Association between Arginine 16 Polymorphism of B_2 Adrenergic Receptor and Bronchial Asthma in Children

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ABSTRACT

Background: Asthma is one of the most prevalent health problems. The relationship between β_2AR genotypes and response to β_2AR agonist therapy is controversial. Some studies have found that the Arg-16 genotype is associated with reduced response to β_2AR agonists**,** whereas others have found that the Gly-16 genotype is associated with reduced response.

Objective: to detect gene polymorphism and to determine the association between gene polymorphism and bronchial asthma susceptibility as a risk factor, and to evaluate of the degree of severity of bronchial asthma among cases having a defect in studied gene and those without defect, and also to assess of drug response to β2 agonists concerning gene polymorphism.

Methods: This study was carried out at Zagazig university hospitals. 100 Egyptian children with ages ranging from 5 to 12 years were enrolled. They were divided into 2 groups, the asthmatic group included 50 asthmatic children who were diagnosed according to GINA guidelines (2016), and a control group of 50 age and sex-matched healthy children.

Results: Arg16Gly heterozygous represents 48%, Arg16 homozygous represents 38% and Gly16 homozygous represents 16% of asthmatic patients, while among the control group Arg16heterozygous represents 38%, homozygous represents 14% and 48% for Gly16 homozygous. There was no significant difference between Arg16Gly genotypes and asthma severity. Also, Arg 16 homozygous showed the best response to treatment with inhaled short-acting beta 2 agonists.

Conclusion: there is an association between beta 2 adrenergic receptor polymorphism and the occurrence of bronchial asthma and also between this polymorphism and response to treatment. Also that the polymorphism at codon 16 of the β2 adrenergic receptor gene not a determinant of asthma severity in the Egyptian children.

INTRODUCTION

Asthma is a chronic disease of childhood and adolescence characterized by reversible airway obstruction, with or without treatment, resulting from an underlying chronic inflammatory process that typically involves infiltration of various cell types **(1).** Asthma is one of the most prevalent chronic childhood causes of hospitalization among children **(2).**

Genetic factors controlling the β2-adrenergic receptor (β2AR) function may be a very important determinant of response to bronchodilator therapy and thus of severity and duration of asthmatic symptoms **(3).**

Inhaled selective β2-agonists were the most widely used treatment for the acute relief of asthma symptoms. The β2AR mediates the physiologic responses of the airways, including broncho-protection (reduced responsiveness to non-specific contractile stimuli), bronchodilatation (improvement in lung mechanics), enhanced mucociliary clearance, suppression of microvascular leakage, inhibition of cholinergic neurotransmission and inhibition of mediator release from basophils and mast cells**.** A number of single-nucleotide polymorphisms (SNPs) in the β2AR gene had been detected in many populations. The most common SNPs were due to two missense mutations, which occur in the coding region of the β2AR gene. Although these polymorphisms were not considered to be susceptibility genes for asthma; they had been reported to be associated with functional changes in the β2AR in the respiratory system **(4).**

An important factor studied in asthma-related research is the beta-2-adrenergic receptor, which is encoded by the ADRβ2 gene. Different polymorphic loci of this gene have been associated with an asthma diagnosis, nocturnal asthma, asthma exacerbations, and response to beta-2 agonists in asthma treatment **(5)**.

Although this gene is the most studied in asthma pharmacogenetics, no clear conclusion as regards its functional effects has been reached. Although the Arg/ Gly16 polymorphism has been linked to the level of lung function, it has not been linked to change in lung function with either SABAs or LABAs**.** The substitution of arginine (Arg) for glycine (Gly) at codon 16 of the β2AR gene is responsible for differences in response to short-acting beta-2 agonists **(6).**

Inhaled β2-adrenergic receptor agonist medications are the foundation of therapy for acute asthma exacerbation. The β2AR protein is expressed on bronchial smooth muscle cells and mediates physiologic responses including bronchodilation, vasodilatation, and lipolysis **(7).**

The objectives of this study were to: detect gene polymorphism and to determine the association

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between gene polymorphism and bronchial asthma susceptibility as a risk factor, and to evaluate the degree of severity of bronchial asthma among cases having a defect in studied gene and those without defect, and also to assess of drug response to β2 agonists with gene polymorphism.

PATIENTS AND METHODS

This study was conducted in Zagazig University Hospital, Pediatric Pulmonology Unite, in cooperation with Zagazig Scientific and Medical Research Center during the period from July 2016 to July 2018.

This study was a case-control study including fifty asthmatic patients (25 males and 25 females), their mean age was (8.10 ± 2.52) years, This group was classified into 4 subgroups: mild intermittent, mild persistent, moderate persistent and severe persistent [8].

Fifty clinically healthy control participants with matched age and sex(25 male and 25 female) and their mean age was (8.44±2.40) years, were thoroughly evaluated, selected after careful clinical examination, they were completely free from any disease, not siblings to asthmatic patients.

Inclusion criteria:

1- Age ranging from 5 to 12 years old.
2- Presence of typical asthma symptoms according to Global Initiative for Asthma Management and prevention guidelines [9].
3- Confirmed variable expiratory airflow obstruction as evidenced by improvement in prebronchodilator FEV1 of > 15% predicted after salbutamol 200 µg administration [9].
4- They had no history of corticosteroid treatment within 6 weeks, oral beta-adrenergic agonists within 1 week, inhaled beta-adrenergic agonists within 6 hours, antihistamines within 72 hours and leukotriene modifiers within 4 weeks [10].

Exclusion criteria: Asthmatic patients with co-morbidities such as; cardiovascular diseases and chronic pulmonary diseases. History of respiratory tract infection within the past 4 weeks or a history of allergy or dermatitis.

Consent: Informed consent was obtained from all caregivers of patients and healthy control.

Methods:

Patients and controls underwent the following:

1- History taking and clinical examination.
2-Chest x-ray.
3- Pulmonary function test for cases (FEV1, FVC, and PEF) before and after Albuterol administration.
4- Total serum IgE level assessment.
5- Genetic analysis of ADRB2 to detect Arg/Gly gene polymorphism at codon 16.

ADRB2 genetic analysis by allele-specific polymerase chain reaction technique (AS-PCR).

Ethical approval

The study was approved by the Ethics Board of Zagazig University and an informed written consent was taken from each participant in the study.

Statistical Analysis

Data were coded, inputted, and analyzed using SPSS version 20 (SPSS Inc, USA). All numeric variables were outlined as mean ± standard deviation (SD). Mean, standard deviation, range, frequency, and percentage were used as descriptive statistics. The results were represented in tabular and diagrammatic forms then interpreted. The following tests were used: Chi-square test (X^2), Student test. Student's paired t-test, ANOVA (F test): P-value was considered significant as the following: For all tests, a probability (p) more than 0.05 was considered non-significant, less than 0.05 was considered significant.

RESULTS

Table (1): Demographic characteristics of the studied groups.

			Patients (No.=50)	Control (No.=50)	t-test	P-value
Age	**Mean±SD**		8.10±2.52	8.44±2.40	0.689	0.492
Sex	**Male**	**No.**	25	25	X^2 0.000	1.000
		%	50	50		
	Female	**No.**	25	25		
		%	50	50		

No significant differences were found between asthmatic children and healthy control as regard age and sex. **(Table 1)**

Table (2): Comparison between grades of asthma severity regarding Total IgE level.

		Mild asthma	Moderate asthma	Severe asthma	F	P-value	
Total IgE level	**Mean±SD IU/ml**	215.22±33.55	719.72±77.42	635.18±52.80	3.90	0.014	P1=0.002* P2=0.761 P3=0.042*

There was a statistically significant difference between mild and moderate asthma and also between mild and severe asthma regarding Total IgE level (P. value = 0.002 and 0.042 respectively) and no statistically significant difference between moderate and severe asthma regarding total IgE level (p. value = 0.761).

(Table 2)

Table (3): Comparison between Pre and post neubilized B2 agonist as regard FEV1% among asthmatic patients.

		Patients	Paired sample t. test	P. value
Pre neubilized B2 agonist FEV1 %	**Mean ±SD**	77.34±11.96	13.20	0.00
post neubilized B2 agonist FEV1%	**Mean ±SD**	91.82±9.68		

There was a statistically significant difference between Pre and post neubilized B2 agonist as regard FEV1% among patients. **(Table 3)**.

Table (4): Comparison between asthmatic patients and control regarding Arg/Gly 16 genotypes.

		Patients No. (50)	Control No. (50)	X^2	P-value
Arg 16 homozygous	No	19	7	7.484	0.006**
	%	38%	14%		
Arg/Gly 16 heterozygous	No	23	19	1.020	0.313
	%	46%	38%		
Gly 16 homozygous	No	8	24	10.519	0.001**
	%	16%	48%		

There was a statistically significant difference between patients and control group regarding Arg 16 homozygous (P. 0.006) and Gly 16 homozygous (P. 0.001), as Arg 16 genotype represents about (38%) of the asthmatic group and (14%) of control group and Gly 16 genotype represents (16%) of the asthmatic group and (48%) of the control group. There was no statistically significant difference between patient and control group regarding Arg/Gly 16 heterozygous (p. 0.313), as Arg /Gly 16 heterozygous represents about (46%) of the asthmatic group and (38%) of the control group. **(Table 4).**

Table (5): Comparison between Pre and post neubilized B2 agonist FEV1 among Arg16 Homozygous, Arg/Gly Heterozygous, and Gly 16 Homozygous.

Genotype	Pre neubilized B2 agonist FEV1 % Mean ±SD	post neubilized B2 agonist FEV1% Mean ±SD	P-value	Mean difference
Arg 16 Homozygous	77.936 ± 12.04	94.505 ± 11.03	0.00*	16.57
Arg/Gly Heterozygous	79.38 ± 11.315	91.31 ± 8.609	0.00*	11.93
Gly 16 Homozygous	76.80 ± 11.074	93.20 ± 7.24	0.00*	16.40

Patients with Arg16 genotype are significantly responsive to B2 agonist, followed by Gly 16 genotype, then Arg/Gly heterozygous, as mean differences of FEV1 before and after B2 agonist inhalation are 16.57, 16.40 and 11.93 respectively. **(Table 5).**

Table (6): Description of asthma severity among asthmatic patients regarding Arg/Gly 16 genotypes.

		Mild intermittent (19)	Mild persistent (10)	Moderate (16)	Sever (5)	X^2	P. value
Arg 16 homozygous	No	5	4	7	3	1.849	0.397
	%	26.3%	40%	43.8%	60%		
Arg/Gly 16 heterozygous	No	12	1	8	2	0.68	0.71
	%	63.2%	10%	50%	40%		
Gly 16 homozygous	No	2	5	1	0	3.515	0.173
	%	10.5%	50%	6.2%	0.0%		

There is no statistically significant difference between asthma severity among patients regarding Arg 16 homozygous, Arg/Gly 16 heterozygous, and Gly 16 homozygous. **(Table 6).**

DISCUSSION

This study showed that there was no statistically significant difference between cases and controls regarding age and sex. This agreed with **Hoshino *et al.*** [11] who found that no significant differences in age and gender were observed between the two groups**.**

This study showed that positive family history was found in (54%) of patients. This result was in accordance with **Magdy *et al.*** [12] who stated that a positive family history of asthma was a risk factor for asthma.

This study showed that smoke exposure was found among (38%) of patients. This result agreed with **Vargas *et al.*** [13] who found that environmental tobacco smoke exposure has been associated with the increased use of the emergency department for acute asthma care.

This study showed that there was a statistically significant increase in the Total IgE level between mild and moderate asthma (p 0.002) and between mild and severe asthma (p 0.042). This agreed with **Kovack *et al.*** [14] who concluded that asthmatic children with higher asthma severity have higher serum concentration of IgE

There was a statistically significant increase in post neubilized B2 agonist FEV1% among patients (mean 91.8±9.68) than Pre neubilized B2 agonist FEV1 % (mean 77.34±11.96). This is in agreement with **Bandaru *et al.*** [3]**.**

Genetic assessment of the study population revealed that, at amino acid position 16 of the β_2AR gene, 19 (38%) asthmatic children were homozygous for Arg (Arg-16), 8 (16%) were homozygous for Gly (Gly-16), and 23 (46%) were heterozygous (Arg16Gly). On the other hand, 7 (14%) healthy children were homozygous for Arg16 and 24(48%) were homozygous for Gly16, and 19 (38%) were heterozygous (Arg16Gly).

In a study on the Egyptian population, **Salama *et al.*** [15] found that the frequencies of β_2AR genotypes at position 16 among healthy children, was 52.6% for homozygous Arg16, 5.3% for heterozygous Arg16Gly and 42.1% for homozygous Gly16. In asthmatic children, these genotypes frequencies were different; 17.5% for homozygous Arg16, 45% for heterozygous Arg16Gly, and 37.5% for homozygous Gly-16. Although distribution frequencies are different from our results, yet the conclusion was the same higher frequency of Arg16Gly and Gly-16 genotypes in asthmatic children.

Salah *et al.* [16] found there was a significant increase of carriers of Arg/Gly and Gly/Gly genotypes among asthmatic children in comparison to controls. On the other hand, there was a lower frequency of Arg/Arg genotype in asthmatic children than in controls.

Alghobashy *et al.* [17] revealed that at amino-acid position 16 of the β_2AR gene 44.2% of asthmatic children were homozygous for Arg–Arg, 9.6% were homozygous for Gly–Gly, and 46.2% were heterozygous Arg–Gly. On the other hand, 78.8% of healthy children were homozygous for Arg–Arg, 9.7% were homozygous for Gly–Gly, and 11.5% were heterozygous Arg–Gly. From their findings, observe that there was a statistical significance between cases and control groups in codon 16 with the higher frequency of Arg–Gly genotypes among asthmatic children and higher frequency of Arg–Arg among the control group.

Regarding the response to treatment, patients with Arg-16 showed a significant improvement in FEV1% post-bronchodilator in comparison to Gly16 and Arg16Gly genotypes, mean (94.505±11.03) (93.20±7.24) and (91.31±8.60) respectively

This is in agreement with **Finkelstein *et al.*** [18] who found a significant association between favorable therapeutic response to inhaled β2-adrenergic agonists and the Arg/Arg genotype.

This was reinforced by **Small *et al.*** [19] physiological concentrations of endogenous agonists result in desensitization and tolerance of receptors, particularly for Gly-16, which in vitro exhibits greater degrees of downregulation. For Arg-16, there is relative preservation of receptor function. Thereafter, exposure to single doses of

agonist at pharmacological concentrations results in a greater immediate response for Arg-16 compared with Gly-16.

Accordingly, individuals carrying the Gly-16 genotype might be more sensitive to stimuli resulting in bronchoconstriction and therefore have more reactive airways than individuals carrying the Arg-16 allele.

On the other hand, **Taylor, [20]** found that long-term treatment with inhaled short-acting beta2 agonist, the genotype difference in downregulation (90% for Gly-16 vs 75% for Arg-16, a difference of 15%) was noticeably less than the overall effect size that occurred with the genotype, which allegedly confers resistance to downregulation (Arg-16; 75%) This indicates that the genetic effect on downregulation is a relative rather than an absolute phenomenon.

However, **Wechsler *et al.* [21]** stated that, although this gene is the most studied in asthma pharmacogenetics, no clear conclusion as to its functional effects with either SABAs or LABAs has been reached.

So, asthmatic patients carrying the Arg-16 form may benefit by minimizing the use of both short- and long-acting β2-agonists who may not derive benefit from the acute administration of salmeterol after chronic use of long-acting β2-agonists

But on the contrary to these results, **Bleecker *et al.* [22]** suggest that Arg-16 individuals have more frequent exacerbations independent of beta-agonist use**.**

In the previously discussed findings by **Carroll *et al.* [23]** found African Americans in whom Arg-16 is more prevalent that, there was a poorer response to the repeated doses of albuterol that are typically given in the emergency department and the hospital for severe asthma exacerbations than Americans in whom Gly-16 is more prevalent**.**

A possible explanation for these inconsistencies may lie in complex gene-environment interactions. The race is both a biologic and a social construct and, as such, is a poor substitute for genetics. Race constitutes not only genetic differences in individuals, but also the behaviors, beliefs, and experiences that vary among races.

Studies also suggest that the genotype-phenotype correlations may differ significantly across different ethnic groups **Tsai *et al.* [24]** and replication studies are needed to validate the differential role of single nucleotide polymorphism on drug response in subjects of different ethnic background.

Our study showed that there was no statistically significant difference between asthma severity among patients regarding Arg 16 homozygous, Arg/Gly 16 heterozygous and Gly 16 homozygous (p 0.0397), (p 0.71), (p 0.173) respectively. This agreed with **Salah *et al.* [16]** who found no difference in the distribution of Arg/Gly, Gly/Gly genotype was noticed among mild and moderate/severe asthmatics (P> 0.05).

In concordance with these results, **Salama *et al.* [15]** stated that there was a strong association of heterozygous Arg16Gly of β_2AR with severe asthmatics rather than that in control subjects, and it was concluded that heterozygous Arg16Gly of β_2AR gene appeared to be an important genetic factor in the expression of asthma severity.

CONCLUSION

Our study high lightened that there is an association between beta 2 adrenergic receptor polymorphism and the occurrence of bronchial asthma and also between this polymorphism and response to treatment, also that the polymorphism at codon 16 of the β2 adrenergic receptor gene not a determinant of asthma severity in the Egyptian children. However, the genetics of drug response traits is complex and codon 16 of the β2 adrenergic receptor gene may be a reasonable target for new therapy of asthma in the future.

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