B_2 adrenergic receptor gene polymorphism effect on childhood asthma severity and response to treatment

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BACKGROUND: Alterations of B2 adrenergic receptor (β_2AR) can modulate the severity of asthma and the response to treatment. Therefore, we aimed to evaluate β_2AR gene polymorphism at codons 16 and 27 and their effect on asthma severity and response to treatment in asthmatic children.

METHODS: Case–control study was conducted on 156 children; 104 of them had bronchial asthma and 52 were healthy children (control group). Subjects of the study underwent history taking, clinical examination, pulmonary function tests, serum IgE level assessment, and identification of β_2AR-16 A46G and β_2AR-27 C79G polymorphism using PCR-Restriction Fragment length polymorphisms (RFLP) test.

RESULTS: There was a higher frequency of Arg–Gly genotypes (odds ratio (OR) = 6.57; confidence interval (CI): 2.42–18.81, $P < 0.001$) and lower frequency of Arg–Arg (OR = 4.7; CI: 2.05–10.95, $P < 0.001$) among asthmatic children compared with that among controls at codon 16. The presence or absence of Gly16 or Glu27 either homozygous or heterozygous for both correlated with the grade of asthma severity. The presence of heterozygous Arg–Gly and Gln–Glu gives a better response to drug therapy than the presence of Gly–Gly and Glu–Glu genotypes at codons 16 and 27.

CONCLUSION: Polymorphism of β_2AR at codons 16 and 27 correlates with asthma severity and response to treatment in asthmatic children.

Asthma is a chronic disease of children characterized by reversible airway obstruction, with or without treatment, due to chronic inflammatory process with cellular infiltration of the small airways (1). Its high prevalence around the world is the result of a complex interaction between genetic and environmental factors. The genetic aspects of susceptibility, severity, and response to treatment in asthma are of great scientific interest (2). The B_2 adrenergic receptor (β_2AR) is highly expressed in lung tissue and has an important role in regulating the pulmonary functions. Alterations of β_2AR can modulate the severity of asthma and the response to treatment, which can be highly variable and difficult to predict (3). The gene located on chromosome 5q31-q32 and encodes the β_2AR; a G-protein-coupled receptor is the one expressed in airway smooth muscle and induces bronchial relaxation (4). Polymorphisms in the β_2AR gene have been associated with intermediate phenotypes of asthma and variations in therapeutic responsiveness (5).

Four missense mutations (Arg16Gly, Gln27Glu, Val34Met, and Thr1641I1) and the Arg19Cys polymorphism in the 5' Leader Cintron of β_2AR mRNA have been identified as being potentially clinically relevant (6). Asano *et al.* have investigated possible associations between asthma and polymorphisms in the coding region of β_2AR, particularly Arg16Gly and Gln27Glu (7). Zhang *et al.* reported that these polymorphisms may be associated with asthma severity, the persistence of asthmatic symptoms, nocturnal asthma, and response to treatment (8). In a multicenter study, asthmatics with Arg–Arg homozygous genotype, receiving inhaled corticosteroids (ICSs), experience no change in hyper-responsiveness status even when long-acting β_2 agonists were used (9). Caucasian children homozygous and heterozygous for Arg16 had higher bronchodilator responses than Gly16 homozygote patients (10). The current study was focused on the β_2AR gene polymorphism at codons 16 and 27 and on their effect on the severity of asthma and response to treatment in asthmatic children.

METHODS

A prospective case–control study was conducted in the Pediatric Department of Zagazig University Hospital from inpatient department and outpatient clinic in the period from February 2015 to February 2016. The study included 156 children; 104 of them had bronchial asthma and 52 were healthy children age- and sex-matched as a control group. Written informed consents were obtained from parents of each child participating in this study after informing them about the steps of study, the complications, and the capability to withdraw at any time after approval of Ethical Committee in Faculty of Medicine, Zagazig University.

Subjects

This study included 104 asthmatic children diagnosed and assessed for severity according to the recently established guidelines of the

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global initiative for asthma management and prevention (GINA 2006) (11). Their age ranged from 6 to 12 years to ensure that the child could understand and perform the pulmonary function maneuvers efficiently. The asthmatic children were stable clinically at the examination and were divided into two groups.

Group I. Group I included 52 children with mild intermittent asthma treated with selective beta 2 agonists as salbutamol or terbutaline mostly in inhalation form or in a few cases in oral form three times daily.

Group II. Group II included 52 cases of persistent asthma treated with ICSs with long-acting beta 2 agonists. The cases in this group were classified into mild persistent, moderate persistent, and severe persistent asthma according to the GINA guidelines (11).

The control group (Group III) included 52 healthy children selected from the pediatrics outpatient clinics that came for non-respiratory complaints. The controls had no history of asthma or any allergic diseases and they all had normal lung function tests.

Exclusion Criteria

All children with age below 6 years, other allergic disorders, any chronic disease other than asthma that may affect pulmonary function, the restrictive pattern in Spirometry, or diseases associated with β_2AR polymorphism such as rheumatoid arthritis, hypertension, and obesity were excluded from the study. None of the subjects had received antihistamines or systemic corticosteroids in the last 3 weeks.

Methods

All studied groups underwent the following:

- *History taking*: about symptoms of asthma, provocation factors for bronchoconstriction (upper respiratory tract infection, exercise, passive smoke exposure, and home environment), allergic diseases either in the cases or in the family (rhinitis and eczema), and treatment stressing on type, doses, and methods of intake.
- *Full clinical examination:* for all children with measuring of weight, height, and BMI. All children of the study had performed pulmonary function tests using forced spirometry by D-97024 Hochberg, Germany, which is a program that allows a fast and reliable determination of the respiratory resistance on the basis of a tidal breathing analysis. The pulmonary function tests included forced vital capacity (FVC), forced expiratory volume in 1 s(FEV_1), and FEV_1/FVC ratio.
- *Laboratory investigations*:

1. Assessment of total serum IgE (12).
2. Identification of β_2AR-16 A46G and β_2AR-27 C79G polymorphism using PCR-RFLP test through the following steps:

- *Blood sampling:* Two milliliters of peripheral venous blood samples were collected on potassium EDTA (1 mg/ml) from each subject under complete aseptic conditions for DNA extraction.
- *DNA extraction:* DNA was isolated and purified from the blood sample using the Wizard Genomic DNA Miniprep Kit purchased from Sigma-Aldrich (Madison, WI, USA) (13).
- *Preparation of DNA solution* was done through different steps that included lysis, DNA binding, washing steps, and elution step.
- *DNA quantitation:* For measuring DNA concentration and evaluation of DNA purity (14).
- *DNA amplification:* Gene amplification of β_2AR polymorphic regions encoding positions for the amino acids 16 and 27 was performed according to the PCR protocol of Martinez *et al.* (15). The two polymorphisms were identified using PCR-RFLP test. PCR was performed in a final volume of 50 µl with 300–500 ng of genomic DNA, 0.5 pmol of each primer (Promega), and 1× PCR mix (Taq PCR Master Mix Kit, QIAGEN), containing 200 mmol/l of each d NTP, 5 ml of 10× reaction buffer, 1.25 U of Tag Gold Polymerase, and 4 mmol/l of $MgCl_2$.

The primer pairs used to delineate the two polymorphisms at nucleic acid 46 to detect β_2AR mutations at codon 16 β_2AR 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 two polymorphisms at nucleic acid 79 to detect β_2AR 27 mutations were 5'-GGACCACGACGTCACGCAGC-3' (sense) and 5'-ACAATCCACACCATCAAGAAT-3' (antisense) or the same antisense primer and 5'-GGACCACGACGTCACGCAGG-3' (sense).

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences, release 16 (Chicago, IL, USA). The quantitative variables were expressed as means and SDs. For comparison between two group means, the *t*-test was used. For comparison between three group means, one-way ANOVA was used. Qualitative variables were expressed by frequency and percentage, and were compared using χ^2-test. In addition, odds ratio (OR) and 95% confidence interval (CI) were calculated. For all tests, $P<0.05$ was considered significant and $P<0.001$ was considered highly significant.

RESULTS

Characteristics of all Groups of Asthma Cases and Controls

Asthmatic children in group I included 52 cases (34 males and 18 females) with the mean age of 8.58 ± 1.9 years and in group II included 52 cases (30 males and 22 females) with the mean age of 9.12 ± 2.18 years. The control group included 52 cases (28 males and 24 females) with a mean age of 8.96 ± 1.78 years. There were no statistically significant differences between the three groups in age, BMI, or sex distribution ($P>0.05$). There were statistically significant differences between all the groups in IgE and pulmonary function tests ($P<0.001$). After application of LSD to find the difference between each of the two groups, it was found that the differences present between all the groups ($P<0.001$). In addition, it showed that FEV1, FVC, and FEV1/FVC ratios were least and serum IgE was highest in group II in comparison with the other two groups ($P<0.001$; **Table 1**).

There were no statistically significant differences between the two case groups in family history and causes of asthma (smoking or exercise-induced); however,there was a statistically significant difference between them in the severity of asthma (**Table 2**).

B_2AR Gene Polymorphism at Codon 16 and Codon 27 of the Studied Groups

There were statistically significant differences between case and control groups in Codon 16 gene polymorphism with higher frequency of Arg–Gly genotypes (OR = 6.57; CI: 2.42–18.81, $P<0.001$) and lower frequency of Arg–Arg (OR = 4.7; CI: 2.05–10.95, $P<0.001$) among asthmatic children in comparison with controls. Regarding codon 27, there was no statistically significant difference between case and control groups ($P>0.05$; **Table 3**). There were statistically significant differences between all the groups in Codon 16 gene polymorphism, with Arg–Arg being more frequent in group I, whereas Arg–Gly and Gly–Gly being more frequent in group II ($P<0.001$). Regarding codon 27, there was

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Table 1. Comparison of demographic data, IgE, and pulmonary function tests of all groups of the study

Variable	Group I ($n=52$)	Group II ($n=52$)	Group III (control; $n=52$)	F	P	LSD
Age (years)						
Mean ± SD	8.58 ± 1.90	9.12 ± 2.18	8.96 ± 1.78	1.04	0.36	
Range	6–11	6–12	6–12		N.S.	
BMI (kg/m^2)						
Mean ± SD	16.71 ± 1.38	16.43 ± 1.48	16.35 ± 1.54	0.47	0.63	
Range	14.05–20.71	13.19–18.93	14.27–19.79		N.S.	
Sex						
Male	34 (65.4%)	30 (57.7%)	28 (53.8%)	$\chi^2=1.48$	0.48	
Female	18 (34.6%)	22 (42.3%)	24 (46.2%)		N.S.	
IgE						<0.001**[a]
Mean ± SD	137.73 ± 41.08	190.15 ± 68.55	19.15 ± 3.22	187.17	<0.001**	<0.001**[b]
Range	80–210	80–320	14–24			<0.001**[c]
FEV1						<0.001**[a]
Mean ± SD	83.12 ± 2.02	66.08 ± 6.81	93.77 ± 1.32	583.09	<0.001**	<0.001**[b]
Range	80–88	58–77	91–96			<0.001**[c]
FVC						<0.001**[a]
Mean ± SD	96.77 ± 2.65	78.27 ± 5.8	99.42 ± 0.80	501.16	<0.001**	<0.001**[b]
Range	91–100	69–92	98–100			<0.001**[c]
FEV1/FVC						0.02**[a]
Mean ± SD	85.58 ± 1.92	84.04 ± 5.06	93.96 ± 1.52	140.73	<0.001**	<0.001**[b]
Range	82–89	74–90	91–97			<0.001**[c]

FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity, N.S., not significant.
[a]Group I vs. Group II.
[b]Group I vs. Group III.
[c]Group II vs. Group III.
**Highly significant.

statistically significant difference between two case groups ($P=0.04$) and there was no statistically significant difference between case and control groups ($P>0.05$; Table 4).

Haplotypes at Codons 16 and 27 of β_2AR in Cases and Controls

There were statistically significant differences between case and control groups in some haplotypes at codon 16 and 27 gene polymorphism, with an increased risk of Arg–Arg × Gln–Glu in control 2.47 times more than that in cases and Arg–Gly × Gln–Gln in cases 3.6 times more than that in control. Finally, the risk of Arg–Gly × Gln–Glu increased 7.9 times more in cases than in the control group (Table 3).

The Relation Between β_2AR Gene Polymorphism at Codons 16 and 27 of Cases and Severity of the Disease

Table 5 shows that there is a statistically significant relation between gene polymorphism at both codons 16 and 27 of group II and severity of the disease. Arg–Arg and Arg–Gly at codon 16 were found to be more frequent in moderate persistent asthma, whereas Gly–Gly was more frequent in severe asthma. Gln-Gln and Gln-Glu at codon 27 were found to be more frequent in moderate persistent while Glu-Glu was more frequent in severe asthma

The Relation between β_2AR Gene Polymorphism at Codon 16 and Codon 27 of Cases and IgE and Pulmonary Function Tests

There were statistically significant relations in group I between gene polymorphism at codons 16 and 27 regarding FEV1 and FEV1/FVC ratio with the decreased mean of both among Arg–Arg and Gln–Gln (Table 6). There were statistically significant relations in group II between gene polymorphism at codons 16 and 27 regarding FEV1 and FEV1/FVC ratio. LSD showed the difference present between Gly–Gly and other genes at codon 16 and Glu–Glu and other genes at codon 27. Both showed less FEV1 and FEV1/FVC ratio than other genes (Table 7).

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Table 2. History and clinical data of the two case groups

Variable	Group I ($n=52$)		Group II ($n=52$)		χ^2	P
	N	%	*N*	%		
Family history:						
−ve	17	32.7	12	23.1	1.2	0.27
+ve	35	67.3	40	76.9		N.S.
Exercise-induced asthma						
No	28	53.8	27	51.9	0.04	0.84
Yes	24	46.2	25	48.1		N.S.
Passive smoking						
No	27	51.9	28	53.8	0.04	0.84
Yes	25	48.1	24	46.2		N.S.
Severity						
Mild intermittent	52	100	0	0	104	<0.001**
Mild persistent	0	0	6	11.5		
Moderate persistent	0	0	42	80.8		
Severe persistent	0	0	4	7.7		

−ve, negative; N.S., not significant; +ve, positive.
**Denote highly significant values.

Also in our results, there was a statistically significant relation between family history and gene polymorphism at codon 16 in group I and at codons 16 and 27 in group II. However, there were no relations between gene polymorphism and other clinical data (**Supplementary Tables S1 and S2** online).

DISCUSSION

The most common polymorphisms of the β_2AR gene are at codons 16 and 27, and these polymorphisms may have a modulatory effect on asthma severity and response to therapy (16,17).

Genetic assessment of our study population 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% healthy children were homozygous for Arg–Arg, 9.7% were homozygous for Gly–Gly, and 11.5% were heterozygous Arg–Gly. From these findings, we can 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.

Genetic assessment of our study population revealed that at amino-acid position 27 of the β_2AR gene 42.3% were homozygous for Gln–Gln, 5.8% were homozygous for Glu–Glu,

Table 3. β_2AR gene polymorphism and haplotypes at codons 16 and 27 of the cases and control group

Variable	Cases ($n=104$)		Control ($n=52$)		χ^2	P	Odds ratio (CI)
	No.	%	No.	%			
Codon 16							
Arg–Arg	46	44.2	41	78.8	**16.84**	**<0.001****	**4.7 (2.05–10.95)**
Arg–Gly	48	46.2	6	11.5	**18.35**	**<0.001****	**6.57 (2.42–18.81)**
Gly–Gly	10	9.6	5	9.7	0	1	1 (0.29–3.59)
Codon 27							
Gln–Gln	44	42.3	18	34.6	0.86	0.35	1.39 (0.66–2.93)
Gln–Glu	54	51.9	30	57.7	0.46	0.5	0.79 (0.39–1.63)
Glu–Glu	6	5.8	4	7.7	0.21	0.64	0.73 (0.17–3.28)
Arg–Arg × Gln–Gln	16	15.4	14	27	2.97	0.09	2.03 (0.83–4.92)
Arg–Arg × Gln–Glu	30	28.8	26	50	**6.74**	**0.009***	**2.47 (1.17–5.22)**
Arg–Arg × Glu–Glu	0	0	1	1.9	2.01	0.16	0.09 (0.01–1.71)
Arg–Gly × Gln–Gln	24	23.1	4	7.7	**5.57**	**0.02***	**3.60 (1.09–13.09)**
Arg–Gly × Gln–Glu	24	23.1	2	3.8	**9.23**	**0.002***	**7.9 (2.39–21.63)**
Arg–Gly × Glu–Glu	0	0	0	0	—	—	—
Gly–Gly × Gln–Gln	4	3.8	0	0	2.05	0.25	—
Gly–Gly × Gln–Glu	0	0	2	3.8	1.01	0.31	—
Gly–Gly × Glu–Glu	6	5.8	3	5.8	0	1	1 (0.21–5.3)

β_2AR, B2 adrenergic receptor; CI, confidence interval.
*Denote significant values.
**Denote highly significant values.
Bold values demonstrates significant results.

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Table 4. β_2AR gene polymorphism at codons 16 and 27 of all groups of study

Variable	Group I ($n=52$)		Group II ($n=52$)		Group III (control; $n=52$)		χ^2	P	P within groups
	N	%	N	%	N	%			
Codon 16									
Arg–Arg	34	65.4	12	23.1	41	78.8	**41.79**	**<0.001****	**<0.001**[a]
Arg–Gly	18	34.6	30	57.7	6	11.5			**0.01**[b]
Gly–Gly	0	0	10	19.2	5	9.7			**<0.001***[c]
Codon 27									
Gln–Gln	20	38.5	24	46.2	18	34.6	8.5	**0.04***	**0.02**[a]
Gln–Glu	32	61.5	22	42.3	30	57.7			0.12 N.S.[b]
Glu–Glu	0	0	6	11.5	4	7.7			0.29 N.S.[c]

β_2AR, B2 adrenergic receptor.
[a]Group I vs. Group II.
[b]Group I vs. Group III.
[c]Group II vs. Group III.
*Denote significant values.
**Denote highly significant values.
Bold values demonstrates significant results.

Table 5. Relation between β_2AR gene polymorphism at codons 16 and 27 of group II and severity of disease

Variable	Arg–Arg ($n=12$)		Arg–Gly ($n=30$)		Gly–Gly ($n=10$)		χ^2	P
	N	%	N	%	N	%		
Severity							18.98	0.001**
Mild persistent	2	16.7	4	13.3	0	0		
Moderate persistent	10	83.3	26	86.7	6	60		
Severe persistent	0	0	0	0	4	40		
Variable	Gln–Gln ($n=24$)		Gln–Glu ($n=22$)		Glu–Glu ($n=6$)		χ^2	P
	N	%	N	%	N	%		
Severity							34.44	<0.001**
Mild persistent	2	8.3	4	18.2	0	0		
Moderate persistent	22	91.7	18	81.8	2	33.3		
Severe persistent	0	0	0	0	4	66.7		

β_2AR, B2 adrenergic receptor.
**Denote highly significant values.

and 51.9% were heterozygous Gln–Glu. On the other hand, 34.6% healthy children were homozygous for Gln–Gln, 7.7% were homozygous for Glu–Glu, and 57.7% were heterozygous for Gln–Glu. From these findings, we can observe that there was no statistically significant difference between cases and control group at codon 27.

Genetic assessment of the two asthmatic groups showed that there was a statistically significant difference between the two case groups at codons 16 and 27; as regard codon 16 in group I, homozygous for Arg–Arg were more frequent, whereas in group II homozygous for Gly–Gly and heterozygous for Arg–Gly were more frequent. As regard codon 27 in group I, heterozygous for Gln–Glu were more frequent (61.5%), whereas in group II homozygous for Gln–Gln and homozygous for Glu–Glu were more frequent.

Genetic assessment of haplotypes at codons 16 and 27 of β_2AR in case and control groups revealed that there was statistically significant difference between cases and control group in some haplotypes with an increased risk of Arg–Gly × Gln–Gln in cases four times more than control and an increased risk of Arg–Gly × Gln–Glu eight times in cases more than in control group, whereas Arg–Arg × Gln–Glu frequency increased by three times more than that in control. This means that this haplotype may carry a protective effect against asthma in control.

In an Egyptian study, Salama *et al.* reported a higher frequency of Arg–Gly genotype at codon 16 in asthmatic children in spite of different distribution frequencies of β_2AR genotypes from our results (18). Al-Rubaish showed that, although a significant difference was observed in genotype frequencies at codon 16 Arg–Gly between the asthmatic and control subjects, no statistically significant difference was observed in allele frequencies between the two groups. In addition, no statistically significant differences were observed in genotype and allele frequencies at codon 27 Gln–Glu between the control and asthmatic groups. In addition,

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Table 6. Relation between β_2AR gene polymorphism at codons 16 and 27 of group I and IgE and pulmonary function tests

Variable	Arg–Arg ($n = 34$)	Arg–Gly ($n = 18$)	*T*-test	*P*
IgE				
Mean ± SD	123.24 ± 35.81	119.78 ± 45.30	0.3	0.76
Range	98–200	80–210		N.S.
FEV1				
Mean ± SD	82.53 ± 1.78	84.22 ± 2.05	3.1	0.003**
Range	80–85	81–88		
FVC				
Mean ± SD	96.47 ± 3.08	97.33 ± 1.45	1.12	0.26
Range	91–100	95–99		N.S.
FEV1/FVC				
Mean ± SD	85.41 ± 2.06	86.89 ± 1.64	2.63	0.01*
Range	82–89	84–89		
Variable	Gln–Gln ($n = 20$)	Gln–Glu ($n = 32$)	*t*-test	*P*
IgE				
Mean ± SD	138.0 ± 43.48	137.56 ± 40.22	0.04	0.97
Range	80–210	90–190		N.S.
FEV1				
Mean ± SD	81.6 ± 1.54	84.06 ± 1.70	5.26	< 0.001**
Range	80–84	80–88		
FVC				
Mean ± SD	96.20 ± 2.59	97.13 ± 2.66	1.23	0.22
Range	91–98	91–100		N.S.
FEV1/FVC				
Mean ± SD	84.70 ± 1.84	86.13 ± 1.79	2.76	0.008**
Range	82–88	84–89		

β_2AR, B2 adrenergic receptor; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity, N.S., not significant.
*Denote significant values.
**Denote highly significant values.

their study concluded a poor association of individual single-nucleotide polymorphism with asthma (19). Another Egyptian study on β_2AR gene polymorphism reported the same like our results at codon 16 only (20).

As regards the severity of asthma in group II in the current study, there was a statistically significant relation between the grade of asthma severity and the presence or absence of Gly16 either homozygous or heterozygous. Their frequencies are shown in Table 5. Our finding detected that Arg–Arg and Arg–Gly were presented by mild and moderate asthma, whereas Gly–Gly was presented by moderate and severe asthma. In group I comprising patients with mild intermittent asthma, 65.4% are homozygous for Arg and 34.6% are heterozygous for Arg–Gly.

As regards the severity of asthma in our study, there was a statistically significant relation between the grade of asthma severity and the presence or absence of Glu 27 either homozygous or heterozygous. Frequencies are shown in Table 5. Our finding detected that genotypes Gln–Gln and Gln–Glu were presented by mild and moderate asthma, whereas the Glu–Glu genotype was presented by moderate and severe asthma. As regards the first group comprising patients with mild intermittent asthma, 38.5% were homozygous for Glu–Glu and 61.5% were heterozygous for Gln–Glu.

Larocca *et al.* assessed the polymorphisms at amino acid 16 and 27 of β_2AR in asthmatic patients. They concluded that genetic typing of β_2AR polymorphism may help to identify

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Table 7. Relation between β_2AR gene polymorphism at codon 16 and codon 27 of group II, and IgE and pulmonary function tests

Variable	Arg–Arg ($n=12$)	Arg–Gly ($n=30$)	Gly–Gly ($n=10$)	ANOVA; test	*P*	LSD
IgE						0.36 N.S.[a]
Mean ± SD	173.33 ± 33.26	195.20 ± 73.49	195.2 ± 85.92	0.46	0.63	0.46 N.S.[b]
Range	140–220	90–320	80–310		N.S.	1 N.S.[c]
FEV1						0.13 N.S.[a]
Mean ± SD	65.33 ± 7.07	68.47 ± 6.52	59.8 ± 1.03	**7.82**	**0.001****	**0.04*** [b]
Range	60–72	62–74	58–61			**<0.001**** [c]
FVC						0.28 N.S.[a]
Mean ± SD	76.5 ± 5.81	74.53 ± 5.05	73.6 ± 4.70	0.96	0.39	0.19 N.S.[b]
Range	70–86	71–92	69–81		N.S.	0.61 N.S.[c]
FEV1/FVC						0.79 N.S. [a]
Mean ± SD	85 ± 5.66	84.53 ± 5.25	79.4 ± 4.88	**4.04**	**0.02***	**0.02*** [b]
Range	75–93	77–96	74–88			**0.01*** [c]
Variable	Gln–Gln ($n=24$)	Gln–Glu ($n=22$)	Glu–Glu ($n=6$)	ANOVA; test	*P*	LSD
IgE						0.85 N.S.[d]
Mean ± SD	193.75 ± 61.03	189.91 ± 67.49	176.67 ± 106.71	0.14	0.87	0.59 N.S.[e]
Range	90–320	90–320	80–310		N.S.	0.68 N.S.[f]
FEV1						0.37 N.S.[d]
Mean ± SD	68.75 ± 5.26	70.36 ± 6.68	59.67 ± 1.37	**8.43**	**0.001****	**<0.001**** [e]
Range	60–74	61–77	58–61			**<0.001**** [f]
FVC						0.36 N.S.[d]
Mean ± SD	75.4 ± 4.41	74.18 ± 4.64	73 ± 6.2	2.76	0.07	0.17 N.S.[e]
Range	70–92	75–80	69–81		N.S.	0.61 N.S.[f]
FEV1/FVC						0.25 N.S.[d]
Mean ± SD	86.5 ± 4.46	88.18 ± 5.25	82 ± 6.45	**3.58**	**0.04***	**0.05*** [e]
Range	77–93	75–96	74–88			**0.02*** [f]

β_2AR, B2 adrenergic receptor; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity, N.S., not significant.
[a]Arg–Arg vs. Arg–Gly.
[b]Arg–Arg vs. Gly–Gly.
[c]Arg–Gly vs. Gly–Gly.
[d]Gln–Gln vs. Gln–Glu.
[e]Gln–Gln vs. Glu–Glu.
[f]Gln–Glu vs. Glu–Glu.
*Denote significant values.
**Denote highly significant values.
Bold values demonstrates significant results.

individuals with asthma resistant to β_2-adrenergic agonist or difficult-to-treat asthma and found that Arg/Arg polymorphism at amino acid 16, as well as the Arg/Arg-Gln/Glu haplotype, is common in a healthy population (21). Salah *et al.* revealed the association of Arg–Arg genotype with mild asthma and the association of Gly–Gly genotype with moderate/severe asthma (20). A meta-analysis about 28 studies performed on β_2AR gene polymorphism concluded that Gly–Gly genotypes had a higher risk for nocturnal asthma and asthma severity than Arg–Arg (22). De Paiva *et al.* reported that the Arg16Gly and Gln27Glu polymorphisms in the β_2AR gene are associated with the degree of asthma severity (23). This may occur secondary to the downregulation of β_2AR upon substitution of arginine with glycine at codon 16 Arg16Gly *in vitro*, leading to increased bronchial hyper-reactivity (25).

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In contrast to our results, Salama *et al.* found higher frequencies of Arg16Gly genotype and lower frequencies of Gly16Gly genotype in severely asthmatic children in comparison with mild/moderate asthma (18). Another Egyptian study reported that nocturnal asthma was associated with β_2AR Arg16Gly polymorphisms but not with β_2AR Gln27Glu polymorphisms (25).

Regarding serum IgE in our study, there was a statistically significant difference between all groups with the higher level in case groups more than the control group. This run with Larocca *et al.* who found the same results (21). In our study, IgE was higher in the Arg–Arg group at codon 16 but without a significant difference and show the similar level at codon 27 in different genotype in group I. In group II the IgE level was higher in Arg–Gly and Gly–Gly but without a significant difference at codon 16, whereas in codon 27 Gln–Gln and Gln–Glu had an elevated IgE level than Glu–Glu but also without significant difference. Giubergia *et al.* detected a significant difference of the IgE level among polymorphism at codon 16 with the highest IgE level in Arg–Arg, but at position 27 the result shows no significant difference of the IgE level at different haplotypes; moreover, when population was stratified according to the most common homozygous haplotype no association was found in relation to serum IgE (26).

In the current study, there was a statistically significant relation between family history and gene polymorphism but no statistical relation between gene polymorphism with the history of exercise-induced asthma or history of passive smoking. Wang *et al.* concluded that childhood exposure to tobacco smoke was associated with an increased risk for wheezy chest, and the risk was greater for cases with Arg–Arg genotype or two copies of Arg16–Gln27 Diplo-type (27). However, other studies did not find an association between Arg16 and airway responsiveness in heavy smokers (28).

Pulmonary function tests in our study detected a stepwise reduction in the mean values of FEV_1, FVC, and FEV_1/FVC ratio with a gradual increase in the severity of asthma process in two studied patient groups, and this decrease was more significant in group II in comparison with group I. This indicates that there were correspondences between clinical data of the asthmatic children and results of their pulmonary function tests. Another study reported that the genetic polymorphism of β_2AR does not represent an increased risk for developing asthma, yet are important in therapy outcome (23).

As regards the response to treatment in group I, there was a statistically significant relation between gene polymorphism at codon 16 and codon 27 and both FEV_1 and FEV_1/FVC ratio with the increased mean of both among Arg–Gly and Gln–Glu. In addition, in group II, there were statistically significant relations between gene polymorphism at codon 16 and codon 27 with both FEV_1 and FEV_1/FVC ratio. LSD showed the difference present between Gly–Gly and other genes at codon 16 and Glu–Glu and other genes at codon 27. Both show less FEV_1 and FEV_1/FVC ratio than other genes, with an increasing mean of both among Arg–Gly and Gln–Glu. This means that asthmatic heterozygous at codons 16 and 27 had a better response to treatment with either bronchodilators only or combined therapy. Our data detected that asthmatic patients with Gly–Gly and Glu–Glu genotypes do not respond to bronchodilator but to bronchodilator and ICS.

This run with previous studies that found the asthmatic population with Arg–Gly × Gln–Glu haplotypes showed the greatest change in post-bronchodilator FEV1 increasing therapy effectiveness (7,21,29). In addition, De Paiva et al. found that asthmatic subjects homozygous for Glu 27 have less airway responsiveness than asthmatic homozygous for Gln 27 (23). Other two studies reported no response to inhaled B2 agonist in asthmatic children associated with the Gly allele in comparison with those with the Arg allele at codon 16 of β_2AR. They found no association between clinical response to B2 agonist and β_2AR polymorphism at codon 27 ((refs 30,31)). Other studies reported that asthmatic patients carrying the Arg–Arg genotype at codon 16 of β_2AR achieve better asthma control with long-term use of combined treatment with long-acting beta 2 agonists and ICS (32,33). Reports suggested that 60% of asthmatic patients who are 16 Arg–Arg may respond favorably to albuterol compared with only 13% of individuals who are 16 Gly–Gly (10,34,35).

However, different studies found that asthma patients carrying the Arg–Arg or Gly–Arg genotype form may have a mild benefit or even no benefit compared with Gly–Gly subjects after salmeterol inhalation was initiated either in the absence or presence of concurrent ICS use (9,36,37,38). Some authors suggest that the Arg16Gly and Gln27Glu polymorphisms are not markers of susceptibility or severity of asthma and do not affect β_2AR gene expression during the rescue therapy (9,39–41). The reason that Arg–Arg patients failed to achieve a satisfactory response to salmeterol is unclear. Some explained this by the difference in receptor downregulation between the polymorphic variant of β_2AR (24).

The different results reported on β_2AR gene polymorphism, and its effect on severity and response to treatment were because of many factors. First, the difference in the sample size of cases, age, and severity. Second, the difference in B2-agonists used in the studies. Third, the use of different outcome measures and end points to evaluate the response to treatment. Fourth, the probability of the presence of different β_2AR haplotypes rather than single allele polymorphism. Finally, the racial and environmental factors (30).

CONCLUSION

It was revealed that there was a higher frequency of Arg–Gly genotypes among Egyptian asthmatic children and higher frequency of Arg–Arg among control group at codon 16. The presence or absence of Gly 16 or Glu 27 either homozygous or heterozygous for both correlated with the grade of asthma severity. Asthmatic patients with Gly–Gly and Glu–Glu genotypes do not respond to bronchodilator but respond to bronchodilator and ICS. However, we cannot exclude the possibility that other polymorphisms or complex haplotypes within the promoter and coding regions of the β_2-AR gene or adjacent genes might contribute to the present results.

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Original article: Case-control study conducted in the Pediatric department of Zagazig University Hospital, Egypt.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

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