicity [8]. T.Y. Shumna et al. (2019) [15] determined that the *C589C* genotype of the *IL-4* gene is linked to bronchial asthma (OR=4.31; 95% CI 1.63–11.36; *p*=0.002) and allergic rhinitis (OR=4.421; 95 % CI 1.04–7.81, *p*=0.04).

Our study of the association between the development of bronchial asthma and the distribution of alleles of the polymorphic variant of *IL-6 (G-174C)* found that the *G174G* genotype increases the risk of developing the virus-induced phenotype of bronchial asthma by 1.93 times with an OR of 1.49 and, with the allergen-induced phenotype, allele *G* increases the risk of developing this phenotype by 2.71 times. Y.L. Liu et al. (2016) [13] confirmed the importance of *IL-6* signaling in dendritic cells for the uptake of allergens and the initiation of Th2/Th17-mediated inflammation in airways in the development of allergic bronchial asthma in animal models.

E. Babusikova et al. (2017) [7], studying the predictive significance of *IL-6 (G-174C)* gene polymorphisms for bronchial asthma among Slovak children, found *GG, GC,* and *CC* genotypes in 37.90; 45.80, and 16.30% of those with the disease and 20.00, 50.80, and 29.20% of healthy peers, respectively, concluding that the dominant *GG* genotype is a risk factor for asthma (OR=3.4; 95% CI 2.045–5.638; p <0.001). In our study, in contrast to the data presented above, the occurrence of heterozygous genotype *G174C of IL-6 gene* among children with bronchial asthma and healthy children practically did not differ.

The frequency of allele distribution in groups with allergen-induced and virus-induced phenotypes differs statistically significantly from those in the control group of healthy peers, according to an analysis of the distribution structure of *IL-17A (G-197A)* genotypes. Among children with allergen-induced phenotype of bronchial asthma, there is a high proportion of the *AA* genotype and a low proportion of the *GG* allele; it was also estimated that the carriage of the mutant allele *A* increases the risk of developing bronchial asthma by *2.62* times. Among children with virus-induced phenotype and healthy children, the mutant allele *A* was found in 26.70 and 10.00% of cases, respectively. The research done by A.M. Ammar et al. (2020) [9] focused on the association of single nucleotide polymorphisms of *IL-17A (G-197A) (rs2275913)* and the development of sensitization to house dust mites and discovered that the occurrence of heterozygous genotype and the frequency of carriage of the *A* allele are higher among patients with bronchial asthma ($p < 0.01$), whereas the carriage of the *G* allele was more prevalent in the control group.

After analyzing *TNF-α (G-308A)* genotype, we discovered an exclusive relationship between the homozygous *A308A* genotype and the risk of developing bronchial asthma. The prevalence of mutant *A* and wild *G* alleles in patients with allergen-induced phenotype of bronchial asthma and the control group of children was 56.70 and 40.00% and 43.30 and 60.00%, respectively, and according to the OR of carrying a functional unfavorable allele *A G308A* polymorphism of *TNF-α* gene causes a 1.96-fold risk of developing bronchial asthma and the realization of virus-induced phenotype of the disease. I.C. Bocsan et al. (2020) [6], studying the correlation of polymorphisms of *IL-6, IL-10, TNF-α* genes and risks of developing bronchial asthma and allergic rhinitis, registered the dominance of the *G* allele carriage in both subgroups with *IL-6 (G-174C)* polymorphism, and *A308A* genotype of *TNF-α* gene was found only in patients with bronchial asthma.

Study limitations

During the planning and execution of the study, the sample size for achieving the required statistical power of the results was not calculated, but the sample obtained during the study can be considered sufficiently representative, allowing extrapolating the results and their interpretation to the general population of similar patients outside of the study.

CONCLUSION

The study provides data on the importance of functional polymorphisms of cytokine genes as predictors associated with the risk of developing various asthma phenotypes in children.

Significant differences in the structure and occurrence of cytokine gene polymorphisms in children with allergen-induced and virus-induced bronchial asthma were determined depending on the manifested disease phenotype. The carriage of mutant *IFN-γ (A-874A), IL-4 (T-589T), IL-6 (G-174G), IL-17A (A-197A),* and *TNF-α (A-308A)* alleles have been identified as genetic predictors of bronchial asthma development: the OR is higher in the presence of *IFN-γ (A-874A), IL-4 (T-589T),* and *TNF-α (A-308A)* mutant alleles for the realization of virus-induced phenotype and in the presence of *IL-6 (G-174G), IL-17A (A-197A)* for allergen-induced phenotype of the disease.

ADDITIONAL INFORMATION

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Authors' contribution. E.V. Prosekova — created concept and designed the study, editing; M.S. Dolgopolov, A.I. Turyanskaya — collection and processing of material; M.S. Dolgopolov, O.L. Zhdanova — analyzed data; E.V. Prosekova, V.A. Sabynych, M.S. Dolgopolov — wrote the manuscript with input from all authors. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

REFERENCES

1. Kurbacheva OM, Zhestkov AV, Nagatkin DA, et al. Modern view on the immunopathogenesis of bronchial asthma. *Russ Allergol J.* 2016;(2):10–14. (In Russ).

2. Kurbacheva OM, Pavlova KS. Phenotypes and endotypes of bronchial asthma: from pathogenesis and clinical features to therapy. *Russ Allergol J.* 2013;(1):15–24.(In Russ).

3. Smolnikova MV, Freidin MB, Smirnova SV. Cytokine genes as genetic markers of controlled and uncontrolled atopic bronchial asthma. *Med Immunol.* 2017;19(5):605–614. (In Russ).

4. Lambrecht B. The immunology of asthma. *Nat Immunol.* 2015; 1(1):46–52. doi: 10.1038/ni.3049

5. Global strategy for asthma management and prevention. Global Initiative for Asthma. (GINA) 2019. Available from: http://www.ginasthma.org. Accessed: 25.11.2021.

6. Bocsan IC, Pop RM, Pasca S, et al. Genetic polymorphisms of cytokines in asthma and allergic rhinitis. *World Allergy Organ J.* 2020;13(8):100195. doi: 10.1016/j.waojou.2020.100195

7. Babusikova E, Jurecekova J, Jesenak M, Evinova A. Association of gene polymorphisms in interleukin 6 in infantile bronchial asthma. *Arch Bronconeumol.* 2017;53(7):381–386. doi: 10.1016/j.arbres.2016.09.012

8. Kousha A, Gorabi AM, Forouzesh M, et al. Interleukin 4 gene polymorphism (-589C/T) and the risk of asthma: a meta-analysis and met-regression based on 55 studies. *BMC Immunol.* 2020;21(1):55. doi: 10.1186/s12865-020-00384-7

9. Ammar AM, Zayyat AE, Khayyal AS, et al. Role of interleukins 12B and 17A genetic variation in house dust mites allergy. *Egypt J Med Human Gen.* 2020;21(60):2–14. doi: 10.1186/s43042-020-00098-w

10. Alyasin S, Kanannejad Z, Esmaeilzadeh H, et al. Cytokine levels and polymorphisms in childhood asthma among the iranian population. *J Pediatr Rev.* 2021;9(1):19–26. doi: 10.32598/jpr.9.1.891.2

11. Smolnikova MV, Smirnova SV, Konopleva OS. Cytokines and genes polymorphism in the promoter regions (C-590t IL-4 and C-597a IL-10) as markers of uncontrolled atopic bronchial asthma in children. *Sib Sci Med J.* 2015;35(3):4–8. (In Russ).

12. Nie W, Zhu Z, Pan X, Xiu Q. The interleukin-4 -589C/T polymorphism and the risk of asthma: a metaanalysis including 7,345 cases and 7,819 controls. *Gene.* 2013;520(1):22–29. doi: 10.1016/j.gene.2013.02.027

13. Liu Y, Zhuo A, Liu W, et al. The -33C/T polymorphism in the interleukin 4 gene is associated with asthma risk: a meta-analysis. *J Investig Allergol Clin Immunol.* 2014;24(2):114–121.

14. Hussein RH, Auda IG, Brakhas SA, Ali EN. Occurrence of +874T/A gene polymorphism of interferon-gamma in Iraqi atopic patients. *Meta Gene.* 2020;(24):124–137. doi: 10.1016/j.mgene.2020.100677

15. Shumna TY, Fedosieieva OS, Zinchenko TP, et al. Characteristics of interleukin-4 gene (C-589T, rs2243250) polymorphism in children with bronchial asthma and allergic rhinitis with isolated or allergic rhinitis-induced comorbid malocclusion. *Zaporozhye Med J.* 2019;21(6):723–732. doi: 10.14739/2310-1210.2019.6.186484

СПИСОК ЛИТЕРАТУРЫ

1. Курбачева О.М., Жестков А.В., Нагаткин Д.А., и др. Современный взгляд на иммунопатогенез бронхиальной астмы // Российский аллергологический журнал. 2016. № 2. С. 10–14.
2. Курбачева О.М., Павлова К.С. Фенотипы и эндотипы бронхиальной астмы: от патогенеза и клинической картины к выбору терапии // Российский аллергологический журнал. 2013. № 1. С. 15–24.
3. Смольникова М.В., Фрейдин М.Б., Смирнова С.В. Гены цитокинов как генетические маркеры атопической бронхиальной астмы с контролируемым и неконтролируемым течением // Медицинская иммунология. 2017. Т. 19, № 5. С. 605–614.
4. Lambrecht B. The immunology of asthma // Nat Immunol. 2015. Vol. 1, N 1. P. 46–52. doi: 10.1038/ni.3049
5. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. 2019. Режим доступа: http://www.ginasthma.org. Дата обращения: 25.11.2021.
6. Bocsan I.C., Pop R.M., Pasca S., Boarescu I. Genetic polymorphisms of cytokines in asthma and allergic rhinitis // World Allergy Organization Journal. 2020. Vol. 13, N 8. P. 100195. doi: 10.1016/j.waojou.2020.100195
7. Babusikova E., Jurecekova J., Jesenak M., Evinova A. Association of gene polymorphisms in interleukin 6 in infantile bronchial asthma // Arch Bronconeumol. 2017. Vol. 53, N 7. P. 381–386. doi: 10.1016/j.arbres.2016.09.012
8. Kousha A., Gorabi A.M., Forouzesh M., et al. Interleukin 4 gene polymorphism (-589C/T) and the risk of asthma: a meta-analysis and met-regression based on 55 studies // BMC Immunol. 2020. Vol. 21, N 1. P. 55. doi: 10.1186/s12865-020-00384-7
9. Ammar A.M., Zayyat A.E., Khayyal A.S., et al. Role of interleukins 12B and 17A genetic variation in house dust mites allergy // Egypt J Med Human Gen. 2020. Vol. 21, N 60. P. 2–14. doi: 10.1186/s43042-020-00098-w
10. Alyasin S., Kanannejad Z., Esmaeilzadeh H., et al. Cytokine levels and polymorphisms in childhood asthma among the iranian population // J Pediatr Rev. 2021. Vol. 9, N 1. P. 19–26. doi: 10.32598/jpr.9.1.891.2
11. Смольникова М.В., Смирнова С.В., Коноплева О.С. Цитокины и полиморфизм промоторных регионов генов (C-590T IL4, C-597A IL10) как маркеры неконтролируемого течения атопической бронхиальной астмы у детей // Сибирский научный медицинский журнал. 2015. Т. 35, № 3. С. 4–8.
12. Nie W., Zhu Z., Pan X., Xiu Q. The interleukin-4 -589C/T polymorphism and the risk of asthma: a metaanalysis including 7,345 cases and 7,819 controls // Gene. 2013. Vol. 520, N 1. P. 22–29. doi: 10.1016/j.gene.2013.02.027
13. Liu Y., Zhuo A., Liu W., et al. The -33C/T polymorphism in the interleukin 4 gene is associated with asthma risk: a meta-analysis // J Investig Allergol Clin Immunol. 2014. Vol. 24, N 2. P. 114–121.
14. Hussein R.H., Auda I.G., Brakhas S.A., Ali E.N. Occurrence of +874T/A gene polymorphism of interferon-gamma in Iraqi atopic patients // Meta Gene. 2020. Vol. 24. P. 124–137. doi: 10.1016/j.mgene.2020.100677
15. Shumna T.Y., Fedosieieva O.S., Zinchenko T.P., et al. Characteristics of interleukin-4 gene (C-589T, rs2243250) polymorphism in children with bronchial asthma and allergic rhinitis with isolated or allergic rhinitis-induced comorbid malocclusion // Zaporozhye Med J. 2019. Vol. 21, N 6. P. 723–732. doi: 10.14739/2310-1210.2019.6.186484

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