ADRB2 gene polymorphisms and salbutamol responsiveness in Serbian children with asthma

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ABSTRACT

Background: Inhaled beta-2 adrenergic receptor (β2-AR) agonists are mainstay of asthma therapy. The β2-AR protein is encoded by the ADRB2 gene and variants within this gene can have significant consequences for modulating the response to asthma therapy. Methods: This cross-sectional study performed at the University Children's Hospital in Belgrade, included 54 children with asthma. The subjects were genotyped for ADRB2 +46A>G (Arg16Gly, rs1042713) and +79C>G (Gln27Glu, rs1042714) polymorphisms and the association with asthma severity and response to inhaled salbutamol was examined. Results: In Serbian asthmatic children, allele +46A was detected with a frequency of 41.7% and allele +79G was detected with a frequency of 23.1%. Allele +46G was found to be associated with a better response to inhaled salbutamol ($p<0.05$) and with mild form of asthma ($p<0.05$).

Conclusions: Polymorphism ADRB2 +46A>G may be a determinant of asthma severity and response to salbutamol in children with asthma. We did not find association of +79C>G polymorphisms with the asthma severity and bronchodilator response to inhaled salbutamol. The results of this study can be potentially useful for personalization of asthma treatment.

Key words: ADRB2 gene, Asthma, bronchodilatator response, polymorphism

INTRODUCTION

Inhaled β2-AR agonists are drugs that form the basis of asthma therapy[1]. They are administered periodically or continuously, and during disease exacerbations. The absence of response to the applied therapy and the occurrence of severe exacerbation of the disease require admission to the hospital, and in the most severe cases, to the pediatric intensive care unit. Therefore, prediction of response to specific therapy is of great importance in the treatment of asthma exacerbations in children[2].

The β2-AR is encoded by the ADRB2 gene and its variations can significantly modulate the response to asthma therapy[3]. The ADRB2 gene is located on chromosome 5q31-q32, in a region associated with asthma. Several polymorphisms in the ADRB2 gene have been described[4]. Beta-2 receptors are present in the respiratory tract, especially in the smooth muscle cells. The most important clinical effect of activation of β2-AR by its agonists is relaxation of the lung smooth muscles. Chronic exposure to the agonists leads to a significant reduction in the number of β2-AR on the surface of the cell[4].

The two most common polymorphisms in the ADRB2 gene are +46A>G (Arg16Gly, rs1042713) and +79C>G (Gln27Glu; rs1042714)[5]. There is evidence that +46A>G and +79C>G polymorphisms alter the functioning of the receptor, leading to down-regulation of β2-AR and thereby induce a resistance to the effect of β2-agonists[6]. A significant correlation was found between the positive therapeutic response to inhaled β2-agonists in children with asthma and +46AA genotype in comparison with +46AG and +46GG genotypes[7,8]. Polymorphism +46A>G can be an important factor in the overall genetic risk of developing asthma, while polymorphism +79C>G is described as a risk factor for asthma in adults in some ethnic groups[9-11]. A previous study in Serbian population has shown that adult carriers of allele +79C and genotype +79CC are at increased risk of developing asthma[12].

The aim of this study was to analyze the incidence of +46A>G and +79C>G polymorphisms/variants in Serbian children with asthma, and to investigate their influence on the severity of the disease and the response to inhaled β2-agonists.

MATERIAL AND METHODS

Subjects

The cross section study was conducted at the University Children's Hospital in Belgrade during the period from October 2016 to May 2017 and included 54 children Serbian ethnicity with asthma (6-18 years old). The diagnosis of asthma and disease severity were set in accordance with the Global Initiative for Asthma (GINA) 2016 guidelines. Severity is assessed retrospectively from the level of treatment required to control symptoms and exacerbations. Subjects were classified into three subgroups: mild, moderate and severe asthma. Children with asthma who had some other illnesses that may affect lung function were excluded from the study, as well as children with any chronic disease other than asthma such as bronchopulmonary dysplasia, tracheobronchial malacia, congenital heart disease. The allergic classification is defined by co-occurrence of positive prick skin tests for inhalation allergens, increased serum IgE levels, more than 4% eosinophils in peripheral blood in the absence of parasites (three negative stool analyzes for parasites three months prior to testing) and co-occurrence of atopic dermatitis as a cumulative nature and which was not present during the study period nor in the previous 12 months. Neither of the subjects had acute exacerbation of asthma. The study was approved by the Ethical Committee of the Faculty of Medicine University of Belgrade (decision No: 29/III-30, 28/3/2016).

Detailed anamnesis, physical examination and lung function tests were performed in all subjects. Pulmonary function tests were performed using a spirometric unit GANSHORN-

SCHILLER SpiroJet (140091, Germany). Spirometry was performed according to the standards of the American Thoracic Society[13]. Spirometric measurements included expiratory volume in the first second (FEV1), forced vital capacity (FVC), and peak expiratory flow (PEF). The results of pulmonary function tests are expressed as a percentage of predicted values. Children with asthma received an instruction to stop the systemic bronchodilator or corticosteroid therapy for 72 hours prior to testing, as well as the use of short-acting β2-agonists 12 hours prior to testing. Response to a short-acting bronchodilator was assessed by applying a single dose of salbutamol (0.15mg/kg) using the Omron Nebuliser (NE-C28P-E, Japan), and by performing the lung function test before and 15 minutes after administration of nebulized salbutamol. The response to salbutamol was measured by recording a change in the percentage of FEV1 obtained before and after salbutamol administration[13].

Genotyping of ADRB2 +46G>A and +79C>G polymorphisms/variants

Genomic DNA was extracted from peripheral blood using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific). The presence of +46A>G and +79C>G polymorphisms /variants was determined by direct sequencing of PCR products obtained with the following primers: 5'-CTGAATGAGGCTTCCAGGCGT-3' and 5'-ACAATCCACACCATCAGAAT-3'. The PCR was conducted in a 50µL reaction mixture containing: 1X KAPA Taq Buffer A (KAPA Biosystems), 0.3mM $MgCl_2$, 0.2mM each dNTP, 10pmol of each primer, 2U of KAPA Taq DNA Polymerase (KAPA Biosystems) and approximately 300ng of DNA. The amplifications were performed as follows: initial denaturation for 5min at 94°C; 35 cycles consisting of 30sec at 94°C, 30sec at 60°C and 30sec at 72°C; final extension for 10min at 72°C. The obtained PCR fragments (584bp long) were purified with PureLink PCR Purification Kit (Thermo Fisher Scientific) and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and the same primers as for the amplification. Products of

sequencing reaction were analyzed by capillary electrophoresis on 3130 Genetic Analyzer (Applied Biosystems) and Sequencing Analysis Software (Applied Biosystems).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences 20.0 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as percentages and means±standard deviation (SD) for continuous variables and percentages for categorical variables. To test the normality of parameters one sample Kolmogorov-Smirnov test was used. Differences between groups for categorical data were tested by χ^2 analysis, while for continuous data Independent Samples Mann Whitney U test and Kruskal Wallis test were used. Hardy-Weinberg equilibrium was analyzed by the Arlequin software. P value less than 0.05 was considered statistically significant.

RESULTS

The study has included a group of 54 asthmatic children (6-18 years old, 22 girls and 32 boys), whose demographic and clinical characteristics are presented in Table 1. All asthmatic children were Serbian ethnic group. The patients were divided in accordance with GINA 2016 guidelines into three groups according to the asthma severity: mild, moderate and severe asthma. There was no association of age or gender with asthma severity in this group of patients. All patients were genotyped for ADRB2 gene polymorphisms +46A>G and +79C>G by direct DNA sequencing. Allele +46A was detected with a frequency of 41.7%, while allele +79G was detected with a frequency of 23.1% (Table 1).

Table 1. Demographic and clinical characteristics of patients and ADRB2 genotype distribution

Age, years (mean±SD)	11.9±2.7
Male/female, %	59.3/40.7
FEV1 before salbutamol, % (mean±SD)	87.1±11.3
FEV1 after salbutamol, % (mean±SD)	98.8±9.1
Asthma severity, n (%)	
mild	23 (42.6)
moderate	14 (25.9)
severe	17 (31.5)
Atopic dermatitis, n (%)	79.6
Serum IgE, IU/mL (mean±SD)	143.3±49.5
Blood eosinophils, % (mean±SD)	6.3±2.1
Alleles, n (%)	
+46A>G	
A	45 (41.7)
G	63 (58.3)
+79C>G	
C	83 (76.9)
G	25 (23.1)
Genotypes, n (%)	
+46A>G	
AA	13 (24.1)
GA	19 (35.2)
GG	22 (40.7)
+79C>G	
CC	34 (63.0)
CG	15 (27.8)
GG	5 (9.2)

SD: Standard deviation; FEV1: Forced Expiratory Volume in first second

Response to salbutamol (measured by dFEV1) and severity of the disease were compared between carriers of different ADRB2 genotypes (Table 2). There was no significant difference in response to salbutamol between boys and girls. The presence of +46G allele in the ADRB2 gene correlates with better bronchodilator response to salbutamol (p=0.044). This allele was also associated with mild form of the disease (p=0.010). No significant association was found between the +79C>G polymorphism and asthma severity (p=0.955) or better bronchodilator response to salbutamol (p=0.316). In the analysis of ADRB2 gene polymorphisms distribution in respect to the clinical characteristics of asthmatic children no significant association was found between carriers of different genotypes (Table 2).

Table 2. Comparison of response to salbutamol, severity of the disease and clinical characteristics between carriers of different ADRB2 genotypes

+46A>G	AA	GA	GG	P value
dFEV1, % (mean±SD)	9.4±6.2	10.4±5.8	14.4±6.1	**0.044**
Asthma severity, n (%)				
mild	1 (4.3)	10 (43.5)	12 (52.2)	
moderate	8 (57.2)	3 (21.4)	3 (21.4)	**0.010**
severe	4 (23.5)	6 (35.3)	7 (41.2)	
Atopic dermatitis, n (%)	12(27.9)	13(30.2)	18(41.9)	0.244
Eosinophils in peripheral blood, % (mean±SD)	6.0±2.5	6.3±1.7	6.4±2.3	0.600
Serum IgE, IU/mL (mean±SD)	152.4±47.5	128.0±53.3	148.9±49.9	0.440
+79C>G	CC	CG	GG	P value
dFEV1, % (mean±SD)	11.6±5.9	11.7±7.2	13.2±7.5	0.955
Asthma severity, n (%)				
mild	12 (52.2)	8 (34.8)	3 (13.0)	
moderate	12 (85.7)	2 (14.3)	0 (0)	0.316
severe	10 (58.8)	5 (29.4)	2 (11.8)	
Atopic dermatitis, n (%)	26(60.5)	13(30.2)	4(9.3)	0.716
Eosinophils in peripheral blood, % (mean±SD)	5.9±1.9	7.1±1.9	6.2±3.4	0.089
Serum IgE, IU/mL (mean±SD)	141.2±45.9	134.4±64.7	172.8±42.4	0.543

dFEV1: Change in Forced Expiratory Volume in first second after administration of salbutamol; SD: Standard deviation

The distribution of observed genotypes for +46A>G and +79C>G polymoprhisms were consistent with Hardy-Weinberg equilibrium (p=0.050 and p=0.359, respectively). The three allele combinations were identified in our group of patients: +46A/+79C (41.7%), +46G/+79C (35.2%) and +46G/+79G (23.1%). The response to salbutamol and asthma severity were compared between carriers of these allele combinations. We found no statistically significant difference in severity of asthma. In the group of children with +46A> G polymorphism and severe asthma, 41.2% of cases are carriers of the +46GG genotype that is associated with the best bronchodilator response. In patients with +46A/+79C combination, the response to salbutamol was significantly worse than in patients with the other two allele combinations (dFEV1 9.4±6.2% vs 14.4±6.1%, p=0.026). There was no significant difference in response of homozygous and heterozygous carriers of +46A allele.

DISCUSSION

The main finding of the study is the association of the +46G allele in the ADRB2 gene with mild form of asthma and better response to salbutamol. The finding that carriers of ADRB2 +46G allele tend to develop mild form of asthma is in correlation with findings of other studies. In meta-analysis of 28 studies, authors concluded that carriers of +46AA genotype have higher risk of developing severe and nocturnal asthma than carriers of +46GG genotype[14]. On the other hand, Egyptan study in school-age children with asthma had shown an association of +46GA genotype with severe asthma[15].

Genetic variation in ADRB2 gene may have important effect on modulating responses to inhaled β2-agonists as the mainstay of asthma therapy. Previous studies have dealt with +46A>G and +79C>G polymorphisms and their impact on differential agonist-stimulated down-regulation of the receptor in transfected cells, including airway smooth muscle cells in humans and which can be associated with a different bronchodilator response to β2-agonists[10,11,16,17,18,19]. In our study, the presence of +46G allele in the ADRB2 gene was associated with a better response to the bronchodilator effect of inhaled short-acting β2-agonists (salbutamol). As noted above, our study showed the association of this genotype + 46GG with the phenotype of mild asthma. However, it was noticed in the subgroup of asthmatic children with + 46A> G polymorphism, the highest percentage of children with severe asthma are the carriers of the +46GG genotype. Since this genotype is associated with the best response to bronchodilators, we can expect a good clinical response to salbutamol in this subgroup of patients (severe asthma phenotype that are carriers + 46GG genotype). Monitoring of FEV1 following administration of salbutamol as a response measure for bronchodilator use is the most objective, immediate and most frequently studied pulmonary function parameter in the previous trials[13]. The relationship between ADRB2 genotypes and response to inhaled β2-agonists is controversal and discordant findings have been reported. In early studies, authors showed better

bronchodilator response in children with the +46GG genotype[20]. Later, several studies showed similar results[21,22]. The meta-analysis showed a significant association between better therapeutic response to inhaled β2-agonist and the +46GG genotype[6]. However, a few studies have shown opposite results. Carroll found that children with +46AA genotype had a more rapid response to inhaled β2-agonist[23]. Examining ethnic differences, Choudhary showed better salbutamol response in Mexican children with +46AA genotype but not in Puerto Ricans ethnic group[24]. The only study conducted in Serbia included adults and showed a better bronchodilator response in carriers of the +79C allele in asthmatics younger than 50 years[12]. Some larger studies have shown the absence of association of genetic variation of ADRB2 and the response to inhaled β2-agonist[25,26]. Several reasons may explain the discordant results reported by different authors. The studies were not coherent in terms of the age of the subjects and the severity of their illness. Authors had also used different β2-agonist and different outcome measures to assess drug responsiveness[27]. Some authors studied the associations of certain haplotypes with therapeutic response to a particular drug and made a conclusion by which different results can be explained by specific combinations of polymorphisms that are most commonly inherited together, rather than individual polymorphisms.

The main limitation of our study is relatively small number of subjects. On the other hand, we have applied strict criteria for the selection of subjects to avoid the results being influenced by any of the non-genetic factors. Children with asthma and other associated illness were not included in the study. The study included children of Serbian ethnicity, although in Serbia there are members of another ethnic community (eg Hungarian, Croatian, Roma). We cannot exclude the possibility that adjacent genes or other polymorphisms within promoter and coding regions of the ADRB2 gene can contribute to the results. The fact is that there are multitude of polymorphisms of ADRB2 gene and certain set of alleles are more likely to be inherited together as a block. The protective effect of one polymorphism may mask the adverse

effect of another polymorphism when inherited together. Hence, association of ADRB2 haplotypes with bronchodilator response may be more relevant than single polymorphisms. For the extrapolation of these results in our population, a larger sample is needed and ethnicity should be taken into consideration. The study covered the acute use of short-acting bronchodilators and the results can not be correlated in light of the effects of their long-term use or, possibly, effects of long-acting bronchodilators.

To conclude, the polymorphism +46A>G of ADRB2 gene may be a determinant of asthma severity and +46G allele is a potential predictive marker of response to salbutamol in Serbian children with asthma. The results of our study can help establish future research strategies regarding the role of ADRB2 gene in asthma and response to therapy, and are of potential use for personalized asthma treatment in children.

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