

Variability of the pharmacological response in children with different ADRB2 gene polymorphisms in recurrent bronchial obstruction and bronchial asthma

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Abstract. To date, methods of studying the effects of drugs at the molecular-genetic level are widely used in pediatric practice. The aim of our work was to evaluate the effectiveness of the effect of β_2 -agonists on the clinical course of recurrent bronchial obstruction (RBO) in children, depending on the variants of polymorphism of the ADRB2 gene. The effectiveness of salbutamol in children with RBO and bronchial asthma (BA) was evaluated depending on the distribution of alleles and genotypes of the ADRB2 Arg16Gly and Gln27Glu genes according to the degree of reversibility of bronchial obstruction. In the group of children with RBO carriers of the heterozygous A/G genotype of the Arg16Gly locus and the Gln27Glu locus of the ADRB2 gene, high efficacy of salbutamol therapy as a bronchodilator was noted. At the same time, in children with RBO representatives of the G/G genotype, the effectiveness of using the drug is low. In children with BA, the change in functional activity is associated with the polymorphism of the Gln27Glu gene locus and is associated with a low therapeutic response to β_2 -agonists in children with the G/G mutational genotype and a good effect in carriers of the homozygous variant with the A/A genotype. Knowledge of the genotype of polymorphic variants of the ADRB2 gene will make it possible to evaluate one of the factors of predisposition and effectiveness of therapy in the recurrent course of bronchial obstruction syndrome and bronchial asthma.

1 Introduction

In recent years, the incidence of bronchitis associated with the syndrome of bronchial obstruction (SBO) among children has been increasing. Among them, obstructive bronchitis with a recurrent course is noted in 50-70% of young children and in many cases the cause is bronchial asthma (BA). The prevalence of recurrent bronchitis with bronchial obstruction syndrome (RBS) among children is a modern problem of a socio-economic nature, and is one of the most frequent pathologies, especially in children, and makes up from 50 to 70%

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of all inflammatory diseases of the bronchi. According to many authors, the high incidence of RBO and the ineffectiveness of treatment is associated with the prevalence and changes in the evolution of bacterial and viral infections, an increase in immunodeficiency in children, and poor environmental factors [1-3]. In the structure of recurrent and chronic bronchial diseases of childhood abroad and in Uzbekistan, the number of children with asthma is the predominant majority, and their number increases with age [4-7].

Currently, a number of studies are being conducted all over the world aimed at identifying clinical and genetic aspects and improving the effectiveness of diagnosis and treatment of chronic respiratory and atopic diseases in children. In this regard, it is necessary to establish the association of gene polymorphisms with the features of the course of bronchial asthma and recurrent bronchitis (RB), to determine the presence of hereditary predisposition in children, to determine genetic markers in the diagnosis of the disease; to identify candidate genes that cause recurrent bronchial obstruction (RBO). At the current level of development, today methods for studying the influence of drugs at the molecular and genetic level are widely used in pediatric practice. The ADRB2 gene is responsible for the functional activity of β_2 -adrenergic receptors, which is characterized by polymorphism. The variability of the response to these drugs in 60-80% of them may be associated with ϕ genotype polymorphism in patients [8-11]. Polymorphism of the ADRB2 gene occurs due to a violation of the sequence of amino acids, which can lead to a decrease or absence of the functional properties of the receptor. This condition provokes a violation of bronchodilation or undesirable side effects. At the same time, the influence of the gene bypasses the necessary reactions of the respiratory tract, due to which the sensitivity of the bronchi muscle receptors decreases (I. V. Vasilevsky. 2019).

To date, timely clinical and genetic diagnosis and prognosis of asthma in children with recurrent bronchial obstruction (RBO) makes it possible to avoid burdensome, unnecessary diagnostic and expensive therapeutic measures and to interest parents in the recovery process [12-14]. Undertreatment, the presence of an incomplete rehabilitation stage in the process of remission of the disease is one of the reasons for the transformation of acute forms of bronchitis into a recurrent course and chronic pathology. Therefore, identification of candidate genes in the development of RBO and AD for early diagnosis, personalization of therapy and timely preventive measures is relevant and timely.

Today, methods of studying the influence of drugs on the molecular and genetic level are widely used in pediatric practice. Advances in genomic technologies have improved our understanding of disease pathogenesis and enabled us to better characterize drug toxicity. This provides important information for personalizing BLD therapy in children and adolescents. Inhaled glucocorticosteroids and beta-2-adrenergic receptor agonists are currently widely used in the treatment of RBO and asthma. Among the widely used beta-2-adrenergic agonists are the drugs salbutamol (ventolin) and fenoterol (berotek) of prolonged action. They are used as bronchoprotective agents for basic anti-inflammatory therapy in combination with inhaled glucocorticosteroids. The therapeutic effect of β_2 -agonists occurs through β_2 -adrenergic receptors, which are located in large numbers in the smooth muscle and glandular cells of the bronchi. In the case of prolonged use of high doses of beta-2 agonists, a decrease in the sensitivity of receptors to them can be observed, which can lead to the development of drug tolerance [2, 3, 12].

The ADRB2 gene has a high degree of affinity for epinephrine, which, when interacting with bronchial receptors, is involved in bronchodilation and a decrease in BO. In this regard, beta-2-receptor agonists are widely used in therapy for the treatment of BO. The use of beta-2-receptor agonists makes it possible to use and reduce the dosage of corticosteroids. Based on modern pharmacogenetics, hereditary factors largely determine the severity of the therapeutic response to drugs. In recent years, many studies have identified new

pharmacogenetic variants that are associated with responses to IGCS and bronchodilators. Thus, Figueiredo R. G. (2021) discovered genetic factors of poor response to treatment in severe asthma. Numerous studies conducted by scientists and practitioners studying the ADRB2 gene, which is located in the region of chromosome 5 (5q31), reveal the pathogenetic mechanisms of bronchial disease development and increases the effectiveness of drug therapy [2, 10].

Authors Eliza M. A. and others (2018) studied the pharmacogenetics of inhaled β -long-acting beta-2 agonists in AD based on the indicators of FVD in children. The association of ADRB2 rs1042713 and rs1800888 with heterogeneity of response to β -long-acting beta-2 agonists was determined.

A large number of studies by scientists are devoted to the study of the ADRB2 gene and its significance in the formation of AD. The authors determined the incomplete efficacy of short-acting beta-2 agonists in carriers of the G/G mutation genotype of the rs1042713 locus of the ADRB2 gene. It turned out that y carriers of the G/G rs1042713 genotype of the ADRB2 gene have a severe course of AD. Representatives from the United States who use short-acting beta-2 agonists, in therapy showed a link between the A allele of the ADRB2 gene and impaired FVD, in contrast to carriers of the homozygous G/G genotype of the ADRB2 gene. Some scientists have concluded that the ADRB2*16Gly-substituted receptor is significantly depleted with continuous use of beta-2 agonists, so patients who are carriers of the homozygous G variant of the ADRB2 gene quickly lose sensitivity to these drugs.

Clenkard M. others (2016, England) investigated the effect of the Arg16 allele of the ADRB2 gene on the effectiveness of combined IGCS (LABA) in patients with moderate and severe asthma. The use of fluticasone with the beta-2-agonist salmeterol in the treatment with the rs1042713 G/G genotype of the ADRB2 gene of European origin gives the effect of increasing EEF with an improvement in PSV. Patients with AD with the Arg16Gly genotype who received fluticasone alone maintained decrease in EEF.

Research results of WuChen and Guo X. (2016) of children with AD showed that the use of long-acting beta-2 agonists is associated with an increase in the number of AD relapses in carriers of the Arg16Gly locus of the homozygous A/A genotype of the ADRB2 gene [11].

Due to the heterogeneity of SBS in children with RBO and BA, an active search is currently underway for clinical and genetic predictors and pharmacogenomics of the disease, which allows not only predicting its further course, but also provides an opportunity to correct therapy. In recent years, many studies have focused on the therapeutic resistance of AD, noting that the optimal clinical response to treatment may vary between racial/ethnic groups or individuals due to genetic differences.

The aim of our study was to determine the role of polymorphism of ADRB2 gene loci in the pharmacological response to beta-2 agonists in children with recurrent bronchial obstruction (RBO) and AD.

2 Material and methods of research

The material for the study was children aged from 1 to 15 years of Uzbek ethnicity, of which 164 patients with recurrent bronchitis occurring with SBR (RBO). The comparison groups included 76 children with bronchial asthma of the same age and ethnicity. The control group included 72 children of the same age and populations without bronchopulmonary pathology and an allergic history. The distribution frequencies of alleles and genotypes (of the Arg16Gly (rs1042713) locus (n=88) and Gln27Glu (rs1042714)

(n=89) of the beta-2-adrenergic receptor (ADRB2) gene in children with RBO, AD, and healthy adults (n=72) were studied by real-time PCR using the SNP-express reagent kit-SHOT "on modern equipment" Rotor Gene 6000 / Q "(Real-time CFX96 C1000 Touch) Bio-Rad (Germany).

We determined the effectiveness of the effect of beta-2 agonists on the clinical course of RBO and AD in children, depending on the variants of the ADRB2 gene polymorphism. The efficacy of salbutamol in children with RBO (n=85) and BA (n=56) was evaluated depending on the distribution of allelic and genotypes of the ADRB2 Arg16Gly and Gln27Glu genes by the degree of bronchial reversibility (BO) during spirometry.

3 Research results

Children with RB were admitted to hospital treatment at different times from the onset of the disease. Most of them were hospitalized on 4-5 days after the onset of the disease – 44.5 %, on 1-3 days-29.3 % and later than 5 days-26.2 % of children. At a later stage, patients who received treatment independently at home and were under the supervision of local doctors, receiving outpatient treatment, applied. It was noted that among children aged 3 to 6 years, the first episode of SBD was less likely to occur in patients with RB (26.8%) compared to the BA group (36.8%). In children with AD, a study of the prevalence of primary AD in children showed the highest occurrence at the age of less than 3 years (47.4%). In this regard, it can be argued that children with an early primary manifestation of SBS are at risk for the formation of a recurrent course of obstructive bronchitis. We analyzed the prevalence of primary bronchial obstruction (PO) in the examined children, depending on their age.

In patients with recurrent bronchitis, the first episode of bronchial obstruction was significantly less frequent in children aged 3 to 6 years (26.8%) versus 65.2 % of children under 3 years ($p<0.05$). The results of our observations showed that most often the first signs of BO were observed on the second or third day after the onset of viral infection or after hypothermia of the child. We noted that relapses of SBS were more often observed in patients with a history of frequent acute respiratory viral infections 7 or more times a year, while in the healthy group the number of episodes was less than 4 times a year. The distribution of children by gender in the examined groups of children had different signs. Among children with RB, the largest number of children belonged to the male sex (64.0% vs. 35.4 %, $p<0.05$), while in the group of OBS there was a small difference (40.0% vs. 49.5%).

We analyzed the number of exacerbations of RB during 1 year, depending on age. It was noted that in children of RB, occurring with SBO, up to 3-4 exacerbations per year were observed more often in the group of children from 3-6 years (30.4%). At the same time, the greatest number of children with exacerbations more than 4 times a year was observed in the group of children aged 6-10 years (Figure 1).

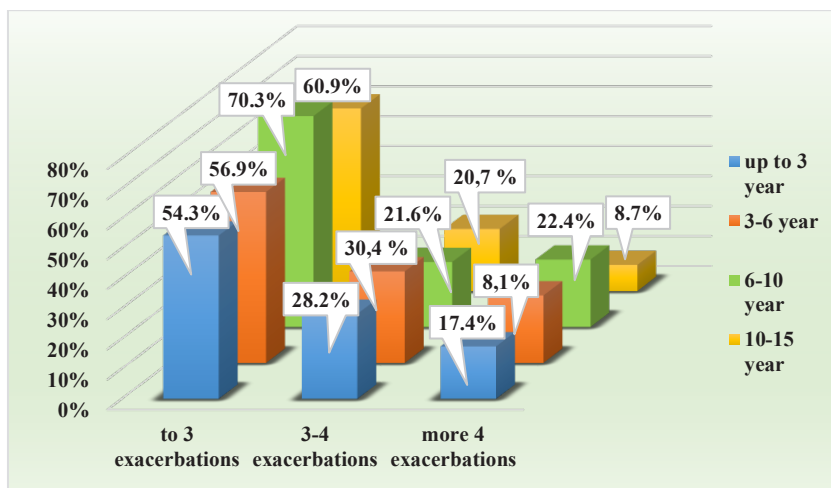


Fig. 1. Number of exacerbations during 1 year depending on age.

More than 4 exacerbations per year were observed most often in the group of children aged 10-15 years (22.4%). Frequent relapses of SBT in children aged 3-6 years are caused by insufficient immunological status and physiological characteristics of the child's body.

The results of the study showed that when studying the rs1042713 locus (Arg16Gly) in the main group, the frequency of Arg(A) alleles is dominant and occurs significantly higher compared to the G allele (61.4% vs. 38.6%, respectively; $\chi^2=8.23$; $p=0.001$). At the same time, among the main group, the carrier of the Gly (G) allele was found with a higher frequency compared to the control group (38.6% vs. 23.6%, $\chi^2=8.23$; $p=0.001$). The calculated relative chance of the presence of this allele in patients compared to the control was $R = 2.04$ with 95% $CI=1.25-3.31$. The results of studies of the frequency of distribution of alleles and genotypes of the Gln27Glu locus of the ADRB2 gene in the main group of children showed: the frequency of the Gln(C) allele was higher compared to the prevalence of Glu(G) (61.8% vs. 38.2%; $p=0.001$); the frequency of the A/A genotype was 2 times higher in the control group compared to from the main group of patients (66.7% vs. 36.0%, respectively $\chi^2=15.02$; $P=0.001$; $OR=0.28$; 95% $CI=0.148-0.534$); among the genotypes, the largest number belongs to the carrier of the heterozygous genotype A/G (51.7%), while the mutacin genotype G /G was found much less frequently (12.4%). At the same time, the G/G mutacin genotype of the Gln27Glu locus of the ADRB2 gene is observed significantly more often in the main group of patients than in healthy ones (12.4% vs. 4.2 %, respectively $\chi^2=3.4$; $P=0.07$; $RR =2.97$; $OR=3.24$; 95% $CI=0.92-11.4$).

In the group of children with RBO, carriers of the Gln27Glu locus of the ADRB2 gene were found (Figure 2): low efficacy of salbutamol in the greatest amount in carriers of the mutational genotype G/G ($45.0\pm 11.1\%$) and heterozygous genotype A/A ($35.0\pm 10.6\%$) in relation to the genotype A/A(20.0 ± 8.9) ($p<0.05$). Comparative analysis showed: high efficiency of the drug salbutamol in carriers of the Arg16Gly A/A locus more often in children with AD $66.6\pm 10.3\%$ compared to patients with RBO- $43.5\pm 6.3\%$; low efficiency was found significantly less often in representatives of the Arg16Gly G/G locus in the group with RB(56.5 ± 10.3) than in patients with AD(71.4 ± 7.6) ($p<0.05$).

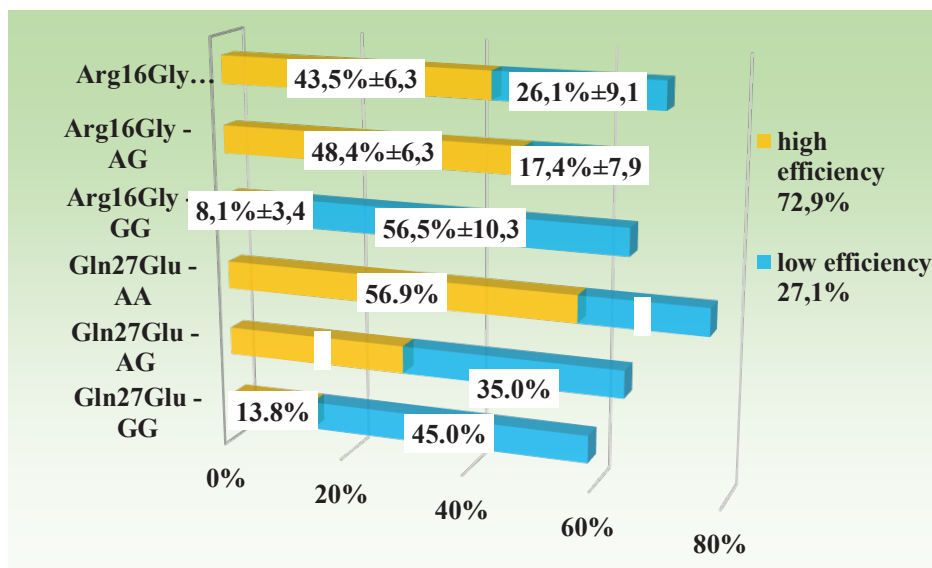


Fig. 2. Polymorphism of Arg16Gly and Gln27Glu of the ADRB2 gene with therapeutic response to beta-agonists in children with RBO.

Among the patients with low drug efficacy, there were children with severe FVD disorders, including RB (72.3%) carriers of the Arg16Gly G/G locus and BA (47.5%) carriers of the Gln27Glu G/G ADRB2 gene locus. Thus, identification of carriers of clinically significant variants of the ADRB2 gene in children with BO will help avoid ineffective treatment and serve as a basis for prescribing alternative therapy.

Results of spirometry and provocation testing in a group of children with AD in carriers of the Gln27Glu locus of the ADRB2 gene, the effectiveness of salbutamol was already found in 26(30.6%) children, while in 59 (64.4%) the effectiveness was low (Table 1).

Table 1. Gln27Glu polymorphism of the ADRB2 gene with therapeutic response to beta-agonists in children with AD, %.

ADRB2 Gene	Genotype	Drug efficacy in patients with in AD patients	
		High efficacy 26(30.6%)	Low efficiency 59 (64.4%)
		M+m,%	M+m,%
Gln27Glu	AA	7(26.9±8.7)	19(32.2±6.1)
	AG	17(65.4±9.3)*	12(20.3±5.2)
	GG	2(7.7 ± 5.2)*	28(47.5±6.5)

Note: * - significant difference ($p < 0.05$) on the t-test in relation to the group with low efficiency

In children with asthma, high efficacy of salbutamol was found significantly more often in carriers of the Gln27Glu ADRB2 gene of the heterozygous A/G genotype (65.4%) versus A/A(26.9%) and G/G(7.7%) ($P < 0.05$). Low efficacy of salbutamol was found in the greatest amount in carriers of the mutational G/G genotype (47.5%) and the homozygous A/A genotype (32.2%) of the Gln27Glu locus of the ADRB2 gene ($P < 0.05$). Consequently, in children with asthma, carriers of the heterozygous A/G genotype of the Gln27Glu locus

of the ADRB2 gene have a high efficiency of therapy, while representatives of the G/G and A/A genotypes have a low efficiency of using salbutamol in therapy.

Thus, in the group of children with RBO, carriers of the heterozygous A/G genotype of the Arg16Gly locus Arg16Gly and the Gln27Glu locus of the ADRB2 gene showed high efficacy of salbutamol therapy as a bronchodilator. At the same time, children with RBO who are representatives of the G/G genotype have a low efficiency of using the drug. In children with AD, the change in functional activity is associated with a polymorphism of the Gln27Glu locus of the Gln27Glu gene locus and is associated with a low therapeutic response to β 2-agonists in children with the G/G mutation genotype and a good effect in carriers of the homozygous variant with the A/A genotype. It should be noted, that long-term use of beta-2 agonists can lead to loss of control over asthma, a decrease in peak expiratory velocity, and prolonged exacerbations of asthma.

The role of genetic factors in the development of bronchopulmonary diseases (BPS), in particular in children with recurrent bronchial obstruction, is one of the least studied problems. The research results obtained by many scientists are mainly devoted to AD and are contradictory: some studies have determined the significance of the influence of polymorphic alleles of the ADRB2 gene in the pathogenesis of AD, as well as in the formation of patients' response to therapy with β 2-agonists, while others have shown that these polymorphic variants of the ADRB2 gene are not associated with AD. According to the studies of some scientists, a comparison of the results on the effectiveness of therapy for exacerbations of asthma in children, depending on the severity of the course, as well as the features of the genotype of the Arg16Gly ADRB2 locus polymorphism, showed that patients with the Gly16Gly locus have a rapid decrease in the sensitivity of β 2-adrenergic receptors to short-acting β 2-agonists. In this regard, scientists have proved that genetic determinism can be responsible for 60-80% of variations in the response to a number of anti-asthmatic drugs (Banadyga N. V., Voloshin S. B., 2016).

Observations showed that in both groups of children with RB and BA with high drug efficacy, the majority were children with mild and moderate BO, while among patients with low efficacy, children with a pronounced degree were found. In children with high efficacy, provocation with salbutamol increased FEV1 and bronchial reversibility by 15% or more. The group of children with low efficacy included children who had been using salbutamol for a long time. Thus, identification of carriers of clinically significant variants of the ADRB2 gene in children with bronchial obstruction will help avoid ineffective treatment and serve as a basis for prescribing alternative therapy.

Analysis of the review of domestic and foreign literature has shown that there are inter-population contradictions in the results of studies of polymorphism of alleles and genotypes of the ADRB2 gene. In this regard, further research of pharmacogenetics for the personalization of therapy in children is timely and relevant today [8, 9, 10].

4 Conclusions

Recent advances in genomic technologies have improved the understanding of the pathogenesis of RBO and AD and have made it possible to better characterize the response and necessity of drugs based on the individual gene pool of an organism. Existing individual polymorphisms of the ADRB2 gene lead to changes in the amino acid sequence of the β 2-adrenergic receptor, which leads to a violation of its functional properties. This phenomenon entails the absence of a bronchodilation effect or adverse side effects.

In the group of children with RBO, carriers of the Arg16Gly locus showed high efficacy (72.9%) of salbutamol in most cases in carriers of the A/G and A/A genotypes,

while low efficacy (27.1%) was found in carriers of the G/G genotype (56.5%) of the ADRB2 gene. Carriers of the Gln27Glu locus showed low drug efficacy in the G/G genotype (45.0%). Among patients with ASTHMA, low efficacy was observed more often among carriers of the Arg16Gly locus in the G/G gene (72.3%), compared with carriers of the Gln27Glu locus of the ADRB2 gene in the G/G gene (47.5%). At SNP the same time, SNP substitutions of the A46G and C79G nucleotides affected the synthesis of partner proteins, and the amino acid substitution in proteins was localized near binding to ligands and reduced the effectiveness of salbutamol to the corresponding mutant receptor. The data obtained allow for an individual approach in the treatment of children with RBO and BA.

Knowledge of the genotype for polymorphic variants of the ADRB2 gene will make it possible to assess one of the predisposition factors and the effectiveness of therapy in RBO and AD. Our studies of ADRB2 gene polymorphism in children with bronchopulmonary diseases expand our understanding of the possibilities of improving the effectiveness of pharmaceutical therapy and personalization.

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