## Original article

# Effects of β2-adrenergic receptor polymorphisms on asthma severity and response to salbutamol in Egyptian children

***Background:*** *Several polymorphisms of the β2-adrenergic receptor (ADRB2) gene have been identified. There is mounting evidence that these polymorphisms are associated with significant variability in response to bronchodilator therapy and thus in severity and duration of asthmatic symptoms.* ***Objectives:*** *to assess the frequency of ADRB2 polymorphisms at codon 16 in Egyptian asthmatic children and to study the association of these polymorphisms with asthma severity and response to inhaled salbutamol.* ***Methods:*** *This case-control study was conducted at pulmonology unit, Zagazig University children's hospital during the period from December 2010 to December 2011. One hundred children (50 asthmatics and 50 controls) were enrolled into the study. For all study population, detailed history taking, systematic physical examination, chest x-ray, pulmonary function testing and ADRB2 genotyping were performed.* ***Results:*** *There was a significant increase in frequencies of Arg/Gly and Gly/Gly genotypes among asthmatic children in comparison with healthy controls (OR = 7.9; CI: 0.94-67.4, P<0.05 and OR= 4.5; CI: 0.91-22.7, P<0.05 respectively). On the other hand, there was a lower frequency of Arg/Arg genotype in asthmatic children than that in healthy controls (OR = 0.14; CI: 0.04-0.55, P<0.05). Moreover, statistical analysis revealed association of Arg/Arg genotype with mild asthma (OR = 5.77; 95% CI: 1.55-21.5, P<0.05) and association of Gly/Gly genotype with moderate/severe asthma (OR=0.057; 95% CI: 0.006-0.516, P<0.05). However, no difference in distribution of Arg/Gly genotypes among mild and moderate/severe asthmatics (OR = 0.79; 95% CI: 0.156-3.99, P>0.05). Regarding bronchodilator responsiveness, Gly/Gly and Arg/Gly genotypes were associated with reduced response, while Arg/Arg genotype was associated with favorable response to nebulized salbutamol.*
***Conclusion:*** *Polymorphisms of ADRB2 at codon 16 may be a determinant of asthma severity and response to salbutamol in Egyptian asthmatic children. Further studies are needed to demonstrate effects of other polymorphisms of ADRB2 gene on these outcomes.*

**Keywords:** Polymorphism; β2-adrenergic receptor (ADRB2) gene; asthma; Egyptian children; bronchodilator therapy.

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## INTRODUCTION

Asthma is a disease characterized by hyper-responsiveness of the airways to various stimuli, which results in airway obstruction that is reversible either spontaneously or as a result of treatment[1]. Asthma prevalence has increased very considerably in recent decades such that it is now one of the commonest chronic disorders in the world[2]. Asthma is estimated to affect 300 million people world-wide, with an expected increase to 400 million worldwide by 2025[3]. In a recent Egyptian study, the overall prevalence of wheezing in the last year was 14.7% and of physician-diagnosed asthma was 9.4%[4].

Asthma aggregates within families and is a complex multifactorial disease with the involvement of environmental and genetic components[5]. Understanding of the genetic basis of asthma may contribute toward identifying better targets for asthma drugs[6].

Inhaled β2-adrenergic agonist medications are the foundation of therapy for acute asthma exacerbation[7]. The β2-adrenergic receptor (ADRB2) protein is expressed on bronchial smooth muscle cells and mediates physiologic responses including bronchodilation, vasodilatation and lipolysis[8]. ADRB2 gene located on chromosome 5q31-32 is one of the candidate genes most consistently identified as being associated with asthma-related phenotypes[9].

Several polymorphisms of ADRB2 have been described in particular at codons 16 Arginine/Glycine and 27 Glutamine /Glutamic acid which alter the receptor function in vitro[10,11]. In adults, genetic variations in this receptor have been linked to asthma severity[12], bronchial hyper-responsiveness[13] and lung function tests[14].

The relationship between ADRB2 genotypes and response to inhaled β2-adrenergic agonists is controversial. Some studies[15-21] have found that the Arg/Arg genotype is associated with reduced response to these medications, whereas others[22-25] have found that the Gly/Gly genotype is associated with reduced response.

Knowledge of the genotype which is associated with favorable response, could significantly affect treatment selection for asthma in children. The current study will focus on the polymorphisms of ADRB2 gene at codon 16 as a previous study[22] found no association between polymorphisms at position 27 and response to inhaled β2-adrenergic agonists in asthmatic children.

So, the objectives of this study were to assess the frequencies of ADRB2 polymorphisms at codon 16 in Egyptian asthmatic children and the effects of these polymorphisms on asthma severity and bronchodilator response to salbutamol.

## METHODS

This case-control study was conducted at pulmonology unit, Zagazig University children's hospital during the period from December 2010 to December 2011. One hundred children were enrolled in the study. They were divided into two groups; Group I and Group II.

Group I (asthmatic group) included 50 children, 26 males and 24 females. Their ages ranged from 5-12 years with a mean value of 6.8 years. The diagnosis of asthma in these patients was based on National Asthma Education and Prevention Program Guidelines[26]. Cases were classified and subgrouped as follow: mild intermittent and mild persistent asthmatics were included into the group of mild asthma and moderate persistent and severe persistent asthmatics were included as the group of moderate/severe asthma. Recruited asthma patients were not in exacerbation state and did not have other concomitant diseases that might affect lung function. Children with any chronic disease (other than asthma) were excluded.

Group II (control group) included 50 healthy children, 26 males and 24 females. Their ages ranged from 5-12 years with a mean value of 7.2 years. Subjects were included as controls only if they reported no history of asthma or allergies, no history or report of having experienced symptoms of coughing, wheezing and shortness of breath in the past 2 years and no other history of lung diseases, chronic illnesses, or medications.

For all study groups, detailed history taking, systematic physical examination, chest x-ray, pulmonary function testing and ADRB2 genotyping were performed. Informed consent was obtained from the children's guardians. The study protocol was approved by the Pediatric Department Ethics Committee of Zagazig University.

**ADRB2 genotyping:**

Three milliliters of venous blood were collected aseptically from every subject in a tube containing EDTA. This blood was incubated at -20°C temperature. After samples were enough, the blood was prepared for DNA extraction and amplification. DNA extraction was done using Axyprep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, USA). DNA amplification was carried out by using polymerase chain reaction (PCR) Master-Mix Gold beads (Bioron, Germany). The nucleotide sequences of the forward and reverse prime used for PCR are:

5'-GCCTTCTTGCTGGCACCCCAT-**3'** and
**5'**-CAGACGCTCGAACTTGGCCATG-**3'**.

Then, for detection of the ADRB2 polymorphisms, restriction fragment assay was done.

**Pulmonary function testing:**

Pulmonary function tests were performed using Lilly Pneumatocho- graph (D-97204 Hochberg, Germany). Spirometry was performed according to the American Thoracic Society standards[27]. Spirometeric measurements included forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and peak expiratory flow (PEF).

Pulmonary function test results were expressed as a percentage of the predicted normal value. Asthmatic children were instructed to stop any systemic bronchodilator or corticosteroid therapy 72 hours before tests and short acting β2-adrenergic agonists were stopped 12 hours before the procedure.

To assess response to a bronchodilator in asthmatic group, one dose of salbutamol (0.15 mg/kg) was administered using ultrasonic nebulizer (eMed A100, Italy) and measurement of lung function was performed before and 15 minutes after salbutamol nebulization. Response to salbutamol was reported as a percent change in FEV1 between baseline and after salbutamol administration. We chose to explore changes in FEV1 after salbutamol

mobilizations as a measure of response to a bronchodilator, because this was the most objective, immediate, and most common endpoint studied by other groups[22].

**Statistical analysis:**

Data were analyzed using Statistical Package for Social Sciences (SPSS), release 16. The quantitative variables were expressed as means and standard deviations. For comparison between two group means, t-test was used. For comparison between three group means, one way ANOVA (analysis of variance) was used. Qualitative variables were expressed by frequency and percentage and compared using chi-square test. Also, odds ratio (OR) and 95% confidence interval (CI) were calculated. For all tests a probability (*P*) less than 0.05 was considered significant and less than 0.001 was considered highly significant.

## RESULTS

The characteristics of the two study groups are shown in table 1. The two groups were similar in age and gender. A highly significant difference was observed in spirometric parameters between asthmatic and healthy children ($P<0.001$). Also, significant difference was observed in genotype distribution between asthmatic and healthy children ($P<0.05$).

Distribution of ADRB2 gnenotypes at codon 16 among asthmatic and non-asthmatic children is illustrated in table 2. There was a significant increase of carriers of Arg/Gly and Gly/Gly genotypes among asthmatic children in comparison to controls (OR = 7.9; CI: 0.94 - 67.4, $P<0.05$ and OR= 4.5; CI: 0.91-22.7, $P<0.05$ respectively). On the other hand, there was a lower frequency of Arg/Arg genotype in asthmatic children than in controls (OR = 0.14; CI: 0.04- 0.55, $P<0.05$).

Relation between polymorph-isms of ADRB2 at codon 16 and asthma severity is shown in table 3. Statistical analysis revealed an association of Arg/Arg genotype with mild asthma (OR= 5.77; 95% CI: 1.55-21.5, $P<0.05$). On other hand, Gly/Gly genotype was less frequent in mild asthma (OR= 0.057; 95% CI: 0.006-0.516, $P<0.05$). However, no difference in distribution of Arg/Gly genotype was noticed among mild and moderate/severe asthmatics (OR=0.79; 95% CI: 0.156-3.99, $P> 0.05$).

Regarding bronchodilator responsiveness, Gly/Gly and Arg/Gly genotypes were associated with reduced response (FEV1 change =11.4% and 9.1%, respectively), while Arg/Arg genotype was associated with favorable response (FEV1change = 17.3%) (Table 4).

**Table 1.** Demographic and clinical data of the study groups.

| Variable | Asthmatic group (n = 50) | Control group (n = 50) | test | P value |
|---|---|---|---|---|
| **Age**( year) **,** Mean ±SD | 6.8±2.54 | 7.2±2.08 | 0.86 | >0.05 |
| **Male gender**, *n* (%) | 26 (52%) | 26 (52%) | 0.0 | >0.05 |
| **Genotype**, *n* (%) | | | | |
| **Arg/Arg** | 35 (70%) | 47 (94%) | | |
| **Arg/Gly** | 7 (14%) | 1(2%) | 9.85 | <0.05 |
| **Gly/ Gly** | 8 (16%) | 2(4%) | | |
| **Spirometric parameters**, Mean±SD | | | | |
| **FEV1 (%)** | 74.78±14.56 | 92.9±1.5 | 8.76 | <0.001 |
| **FVC (%)** | 92.03±8.03 | 99.39±2.26 | 6.24 | <0.001 |
| **PEF (%)** | 75.71±14.28 | 95.20±1.68 | 9.58 | <0.001 |
| **Asthma severity**, *n* (%) | | | | |
| Mild intermittent | 23 (46%) | - | - | - |
| Mild persistent | 8 (16%%) | - | - | - |
| Moderate persistent | 15 (30%%) | - | - | - |
| Severe persistent | 4 (8%%) | - | - | - |

**Table 2.** Distribution of ADRB2 genotypes at codon 16 in asthmatic and healthy children.

| Genotype | Asthmatic group (n = 50) | Control group (n = 50) | OR | 95% CI | P |
|---|---|---|---|---|---|
| **Arg/Arg** | 35 (70%) | 47 (94%) | 0.14 | (0.04 - 0.55) | <0.05 |
| **Arg/Gly** | 7 (14%) | 1(2%) | 7.9 | (0.94 - 67.4) | <0.05 |
| **Gly/Gly** | 8 (16%) | 2(4%) | 4.5 | (0.91 - 22.7) | <0.05 |

**Table 3.** Relation between polymorphisms of β2AR at codon 16 and asthma severity.

| Genotype | Mild asthma (n =31) | Moderate/ severe asthma (n =19) | OR | 95% CI | P |
|---|---|---|---|---|---|
| **Arg/Arg** | 26 (83.8%) | 9 (47.3%) | 5.77 | 1.55-21.5) | < 0.05 |
| **Arg/Gly** | 4 (13%) | 3( 15.7%) | 0.79 | 0.156-3.99) | > 0.05 |
| **Gly/Gly** | 1 (3.2%) | 7 (37 %) | 0.057 | 0.006-0.516 | < 0.05 |

**Table 4.** Relation between polymorphisms of β2AR at codon 16 and response to salbutamol nebulization**.**

| | Arg/Arg (n = 35) | Arg/Gly (n =7) | Gly/Gly (n =8) | P1 | P2 | P3 |
|---|---|---|---|---|---|---|
| **Percentage of change of FEV1 (%)**, Mean±SD | 17.3±7.9 | 9.1± 3.6 | 11.4 ±2.1 | <0.05[a] | <0.05[b] | >0.05[c] |

FEV1: forced expiratory volume in one second , [a] Arg/Arg group vs. Arg/Gly group , [b] Arg/Arg group vs. Gly/Gly group, [c] Arg/Gly group vs. Gly/Gly group

## DISCUSSION

Genetic assessment revealed that asthmatic children had frequencies of Arg/Arg of 70%, Arg/Gly of 14%, and Gly/Gly of 16%, while in the healthy children the frequency of Arg/Arg was 94%, Arg/Gly was 2%, and Gly/Gly was 4%. These results revealed a significant difference of distribution of ADRB2 genotypes at codon 16 among asthmatics and healthy children.

Globally, there are marked interethnic differences in the frequency of ADRB2 polymorphisms. In Caucasian–American and African–American healthy individuals, the Arg/Gly genotype was the most predominant (38.3% and 50.4% respectively), while Arg/Arg genotype was present in lower frequencies (26.6% and 23.6%, respectively)[28]. In another study conducted on 128 Chinese asthmatics by Wang et al*.,* the frequency of Arg/Arg was 40.6%, Arg/Gly was 42.2%, and Gly/Gly was 17.2%, while in the healthy people the frequency of Arg/Arg was 27.9%, Arg/Gly was 47.1%, and Gly/Gly was 25.0% [29].

In Egyptian population, Salama et al.[30] found that the frequencies of ADRB2 genotypes at position 16 among healthy children, was 52.6% for Arg/Arg, 5.3% for Arg/Gly and 42.1% for Gly/Gly. In asthmatic children, these genotype frequencies were different; 17.5% for Arg/Arg, 45% for Arg/Gly and 37.5% for Gly/Gly. Although these frequencies were different from our results, yet the final conclusion was the same: higher frequencies of Arg/Gly and Gly/Gly genotypes in asthmatic children when compared with controls. In another study, the frequencies of ADRB2 genotypes at position 16 among Egyptian healthy individuals, was 32.6% for Arg/Arg, 49% for Arg/Gly and 18.5% for Gly/Gly[31]. These differences reflect the need for wide-based, population studies.

There was an association of Arg/Arg genotype with mild asthma when compared with moderate/severe asthma. On the other hand, Gly/Gly genotype was associated with moderate/severe asthma when compared with mild asthmatics. This indicates that the polymorphism at codon 16 of ADRB2 is a possible determinant of asthma severity.

A meta-analysis including a total of 28 studies performed by Contopoulos-Ioannidis et al.[32], concluded that, Gly/Gly had a much higher risk for nocturnal asthma and asthma severity than the Arg/Arg.

In contrast, Salama et al.[30] found an association between Arg/Gly genotype with severe asthma when compared to mild/moderate asthma. Also, Gly/Gly genotype was present with lower frequencies in severe asthma when compared with mild/moderate asthma.

In vitro functional studies[33,34] indicated that down-regulation of ADRB2 receptors occurs in individuals expressing Gly16 allele in response to circulating catecholamines or exogenously administered $\beta_2$-agonists. The Arg16 allele, which demonstrates resistance to down-regulation, might therefore be expressed at greater levels than the Gly16 allele within airways. Accordingly, individuals carrying the Gly/Gly genotype might be more sensitive to stimuli resulting in bronchoconstriction, and therefore have more reactive airways than individuals carrying the Arg16 allele.

In our study, the Gly allele (Arg/Gly and Gly/Gly genotypes) was associated with resistance to the bronchodilator effect of inhaled short-acting β 2-agonist (salbutamol) when compared with Arg/Arg genotype. The association between ADRB2 genotypes and response to inhaled β2 agonists is controversial, and discordant findings have been reported. Finkelstein et al.[22] conducted a meta-analysis to examine the association between ADRB2 polymorphisms and the response to inhaled

β2 -adrenergic agonists in children with asthma. They included 3 studies[9,35,36] and found a significant association between favorable therapeutic response to inhaled β2-adrenergic agonists and the Arg/Arg genotype. Several studies came up with the same conclusion[23-25].

However, when examining the influence of ADRB2 polymorphisms on the response to long-term and repeated dosages of inhaled β2 -adrenergic agonist therapy, the Arg/Arg genotype has been more closely associated with reduced response[15-20]. Moreover, in children with severe asthma exacerbations, Carroll et al.[21] found that children whose genotypes were Gly/Gly had a more rapid response to inhaled β2 -adrenergic agonists.

Five reasons may underlie the somewhat discordant results reported by different authors. First, studies had included different patient populations, regarding age, disease severity, and ethnic background. Second, authors had administered different β2-agonists to study patients. Third, authors had used different outcome measures and endpoints to assess drug-responsiveness. Fourth, some authors had suggested that the conflicting results among studies could be explained by specific combinations of polymorphisms that are commonly inherited together (ADRB2 haplotypes), rather than by a single allele polymorphism[37]. Finally, race is both a biologic and a social construct and constitutes not only genetic differences in individuals, but also the behaviors, beliefs, and experiences that vary among races.

In conclusion, genetic polymorphisms of ADRB2 gene at codon 16 may be a determinant of asthma severity and response to salbutamol in Egyptian asthmatic children. However, we cannot exclude the possibility that other polymorphisms or complex haplotypes within the promoter and coding regions of the ADRB2 gene or adjacent genes might contribute to the present results. Further studies are needed to demonstrate effects of other polymorphisms of ADRB2 gene on asthma-related phenotypes in our ethnic group.

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