

Association between Genetic Polymorphisms of β_2 Adrenergic Receptors and Nocturnal Asthma in Egyptian children

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■ Abstract

Background: Identification of the genetic basis of asthma may contribute to the discovery of effective asthma drugs.

Objective: Our aim was to estimate the association between β_2 adrenergic receptor (*ADRB2*) polymorphisms and nocturnal asthma in Egyptian children.

Methods: *ADRB2* polymorphisms Gly16 and Glu27 were genotyped in 200 Egyptian children (90 with nocturnal asthma and 110 healthy controls) using allele-specific polymerase chain reaction.

Results: The homozygous (Gly16) genotype significantly increased the risk of nocturnal asthma (odds ratio [OR], 3.2; 95% CI, 1.3-7.7; $P=0.003$), as did the Gly allele (OR, 1.8; 95% CI, 1.2-2.8).

Conclusions: Our study demonstrated that nocturnal asthma was associated with *ADRB2* Arg/Gly polymorphisms but not with *ADRB2* Gln/Glu polymorphisms.

Key words: Nocturnal asthma. Egyptian children. β_2 adrenergic receptor polymorphisms. Allergy. Polymorphism.

■ Resumen

Introducción: La identificación de las bases genéticas del asma puede contribuir al descubrimiento de una terapia antiastmática efectiva.

Objetivo: Estimar la asociación entre polimorfismos del receptor beta2-adrenérgico (RB2A) y el asma nocturna en niños egipcios.

Métodos: Se genotiparon los polimorfismos de RB2A Gly16 y Glu27 en 200 niños egipcios (90 con asma nocturna y 110 controles sanos), mediante la técnica de la reacción en cadena de la polimerasa.

Resultados: El genotipo homocigoto (Gly16) incrementa el riesgo de padecer asma nocturna (OR: 3.2, 95% CI, 1.3-7.7) ($p=0.003$) y el alelo Gly se asocia significativamente con el riesgo de padecer asma nocturna (OR: 1.8, 95% CI, 1.2-2.8).

Conclusión: Este estudio demuestra una asociación de polimorfismos Arg/Gly del RB2A con el asma nocturna y una falta de asociación del polimorfismo Gln/Glu con dicho tipo de asma.

Palabras clave: Asma nocturna. Niños egipcios. Polimorfismos receptor beta2-adrenérgico. Alergia. Polimorfismo.

Introduction

Asthma is a complex disease caused by interactions between many genes and environmental factors. Subsets of asthmatic patients have nocturnal asthma, which is characterized by airflow obstruction symptoms during the night [1].

According to a study published in Egypt in 2010, the overall prevalence of asthma among children is 14.7% while that of physician-diagnosed asthma is 9.4% [2].

The adrenoceptor beta 2, surface gene, *ADRB2*, an intronless gene on chromosome 5q31-q32 encoding 413 amino

acids, is expressed on the surface of airway smooth muscle cells and plays a key role in the reactivity of the airways [3]. Catecholamines or β_2 -agonists activate *ADRB2* receptors in the airways, causing bronchodilatation and relief of wheezing symptoms. Defects in this receptor might have a pathogenic role in asthma [3].

A number of single nucleotide polymorphisms (SNPs) have been reported in *ADRB2*. Four of these result in amino acid substitutions at amino acids 16, 27, 34, and 164, whereas there are 5 silent mutations located at amino acids 84, 175, 351, 366, and 413 [4].

The most common SNPs result from 2 missense mutations in the coding region of *ADRB2*. The first SNP (A>G), at nucleotide 46, causes the substitution of glycine (Gly) for arginine (Arg) at codon 16, while the second one (C>G), at nucleotide 79, results in the substitution of glutamic (Glu) acid for glutamine (Gln) at codon 27. It has been reported that there are ethnic variations in the prevalence of these SNPs [5].

The aim of the present study was to investigate whether *ADRB2* polymorphisms at amino acids 16 and 27 might modify relative risk for the development of nocturnal asthma in Egyptian children.

Material and Methods

Participants

We studied 90 children with nocturnal asthma and 110 apparently healthy control children. All the patients were recruited among children seen and followed in the pediatrics department of the Faculty of Medicine of Zagazig University in Zagazig, Egypt.

Following the American Thoracic Society guidelines [6], asthma was confirmed by a history of chest tightness and wheezing during the previous 12 months and reversibility of more than 12% in forced expiratory volume in the first second (FEV₁), either spontaneously or after β_2 -agonist inhalation. All asthma patients were clinically stable. None of the participants had received antihistamines or systemic or topical corticosteroids in the 3 weeks prior to clinical evaluation. FEV₁ and peak expiratory flow rates (PEFRs) were measured using a portable spirometer (Masterlab Jaeger). Nocturnal asthma was defined as a documented fall in PEFR of 20% or more on at least 4 of 7 nights of testing at home [7] or based on a history of early morning awakening, dyspnea, wheezing, and cough between 2 and 6 AM on 3 consecutive days.

The control group consisted of 110 children seen at the pediatrics department of Zagazig University Hospital for nonrespiratory complaints. The controls had no history of asthma and they all had normal lung function tests. The study was approved by the ethics committee of the Faculty of Medicine of Zagazig University and written informed consent

was obtained from all participants. The characteristics of the participants are shown in Table 1.

DNA Extraction

DNA was isolated and purified from whole blood (EDTA) using the protocol provided by the manufacturer (Qiagen GmbH). The purified DNA was safely stored at -20 °C for later use.

Detection of *ADRB2* Polymorphisms

Polymorphisms in the *ADRB2* coding block were delineated using allele-specific polymerase chain reaction (PCR) [8]. Allele-specific PCR was performed to assess polymorphisms at nucleic acids 46 and 79, which result in changes in the encoded amino acids at positions 16 and 27 of the receptor protein. The genotypes are abbreviated as Arg16, Gly16, Gln27, and Glu27.

The primer pairs used to delineate the 2 polymorphisms at nucleic acid 46 to detect *ADRB2* mutations at codon 16 (*ADRB2* 16) were 5'-CTTCTTGCTGGCACCCAATA-3' (sense) and 5'-CCAATTTAGGAGGATGTAAACTTC-3' (antisense) or the same antisense primer and 5'-CTTCTTGCTGGCACCCAATC-3' (sense). The corresponding pairs used for the 2 polymorphisms at nucleic acid 79 to detect *ADRB2* 27 mutations were 5'-GGACCACGACGTCACGCAGC-3' (sense) and 5'-ACAATCCACACCATCAAGAAT-3' (antisense) or the same antisense primer and 5'-GGACCACGACGTCACGCAGG-3' (sense).

For both polymorphisms, the 25- μ L PCR reaction mixtures included 10 μ g of genomic DNA, 0.5 μ mol of each primer, (Promega), and 1X PCR mix (Taq PCR Master Mix Kit, QIAGEN), containing 200 μ mol/L of each dNTP, 5 μ L of 10 \times reaction buffer, and 1.25 U of Taq Gold Polymerase, and 4 mmol/L of MgCl₂. Amplification was carried out using a PTC-100 thermal cycler (Perkin-Elmer).

Temperature cycling was 98°C for 30 seconds, 66°C to 68°C for 45 seconds, and 72°C for 45 seconds for 30 cycles. Twenty μ L of the PCR mixture was then electrophoresed on 1% agarose gels and visualized with ethidium bromide staining and ultraviolet illumination. The sizes of the PCR products were 913 base pairs (bps) for *ADRB2* 16 and 442 bps for *ADRB2* 27.

Statistical Analysis

The results for continuous variables are expressed as means (SD). Mean results for the groups were compared by the *t* test, and the statistical significance of differences in frequencies of variants between groups was tested using the χ^2 test. In addition, odds ratios (ORs) and 95% CIs were calculated as a measure of the association between *ADRB2* alleles and nocturnal asthma. Genotype frequencies in patients and controls were tested for Hardy-Weinberg equilibrium, and any deviations between observed and expected frequencies were tested for significance using the χ^2 test. Differences were considered significant for *P* values of less than .05. Data were analyzed using SPSS software, version 11 [9].

Table 1. Characteristics of Nocturnal Asthma Patients and Controls

	Patients (n=90)	Controls (n=110)
Age, mean (SD), y	10.3 (2.4)	9.8 8 (2.8)
Boys, % of patients	35 (38.8)	48 (43.6)
Girls, % of patients	55 (61.2)	62 (56.4)
FEV ₁ , mean (SD), % predicted	65.1 (18.2)	Normal
PEFR, mean (SD), L/min	184.5 (50.5)	Normal

Abbreviations: FEV₁, forced expiratory volume in the first second; PEFR, peak expiratory flow rate.

Results

Characteristics of Nocturnal Asthma Patients and Controls

Of the 90 children in the nocturnal asthma group, there were 35 boys and 55 girls, with a mean age of 10.3 (2.4) years. In the healthy control group, there were 48 boys and 62 girls, with a mean age of 9.8 (2.8) years. The mean FEV₁ predicted value at baseline was 65.1% (18.2%) and the mean PEF_R was 184.5 (50.5) L/min (Table 1).

Genotype Distributions and Allele Frequencies of ADRB2 Arg/Gly Polymorphisms at Codon 16 in Patients and Controls

ADRB2 16 genotype frequencies conformed to the Hardy-Weinberg equilibrium in both patients ($P=.7$) and controls ($P=.29$). In the nocturnal asthma group, the frequencies were 25.6% for Arg/Arg homozygous individuals, 44.4% for Arg/Gly heterozygous individuals, and 30% for Gly/Gly homozygous individuals, while in the control group they were 40%, 45.4%, and 14.6% respectively. The Arg/Arg wild-type genotype and the Arg wild-type allele were taken as references for the risk of nocturnal asthma. The analysis showed that the

homozygous mutant genotype Gly/Gly significantly increased the risk of nocturnal asthma (OR, 3.2; 95% CI, 1.3-7.7) ($P=.003$), as did the Gly allele (OR, 1.8; 95% CI, 1.2-2.8). No significant associations were observed between the Arg/Gly genotype and the risk of nocturnal asthma (OR, 1.5; 95% CI, 0.7-3.1) (Table 2).

Genotype Distributions and Allele Frequencies of ADRB2 Gln/Glu Polymorphisms at Codon 27 in Patients and Controls

The distribution of ADRB2 27 genotypes was also consistent with Hardy-Weinberg equilibrium in patients ($P=.9$) and controls ($P=.14$). In the nocturnal asthma group, the genotype frequencies were 48.9% for Gln/Gln homozygous individuals, 42.3% for Gln/Glu heterozygous individuals, and 8.8% for Glu/Glu homozygous individuals. In the control group, the respective frequencies were 58.2%, 32.7%, and 9.1%. The Gln/Gln wild-type genotype and Gln wild-type allele were taken as references for the risk of development of nocturnal asthma. The analysis showed an absence of increased risk for nocturnal asthma in patients who were Gln/Glu heterozygotes (OR, 1.5; 95% CI, 0.8-2.9) and Glu/Glu homozygotes (OR, 1.1; 95% CI, 0.3-3.5); there was also an absence of increased risk for the Glu allele (OR, 1.2; 95% CI, 0.8-2.00) (Table 3).

Table 2. Genotype Distributions and Allele Frequencies of ADRB2 Arg/Gly Polymorphisms at Codon 16 in Pediatric Patients with Nocturnal Asthma and Controls

Polymorphic Site	Patients (n=90) No. (%)	Controls (n=110) No. (%)	OR (95% CI)	P Value
<i>ADRB2 16</i>				
Arg/Arg	23 (25.6)	44 (40)		
Arg/Gly	40 (44.4)	50 (45.4)	1.5 (0.7-3.1)	.2
Gly/Gly	27 (30)	16 (14.6)	3.2 (1.3-7.7)	.003
Allele frequency				
Arg	86 (47.7)	138 (62.7)		
Gly	94 (52.2)	82 (37.2)	1.8 (1.2-2.8)	.002

Table 3. Genotype Distributions and Allele Frequencies of ADRB2 Gln/Glu Polymorphisms at Codon 27 in Pediatric Patients with Nocturnal Asthma and Controls

Polymorphic Site	Patients (n=90) No. (%)	Controls (n=110) No. (%)	OR (95% CI)	P Value
<i>ADRB2 27</i>				
Gln/Gln	44 (48.9)	64 (58.2)		
Gln/Glu	38 (42.3)	36 (32.7)	1.5 (0.8-2.9)	.1
Glu/Glu	8 (8.8)	10 (9.1)	1.1 (0.3-3.5)	.7
Allele frequency				
Gln	126 (70)	164 (74.5)		
Glu	54 (30)	56 (25.5)	1.2 (0.8-2.00)	.3

Table 4. Haplotype Frequencies in Pediatric Patients with Nocturnal Asthma and Controls

	Patients (n=180) No. (%)	Controls (n=220) No. (%)	OR (95% CI)	P Value
AG	67 (37)	88 (40)	1.12 (0.73- 1.7)	.5
AC	13 (7)	18 (8)	0.87 (0.3- 1.9)	.7
GG	59 (33)	75 (34)	0.9 (0.61-1.46)	.7
GC	41 (23)	39 (18)	1.3 (0.8-2.3)	.2

Haplotype Frequencies in Patients and Controls

The estimated frequencies of various haplotypes in both the control and patient groups and the significance of the association with nocturnal asthma are shown in Table 4. The association was nonsignificant in all cases.

Discussion

Patients with nocturnal asthma are a unique subset of asthmatic patients who experience worsening symptoms and airflow obstruction at night. The basis for this phenotype of asthma is unknown, but β_2 -adrenergic receptors are known to decrease overnight in nocturnal asthmatics but not in healthy individuals or in non-nocturnal asthmatics [10].

Two common *ADRB2* polymorphisms—Arg16Gly and Gln27Glu—have been studied for their possible association with asthma-related phenotypes [11,12]. However, conflicting data exist regarding their clinical significance and effect in different populations.

In Egypt, a recent study of the association between Arg16Gly and asthma in schoolchildren showed a significant difference in genotype frequencies at codon 16 (Arg16Gly) between asthmatics and healthy controls and also between asthmatics with severe disease and those with mild/moderate disease, suggesting an association between heterozygous Arg16Gly and severe asthma [13].

To explore the association between *ADRB2* polymorphisms and nocturnal asthma in Egyptian children, we genotyped 2 common polymorphisms of this gene at codons 16 and 27.

The genotype frequencies for both polymorphic sites determined in our group of healthy controls were slightly different from those previously reported [5,14], but this is probably due to ethnic differences between populations.

Our study demonstrated an association between Gly16 and nocturnal asthma. The homozygous Gly/Gly genotype significantly increased the risk of nocturnal asthma ($P=.003$) compared with the Arg/Arg genotype, and the Gly allele was significantly associated with an increased risk of nocturnal asthma (OR, 1.8; 95% CI, 1.2-2.8). These findings are consistent with those of Contopoulos-Ioannidis et al [15] and Yin et al [16], who found a strong association between Gly16 and nocturnal asthma. This may be because the replacement of arginine at codon 16 (Arg16) with glycine (Gly16) (Arg16Gly) in vitro would enhance the downregulation of *ADRB2* and may

therefore possibly increase the sensitivity of the bronchial tree [17,18]. By contrast, a cross-sectional survey provided evidence that carrying homozygous Arg16 may predispose children and young adults to asthma exacerbation [19]. Another group found that PEFr was reduced in patients with homozygous Arg16 [20].

Turki et al [10] reported a higher frequency of the Gly16 allele in children with nocturnal asthma compared with those with non-nocturnal asthma, and Gly16 was significantly associated with nocturnal falls in PEFr in individuals with asthma. Reihnsaus et al [21], in turn, found that Gly16 was associated with more severe asthma, and patients with this polymorphism may show a lack of benefit from β_2 -agonist therapies and be forced to use corticosteroids and/or immunotherapy.

Individuals with the *ADRB2* Gln27 polymorphism may have increased airway hyperresponsiveness to endogenous catecholamine, resulting in increased airway sensitivity to proinflammatory stimuli and leading to some extent of long-term airway inflammation [22]. Also, Lee et al [23] suggested that patients with Glu27 may have an increased response to terbutaline inhalation compared with those with Gln27. However, our results showed a nonsignificant difference in genotype or allele frequencies between patients with nocturnal asthma and healthy controls.

Certain haplotypes of the *ADRB2* gene may play an important role in modifying clinical characteristics of asthma phenotypes [24]. Although the exact relationships have not been well studied, some researchers have argued that assessment of individual SNP effects without consideration of haplotypes may have resulted in inconsistent associations because the SNPs in the *ADRB2* gene are tightly linked [3]. On examining the association between Arg16Gly and Gln27Glu and haplotypes in relation to nocturnal asthma, we found no significant association.

In conclusion, our study has demonstrated a significant association between the *ADRB2* (Gly16) genotype and the presence of nocturnal asthma. Future studies are recommended to understand the possible role of these genetic variations in regulating responses to asthma therapy in the Egyptian population.

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