

ADRB2 GENE POLYMORPHISMS AND SALBUTAMOL RESPONSIVENESS IN SERBIAN CHILDREN WITH ASTHMA

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ABSTRACT

Inhaled β_2 adrenergic receptor (β_2 -AR) agonists are the mainstay of asthma therapy. The β_2 -AR protein is encoded by the *ADRB2* gene and variants within this gene can have significant consequences for modulating the response to asthma therapy. 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. 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 ($p < 0.05$). Polymorphism *ADRB2* +46A>G may be a determinant of asthma severity and response to salbutamol in children with asthma. We did not find any 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.

Keywords: *ADRB2* gene; Asthma; Bronchodilator response; Polymorphism.

INTRODUCTION

Inhaled β_2 adrenergic receptor (β_2 -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 requires admission to the hospital, and in the most severe cases, to the pediatric intensive care unit. Therefore, prediction of response to a specific therapy is of great importance in the treatment of asthma exacerbations in children [2].

The β_2 -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]. The β_2 receptors are present in the respiratory tract, especially in the smooth muscle cells. The most important clinical effect of activation of β_2 -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 β_2 -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 β_2 -AR and thereby induce a resistance to the effect of β_2 -agonists [6]. A significant correlation was found between the positive therapeutic response to inhaled β_2 -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 the 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

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children with asthma, and to investigate their influence on the severity of the disease and the response to inhaled β 2-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 of 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 was 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 and/or congenital heart disease. The allergic classification was defined by co-occurrence of positive prick skin tests for inhalation allergens, increased serum IgE levels, more than 4.0% eosinophils in peripheral blood in the absence of parasites (three negative stool analyzes for parasites 3 months prior to testing) and co-occurrence of atopic dermatitis as a cumulative nature and which was not present during the study period or in the previous 12 months. None of the subjects had acute exacerbation of asthma. The study was approved by the Ethics 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; Ganshorn Medizin Electronic GmbH, Niederlauer, Germany). Spirometry was performed according to the standards of the American Thoracic Society [13]. Spirometric measurements included forced expiratory volume in the first second (FEV1), forced vital capacity (FVC) and peak expiratory flow (PEF). The results of pulmonary function tests were 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 β 2-agonists 12 hours prior to testing. Response to a short-acting bronchodilator was assessed by applying a single dose of salbutamol (0.15 mg/kg) using the Omron Nebuliser (NE-C28P-E; Omron Healthcare Group, Kyoto, Japan), and by performing the lung function test before and 15 min. 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, Waltham, MA, USA). The presence of +46A>G and +79C>G polymorphisms/variants was determined by direct sequencing of polymerase chain reaction (PCR) products obtained with the following primers: 5'-CTG AAT GAG GCT TCC AGG CGT-3' and 5'-ACA ATC CAC ACC ATC AGA AT-3'. The PCR was conducted in a 50 μ L reaction mixture containing: 1 \times KAPA Taq Buffer A (KAPA Biosystems, Waltham, MA, USA), 0.3 mM MgCl₂, 0.2 mM each dNTP, 10 pmol of each primer, 2U of KAPA Taq DNA Polymerase (KAPA Biosystems) and approximately 300 ng of DNA. The amplifications were performed as follows: initial denaturation for 5 min. at 94 °C; 35 cycles consisting of 30 seconds at 94 °C, 30 seconds at 60 °C and 30 seconds at 72 °C; final extension for 10 min. at 72 °C. The obtained PCR fragments (584 bp long) were purified with PureLink PCR Purification Kit (Thermo Fisher Scientific) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and the same primers as for the amplification. Products of sequencing reactions were analyzed by capillary electrophoresis on an ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and sequencing analysis software (Applied Biosystems).

Statistical Analyses. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as percentages and means \pm standard deviation (SD) for continuous variables and percentages for categorical variables. To test the normality of the 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 the Kruskal-Wallis test were used. Hardy-Weinberg equilibrium was analyzed by the Arlequin software. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

The study 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 of a Serbian ethnic group. The

patients were divided in accordance with GINA 2016 guidelines into three groups according to their asthmatic severity: mild, moderate and severe. There was no association of age or gender with asthma severity in this group of patients. All patients were genotyped for the *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).

Response to salbutamol (measured by recording the change in the percentage of FEV1 before and after salbutamol administration and expressed as a percentage difference in FEV1 (dFEV1) after and before salbutamol administration) 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 the +46G allele in the *ADRB2* gene correlates with better bronchodilator response to salbutamol ($p = 0.044$). This allele was also associated with a 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 the *ADRB2* gene polymorphism distribution in respect to the clinical characteristics of asthmatic children, no significant association was found between carriers of the different genotypes (Table 2).

The distribution of observed genotypes for +46A>G and +79C>G polymorphisms were consistent with the Hardy-Weinberg equilibrium ($p = 0.050$ and $p = 0.359$,

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

Age in years (mean±SD)	11.9±2.7
Gender (%)	M: 59.3; F: 40.7
FEV1 before salbutamol (%) (mean±SD)	87.1±11.3
FEV1 after salbutamol (%) (mean±SD)	98.8±9.1
Asthma severity: <i>n</i> (%)	
mild	23 (42.6)
moderate	14 (25.9)
severe	17 (31.5)
Atopic dermatitis: <i>n</i> (%)	43 (79.6)
Serum IgE (IU/mL) (mean±SD)	143.3±49.5
Blood eosinophils (%) (mean±SD)	6.3±2.1
Allele +46A>G: <i>n</i> (%)	
A	45 (41.7)
G	63 (58.3)
Allele +79C>G: <i>n</i> (%)	
C	83 (76.9)
G	25 (23.1)
Genotype +46A>G: <i>n</i> (%)	
AA	13 (24.1)
GA	19 (35.2)
GG	22 (40.7)
Genotype +79C>G: <i>n</i> (%)	
CC	34 (63.0)
CG	15 (27.8)
GG	5 (9.2)

SD: standard deviation; M: males; F: females;

FEV1: forced expiratory volume in the first second.

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

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	<i>p</i> Value
dFEV1 (%) (mean±SD)	9.4±6.2	10.4±5.8	14.4±6.1	0.044
Asthma severity: <i>n</i> (%)				0.010
mild	1 (4.3)	10 (43.5)	12 (52.2)	
moderate	8 (57.2)	3 (21.4)	3 (21.4)	
severe	4 (27.9)	6 (35.3)	7 (41.2)	
Atopic dermatitis: <i>n</i> (%)	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	<i>p</i> Value
dFEV1 (%) (mean±SD)	11.6±5.9	11.7±7.2	13.2±7.5	0.955
Asthma severity: <i>n</i> (%)				0.316
mild	12 (52.2)	8 (34.8)	3 (13.0)	
moderate	12 (85.7)	2 (14.3)	0 (0.0)	
severe	10 (58.8)	5 (29.4)	2 (11.8)	
Atopic dermatitis: <i>n</i> (%)	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 the first second after administration of salbutamol; SD: standard deviation.

significant difference in severity of asthma. In the group of children with the +46A>G polymorphism and severe asthma, 41.2% of cases were carriers of the +46GG genotype that is associated with the best bronchodilator response. In patients with the +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. $14.4 \pm 6.1\%$, $p = 0.026$). There was no significant difference in response of the homozygous and heterozygous carriers of the +46A allele.

DISCUSSION

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

Genetic variation in the *ADRB2* gene may have important effect on modulating responses to inhaled β_2 -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 β_2 -agonists [10,11,16-19]. In our study, the presence of the +46G allele in the *ADRB2* gene was associated with a better response to the bronchodilator effect of inhaled short-acting β_2 -agonists (salbutamol). As noted above, our study showed the association of this +46GG genotype with the phenotype of mild asthma. However, it was noticed in the subgroup of asthmatic children with the +46A>G polymorphism, the highest percentage of children with severe asthma were the carriers of the +46GG genotype. As this genotype was associated with the best response to bronchodilators, we can expect a good clinical response to salbutamol in this subgroup of patients (the severe asthma phenotype who are carriers of the +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 β_2 -agonists was controversial and discordant findings had 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 β_2 -agonist and the +46GG genotype [6]. However, a few studies have shown opposite results. Carroll [23] found that children with the +46AA genotype had a more rapid response to inhaled β_2 -agonist. Examining ethnic differences, Choudhry *et al.* [24] showed better salbutamol response in Mexican children with the +46AA genotype but not in the Puerto Rican ethnic group. The only study conducted in Serbia included adults and showed a better bronchodilator response in carriers of the +79C allele in asthmatic patients younger than 50 years [12]. Some larger studies have shown the absence of association of genetic variation of *ADRB2* and the response to inhaled β_2 -agonist [25,26]. Several reasons may explain the discordant results reported by different authors. The studies were not coherent in terms of age of the subjects and the severity of their illness. Authors had also used different β_2 -agonists 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 was the relatively small number of subjects. On the other hand, we had 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 other ethnic communities (*e.g.*, Hungarian, Croatian, Roma). We cannot exclude the possibility that adjacent genes or other polymorphisms within the promoter and coding regions of the *ADRB2* gene can contribute to the results. The fact is that there are a multitude of polymorphisms of the *ADRB2* gene and a 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 cannot 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 the *ADRB2* gene may be a determinant of asthma severity and the +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 the *ADRB2* gene in asthma and response to therapy, and are of potential use for personalized asthma treatment in children.

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Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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