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ЦИТОКИНОВЫЙ ПРОФИЛЬ Th1/Th2/Th17 В ПЛАЗМЕ И ПОЛИМОРФИЗМ ГЕНОВ (*IL12B, IL13, IL31, IL33*) У БОЛЬНЫХ АСТМОЙ ДЕТЕЙ: МУЛЬТИПЛЕКСНЫЙ АНАЛИЗ

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Резюме. Изучение патогенеза бронхиальной астмы является актуальной проблемой в связи с ее высокой распространенностью и зачастую бесконтрольным развитием тяжелых форм, в том числе в детском возрасте. Первые признаки развития астмы обычно возникают в детстве, что приводит к ухудшению качества жизни пациента и ранней инвалидизации. Поскольку астма является генетически опосредованным процессом, предполагается, что тяжесть заболевания зависит от наличия определенного аллельного варианта в медиаторных (таких как цитокины) генах, участвующих в патогенезе БА. Целью данного исследования был поиск иммуногенетических маркеров тяжелого течения астмы у детей славянского происхождения, проживающих в г. Красноярск. Впервые с помощью метода мультиплексного анализа (xMAP) определены количественные показатели Th1/Th2/Th17-цитокинового профиля у больных бронхиальной астмой (БА) детей с различной степенью тяжести заболевания, в зависимости от полиморфизма генов цитокинов. Изменения цитокинового фона у больных БА укладываются в концепцию о том, что при тяжелых формах астмы возрастает доля нейтрофильного эндотипа заболевания, осуществляющего свои функции посредством Th1 и Th17-лимфоцитов. Кроме этого, получены данные цитокинового профиля в зависимости от наличия сопутствующих острых респираторных инфекций: показан дисбаланс уровня цитокинов, с тенденцией сохранения защитных функций иммунной системы у больных в состоянии ремиссии. Получено распределение в генах цитокинов: аллельные варианты *IL12B* rs321220**G*, *IL13* rs1800925**C*, *IL31* rs7977932**C* и *IL33* rs7044343**T* являются наиболее часто встречающимися в популяционной выборке г. Красноярска. Изучена вероятность ассоциации генотипов полиморфных генов цитокинов (*IL12B, IL13, IL31, IL33*) с состоянием иммунной системы при бронхиальной астме с различной степенью тяжести заболевания у детей: показана достоверная ассоциация генотипа *TT IL12B* rs3212220 с низкой концентрацией IL-12B. Полученные данные можно использовать в комплексе с полученными нами ранее иммуногенетическими маркерами тяжелой степени и неконтролируемого течения астмы у детей с целью персонифицированного прогноза характера заболевания.

Ключевые слова: бронхиальная астма, дети, цитокины, мультиплексный анализ, полиморфизм, тяжесть заболевания

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PLASMA Th1/Th2/Th17 CYTOKINE PROFILE AND CYTOKINE GENE POLYMORPHISMS (*IL12B, IL13, IL31, IL33*) IN ASTHMATIC CHILDREN: A MAGNETIC MULTIPLEX ASSAY

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Abstract. The study of the bronchial asthma pathogenesis is an urgent problem due to its high prevalence and often developing uncontrolled severe ashma, including in childhood. The first signs of asthma development tend to occur in childhood, which causes deterioration in the patient's quality of life and early disability. Since BA is a genetically mediated process, the severity of the disease is assumed to depend on the presence of a specific allelic variant in the mediator (e.g. cytokines) genes involved in the BA pathogenesis. The aim of this study was to search for immunogenetic markers of severe asthma in Slavs children living in Krasnoyarsk city. The quantitative indicators of the Th1/Th2/Th17-cytokine profile in children with bronchial asthma (BA) with varying disease severity, depending on the polymorphism of cytokine genes, using the method of multiplex analysis (xMAP), were first determined. Changes in the cytokine background in BA patients fit into the concept that a percentage of neutrophilic endotype, which performs its functions through Th1 and Th17-lymphocytes in severe asthma, increases. In addition, the cytokine profile data depending on concomitant acute respiratory infections were obtained. There was an imbalance when analyzing the cytokine plasma level, with a tendency to maintain the protective functions of the immune system among patients in remission. Distribution of cytokine genes was obtained: allelic variants of *IL12B* rs321220**G, IL13* rs1800925**C, IL31* rs7977932**C* and *IL33* rs7044343**T* are the most common in the population sampling from Krasnoyarsk. The probability of the genotype association of cytokine genes *(IL12B, IL13, IL31, IL33)* with the state of the immune system in bronchial asthma with varying disease severity in children was studied: a significant association of the *TT* genotype *IL12B* rs3212220 with a low concentration of IL-12B was presented. Our data obtained can be used along with the previously obtained immunogenetic markers of severe and uncontrolled asthma in children for patient-specific prognosis of the disease nature.

Keywords: bronchial asthma, child, cytokines, multiplex assay, polymorphism, disease severity

Introduction

Bronchial asthma (BA) is the most severe disease of the bronchopulmonary system associated with chronic inflammation, mucus hypersecretion, hyperreactivity and remodeling of the airways. There is a need for a detailed study of the developing severe forms and the search for methods to achieve asthma control due to its high prevalence. The first signs of asthma development tend to occur in childhood, which causes deterioration in the patient's quality of life and early disability. Bronchial asthma is a multifactorial disease (MD), i.e. its development appears to depend on both a variety of environmental factors and a genetic component. An unfavorable genetic background for this disorder is triggered by interacting with specific environmental factors and manifested in developing the pathological phenotype. Since BA is a genetically mediated process, the severity of the disease is assumed to depend on the presence of a specific allelic variant in the mediator genes involved in the BA pathogenesis.

The research on medical pathogenetics has been started in the early 1990s, when allelic polymorphism and the significance of individual allelic gene variants in predisposition to various diseases, their influence on the nature, severity and outcome of the disease were established [3]. The most common method of genetic MD studies is the candidate gene approach based on the analysis of a limited number of specific genes, with a choice being based on the identified or intended role of their expression products in the disease development [15]. Genetic analysis is mostly based on comparing frequencies of alleles and genotypes of certain genes in ill and healthy persons coupled to the studied pathology of among unrelated individuals (case-control study design).

Cytokines are important targets for the diagnostics of a wide range of human diseases, and their study is important for better understanding a mechanism of immunological changes observed in patients. Moreover, cytokines play an essential role in all stages of triggering and maintaining allergic inflammation, therefore, the study of their activity and regulation is

of great importance for understanding the molecular basis behind BA pathogenesis. The accumulated data indicate that Th2-cells and their cytokines such as IL-4, IL-5 and IL-13 play a critical role in the development and activation of allergic inflammation in asthma. Th1-lymphocytes, with specific cytokines (IL-2, IL-3, IFNγ, TNFα), regulate the “cellular” antigen-specific immune response, NK-cell function, phagocytic cells, and also participate in regulating IgM and IgG2 gene expression in B-lymphocytes. Cytokines produced by Th2-lymphocytes support IgE production and some other immunoglobulins (humoral immune response), and also participate in developing allergic inflammation by activating mast cells and eosinophils [12]. Recent studies have expanded the Th1/Th2-paradigm and have drawn attention to Th17-cells representing the third $CD4^{+}$T-cell lineage, as they are responsible for a number of pathological processes [18].

To determine the cytokine profile in various biological fluids should be used for evaluating the inflammation activity, the polarization of immune response, the therapy effectiveness, and the disease prognosis. In recent years, the multiplex analysis has become widespread, including various formats for assessing cytokines in biological fluids using flow cytometry: xMAP (Multi-Analyte Profiling). This type of assay is based on binding to monoclonal antibodies fixed on the surface of microbeads conferring high sensitivity and specificity. Because xMAP technology is a method for determining metabolites in a small sample quantity, it makes it optimal for studying the patients in childhood. There is evidence on differences in production of certain cytokines among populations in different ethnic groups [1]. This results from different distribution of cytokine gene alleles in racial groups. In different populations, accurate reproduction of the obtained reliable association between individual polymorphisms and certain medical condition is rarely achieved, suggesting the specificity and population characteristics of the frequency distribution of polymorphic variants of the analyzed genes. Hence, when studying polymorphic gene variants, it is necessary to take into account human population characteristics .

Although BA symptoms in most patients with mild disease are well controlled with current treatment methods, around 10% of patients with severe asthma show poor control and increased risk of developing severe asthma despite a large baseline therapy [6]. Since the spectrum of genes for BA susceptibility cannot be considered tas fully identified, the analysis of hereditary factors is a promising area for allergology, dermatology, and immunogenetics. A comprehensive analysis of cytokine genetic polymorphism and level of relevant protein products, in case of their participation in the BA pathogenesis related to disease severity, seems promising for developing a personalized approach to determine disease susceptibility and severity. Preclinical risk diagnostics for developing multifactorial diseases including asthma, can help to lower cost of expensive treatment as well as to preserve human working capacity preventing disability.

Materials and methods

A total of 546 individuals were involved in the study: 317 bronchial asthma (mean age 13.6±2.5 years) and 229 healthy individuals from the city of Krasnoyarsk. The control group included children (n = 127) and adults with no history of asthma and allergies (n = 102). No statistically significant differences were revealed between the control groups of different ages, which allowed to randomize individuals of different ages into one control group. Children with asthma were divided into groups depending on the severity of the disease: mild asthma (n = 133), moderate asthma (n = 102), severe asthma (n = 82). The diagnostics and severity of the disease were determined in accordance with the recommendations of the GINA working group. All examined patients met the general inclusion criteria for the study: BA diagnosis, severe /moderate course, absence of acute upper respiratory tract infection (URTI) and other acute diseases upon examination, as well as Slavic descent for the last three generations. Inclusion criteria for patients to be included in the control group: apparently healthy individuals, negative allergic history, total IgE level < 100 IU/ml, Slavic descent.

All study participants or their parents provided written informed consent prior to the study. The protocol for examining ill and healthy subjects complied with ethical standards and was approved by the Biomedical Ethics Committee of the Research Institute of Medical Problems of the North, Krasnoyarsk Scientific Center, Siberian Branch, Russian Academy of Sciences.

The research materials were presented by patient serum and DNA extracted from the peripheral blood by the sorbent method using the DIAtom DNAPrep100 kit (OOO Isogen, Russia). Genotyping of polymorphisms *IL12B* (rs321220), *IL13* (rs1800925), *IL31* (rs7977932) and *IL33* (rs7044343) was performed using the real-time polymerase chain reaction (RT-PCR) as well as specific oligonucleotide primers and fluorescently labeled probes (TagMan) (DNA-synthesis, Russia).

Plasma cytokine concentrations were determined with magnetic microsphere test systems MILLIPLEX MAP *Human TH17* (Merck, USA) using multiplex analysis by xMAP technology (Luminex, USA) according to the manufacturer’s instructions. Data registration and analysis were performed with a Luminex MAGPIX device (Luminex, USA).

Differences at the $p < 0.05$ level were considered as statistically significant. The allele and genotype frequencies between groups were compared by using χ-square test and online calculator. An odds ratio (OR) with a 95% confidence interval (CI) was performed to assess a relationship between genetic markers and pathology phenotypes. Statistical significance of differences in quantitative traits was analyzed using the Mann–Whitney test. Data are expressed as median and interquartile range.

Results and discussion

In multiplex analysis, the values of the cytokine concentration produced by many immune cell types in pediatric bronchial asthma with varying severity and different status of disease exacerbation (n = 106) were obtained. We analyzed the concentration of 17 cytokines; significant differences are shown in the Tables.

The development of allergic reactions is known to be affected by a complex interplay in cytokine network with multiple reciprocal relationships [12]. A number of mediator genes and their interaction with environmental factors, hereditary predisposition, response of respiratory and immune systems enable to distinguish acute URTI BA-phenotypes. The most common is neutrophilic asthma (up to 81.8%), especially its severe form. Identifying BA-phenotypes/endotypes provides deeper insights into the essence of disease and is necessary to develop individualized therapy approach. However, currently, no consensus on the criteria for distinguishing phenotypes has been achieved due to the lack of specific biomarkers for the majority of phenotypes.

Our study on serum cytokine profile analyzed the data obtained from children with varying severity of bronchial asthma. Our data on BA patients with diverse cytokine background fit into the concept that a percentage of neutrophilic endotype executing functions through Th-1 and Th17-lymphocytes in severe asthma, increases. The level of cytokines produced by various types of immunocompetent cells (Th1, Th2, Treg, Th17) has been shown to be significantly lower for severe BA patients, i.e decreased expression numerous pro- and anti-inflammatory cytokines associated with disease severity ($p < 0,05$). It suggests increased suppression due to inflammatory processes in the bronchopulmonary system and decreased protective functions as well as transition of the disease to uncontrolled course (Table 1).

One of the main factors in regulating proliferative response of B-lymphocytes and immunoglobulin isotype switching inducing expression of IgG and IgE is provided by IL-4, a growth factor for T-lymphocytes and mast cells, a key factor for the $CD4^{+}$T-cell differentiation into type Th2-cells that affects their functioning [16]. Along with other cytokines such as G-CSF and IL-6, IL-4 can stimulate growth of mast cell and myeloid and erythroid cell precursors. There has been noted that the level of IL-4 in the peripheral blood serum and plasma is increased in patients with asthma and related comorbidities [17]. Nevertheless, the systemic serum amount of IL-4 in BA patients has been observed in numerous studies to reflect disease clinical course, so that its imbalanced level was observed during disease exacerbation [9, 17]. The IL-4 and IL-6 concentration in patients with severe asthma observed in our study was 0.11 and 0.02 pg/ml, respectively, which were significantly lower than in mild asthma ($p < 0.05$).

Neutrophilic inflammation, characterized by an aggressive course, pronounced tissue destruction, low response to corticosteroids, is typical for children with bronchial asthma [7]. This phenotype is associated with the active neutrophilic products such as neutrophil elastase, α1-antitrypsin, IL-8, IL-17, as well as Th17-cells for their maintenance [5]. Th17-cells differs from Th1, Th2 and Treg-cells characterized by the IL-17 family cytokine production. The IL-17 family has been recently described and includes IL-17A (also called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (known as IL-25), and IL-17F, according to the order of discovery [11]. The ability of IL-17A and IL-17F to induce neutrophil migration suggests that they are involved in the pathogenesis of severe BA mainly characterized by neutrophilic airway inflammation [10]. The association between IL-17A and IL-17F and airway inflammation has also been confirmed experimentally in mouse asthma models. The role of these two cytokines has also been shown in development of eosinophilic inflammation. In fact, mice deficient in IL-17 receptors or IL-17 show reduced potential for eosinophils to migrate into the respiratory tract due to low expression of Th2 cytokines such as IL-4 and IL-5 acting as eosinophil chemokines [14]. Thus, the identified functions of IL-17 suggest that the airways may be affected by this potent proinflammatory cytokine in different directions. Understanding the effector functions of Th17-cells during inflammation in the bronchopulmonary system may be a key to unequivocal understanding of the BA pathogenesis [4, 8].

BA exacerbation is known to be aggravated by concomitant acute respiratory infections. The patients without exacerbation (remission) (group 1), patients with concomitant acute URTI (group 2) and patients without concomitant acute URTI (group 3) were involved in the study. An imbalanced cytokine profile between groups of patients was found that tended to maintain immune protective functions among patients in remission. The IL-17A and IL-17F concentration in patients with severe vs. mild BA obtained in the study was lowered (data not shown

TABLE 1. CYTOKINE PLASMA LEVEL IN BRONCHIAL ASTHMA PATIENTS WITH DIFFERENT SEVERITY, Me ($Q_{0.25}$-$Q_{0.75}$), pg/ml

Cytokine	Mild asthma (1), n = 58	Moderate asthma (2), n = 37	Severe asthma (3), n = 11	p
IFNγ	9.69 (6.92-18.06)	9.69 (6.01-18.98)	4.02 (1.05-7.84)	$p_{1,3} = 0.02$ $p_{2,3} = 0.04$
IL-10	12.05 (6.92-16.79)	13.04 (6.12-17.49)	4.66 (2.99-9.79)	$p_{1,3} = 0.01$ $p_{2,3} = 0.01$
IL-9	20.81 (14.10-35.85)	25.98 (7.17-42.91)	12.03 (0-15.46)	$p_{1,3} = 0{,}01$
IL-1β	15.27 (11.02-18.42)	20.68 (15.94-23.82)	18.98 (14.97-28.34)	$p_{1,2} = 0.01$ $p_{1,3} = 0.04$
IL-33	50.93 (36.74-72.87)	65.42 (51.57-73.49)	57.84 (26.78-76.57)	$p_{1,2} = 0.05$
IL-4	0.24 (0.14-0.29)	0.20 (0.11-0.32)	0.11 (0.06-0.19)	$p_{1,3} = 0.01$ $p_{2,3} = 0.02$
IL-6	26.02 (8.43-43.46)	25.75 (9.01-48.97)	0.02 (0.01-16.42)	$p_{1,3} = 0.01$ $p_{2,3} = 0.03$
IL-27	0.83 (0.62-1.13)	1.05 (0.85-1.34)	1.08 (0.96-1.42)	$p_{1,2} < 0.01$ $p_{1,3} = 0.03$
IL-28A	0.88 (0.63-1.43)	1.08 (0.56-1.46)	0.20 (0.00-1.27)	$p_{2,3} = 0.05$

in Table 1, p > 0.05). Regarding disease remission, the concentration of such cytokines was significantly higher than in exacerbations with or without concomitant acute URTI (Table 2).

It was noted above, that the level of protein expression and production was genetically determined and depended on specific polymorphic gene variants. The concentration level of the studied cytokines depending on the genotype of coding cytokine genes exemplified by *IL12B, IL13, IL31* and *IL33* was comprehensively analyzed in our study.

Based on the distribution frequency of cytokine gene polymorphisms in children with BA, features in the allelic variants distribution of the genes *IL12B* (rs321220), *IL13* (rs1800925), *IL31* (rs7977932), *IL33* (rs7044343) specific to Caucasians were shown. Thus, according to the website http://www.ensembl.org, the global Caucasian population seems to carry prevalent alleles *IL12B*G* (90%)*, IL13 *C* (75%), *IL31*C* (84%) и *IL33*T* (63%). In our sampling of Slavic subjects from the Eastern Siberia were found to carry *IL12B*G* (76%)*, IL13 *C* (75%), *IL31*C* (88%) and *IL33*T* (60%), respectively. While analyzing the distribution frequency of the *IL12B* (rs321220) polymorphism, a significant difference in the genotype frequencies between BA patients and control group was demonstrated. Statistically different data on the genotype distribution between the groups with moderate BA and control (OR 0.63 (0.41-0.97), p = 0.035) were obtained. The frequency of the *GG* genotype in the moderate asthma was higher than in the control group (71.6% *versus* 57.2%), whereas the frequency of the *GT* and *TT* genotypes – higher in the control group than in moderate asthma (5.2% *versus* 4,9%; 37.6% *versus* 23.5%, respectively). Comparing the distribution frequency of the *IL13* rs1800925 polymorphism detected significant difference in the genotype frequencies between BA patients and control group. The variant allele T * was found to be more common in patients, regardless of disease severity compared to the control (p = 0.01). The frequency of *CT* and *TT* genotypes in BA patients is significantly higher than in the control group. Moreover, the frequency of *TT* genotype associated with increased IL-13 concentration was higher bt at least 2-fold than in control group of children with severe BA (13.4% *versus* 5.7%, OR 1.67, p = 0.01). Thus, this genotype serves as a BA genetic marker predisposing to the pathologic behavior, i.e. the development of severe BA. At the same time, the *CC* genotype rs1800925 protects against the BA development as well as severe asthma.

While analyzing IL-12B plasma level in children with bronchial asthma, depending on the genotype, significant differences were revealed. In particular, a significant association between the *TT* genotype *IL12B* rs3212220 and low IL-12B concentration was found. Importantly, no published data are available

TABLE 2. CYTOKINE PLASMA LEVEL IN BRONCHIAL ASTHMA PATIENTS WITH DIFFERENT TYPE OF EXACERBATION, Me ($Q_{0.25}$-$Q_{0.75}$), pg/ml

Cytokine	Remission (1), n = 67	Exacerbation with acute URTI (2), n = 15	Exacerbation without acute URTI (3), n = 22	p
IL-17F	0.03 (0.00-0.06)	0.01 (0.00-0.02)	0.01 (0.00-0.04)	$p_{1,2} = 0.04$
IFNγ	11.55 (6.92-18.98)	7.38 (3.38-16.67)	7.84 (2.55-10.62)	$p_{1,3} = 0.05$
IL-17A	10.74 (6.66-13.94)	8.69 (3.62-15.31)	5.77 (3.41-10.51)	$p_{1,3} = 0.01$
IL-1b	15.55 (11.22-23.27)	20.68 (17.28-23.27)	16.81 (14.39-23.82)	$p_{1,2} = 0.05$
IL-6	25.21 (3.75-43.46)	16.42 (0.01-41.43)	27.36 (0.03-60.72)	$p_{1,2} < 0.01$
IL-27	0.83 (0.64-1.05)	1.08 (0.87-1.40)	1.23 (0.99-1.43)	$p_{1,2} = 0.02$ $p_{1,3} < 0.01$

on the association of this polymorphism with the expression level. The IL-12B plasma level in the *TT* genotype patients was 3.96 (2.98-8.42) pg/ml compared to the alternative homozygote GG – 12.73 (8.42-17.11) pg/ml and the heterozygote GT – 10.44 (6.42-15.04) ($p < 0.01$). The association of this polymorphism with the level of intra- and extracellular expression of the pro-inflammatory Th1-cytokine (IL-12B) can be assumed to determine the BA development. Another previously studied *IL12B* polymorphism (rs3212227) affects the expression level, i.e. allelic variant C is associated with decreased production of IL-12B [13].

Analysis of the associative relationships of the genotypes *IL13* (rs1800925), *IL31* (rs7977932) and *IL33* (rs7044343) showed no significant differences in the concentration level of the corresponding cytokines. It is noteworthy that the level of IL-13 in the plasma of BA patients in carrying the CT and TT vs. CC genotypes of *IL13* (rs1800925) is higher. According to the literature mentioned above, the allelic variant T of this SNP is associated with increased production of IL-13, airway hyperresponsiveness most often occurring in severe disease [2].

Conclusion

The immunological profile of multifactorial diseases such as bronchial asthma is a complex network of cytokines, chemokines and growth factors. As a result, it is worth studying asthma by using multiplex analysis as a modern tool for determining metabolite concentration, even in trace amounts that rominently differs from the ELISA.

Polymorphic cytokine genes are one of the most studied genetic markers, as the products of these genes are regulators of the main immune and inflammatory processes in the body. Determining the hereditary factors of BA along with the assessing cytokine level in biological fluids will allow to study disease immunopathogenesis and develop some personalized approaches to determining the disease severity and the appropriate therapy.

In the study, an integrated approach was applied to studying both the polymorphic cytokine genes produced by immune cells and concentration of expressed protein products involved in the BA pathogenesis in children with varying disease severity.

The data obtained for the first time provide insight into the variant distribution in the cytokine genes *IL12B, IL13, IL31, IL33*, produced by different immune cells in patients with bronchial asthma and in the population sampling of the Krasnoyarsk residents. According to the data, the allele and genotype frequencies for the studied polymorphisms in the Slavic subjects of the Eastern Siberia correspond to those in found other Caucasoid populations worldwide. Allelic variants of *IL12B*G, IL13*C, IL31*C* and *IL33*T* are the most common in the population sampling of the city of Krasnoyarsk.

The genetic markers of susceptibility to severe bronchial asthma in children have been identified: allelic variant of *G*IL12B rs321220* and variant allele of *T*IL13 rs1800925*. According to the literature, the products encoded by these genes are involved in allergic asthmatic airway inflammation. Therefore, the genetic polymorphism analysis enables to establish the pathogenetic role in emerging specific clinical BA

course in the Eastern Siberia population. The human populational characteristics should be to be taken into account. In different populations, an accurate reproduction of the obtained reliable associations between individual polymorphisms and a certain pathological state is rarely achieved, suggesting the specificity and populational features in frequency distribution of polymorphic variants for analyzed genes. In addition, there is evidence about differences in produced some cytokines among various ethnic populations.

When analyzing the cytokine profile in BA patients, complex data fitting into the concept that severe asthma is coupled to a percentage of neutrophilic endotype that exerts its functions through Th1 and Th17-lymphocytes. The level of cytokines produced by various immunocompetent cells is significantly lower for severe BA patients, i.e. a decreased expression for several pro- and anti-inflammatory cytokines correlates with the disease severity ($p < 0.05$).

Achieving control of severe asthma is a challenge and requires a multifaceted approach, taking into account the phenotypic characteristics of severe bronchial asthma. Severe asthma is a common problem and significantly reduces the quality of life for patients and their families, leading to frequent and severe exacerbations, decreased lung function, and limited professional and social activity.

To sum up, we can conclude that prognosis criteria for the development of severe bronchial asthma is presented as a combination of genotypes for pro- and anti-inflammatory cytokines obtained in the current study and earlier, as well as the level of concentration of immune response mediators combined with clinical characteristics. To identify general and specific immunogenetic markers it opens up a potential for populational screening, as well as patients with multifactorial diseases, including bronchial asthma, which allows to find out risk factors for developing pathology before clinical manifestations as well as to predict a probability for developing severe and uncontrolled disease and apply personalized approach to treatment strategy of patients.

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