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Beta$_2$-adrenergic receptor variants in children and adolescents with bronchial asthma

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1. ABSTRACT

The region encoding the N-terminal of human β2-adrenergic receptor gene (*ADRB2*) shows several polymorphisms. To this end, we studied change in susceptibility and/or response to therapy in 175 asthmatic children and adolescents by the two most common variants of the *ADRB2* gene namely, rs1042713 (Gly16Arg) and rs1042714 (Gln27Glu). Although, the variants did not correlate with risk of development nor with the severity of the asthma, Gly16/Glu27 haplotype in homozygous individuals conferred protection against development of asthma and was associated with a lower frequency of dyspnea and sputum production. In contrast, the Arg16/Gln27 haplotype was associated with a better response to treatment. These findings show that the risk of development of asthma or response to treatment can be, respectively, deciphered by the detection of both rs1042713 and rs1042714 variants in *ADRB2* gene.

2. INTRODUCTION

Bronchial asthma is the most common chronic respiratory disease in childhood and adolescence. The prevalence of asthma in developed countries is increasing to epidemic proportions (1). In Egypt, asthma affects 15-16 % of children aged 3-15 years and one in four children with asthma fails to attend school regularly because of poor asthma control (2). Asthma is a complex multifactorial disease for which a strong genetic element has been firmly established (3).

Figure 1. Drug targets interactions of β2-AR. Annotated chemical-protein interactions are retrieved from curated chemical interaction data. The network is composed of 90 nodes and 94 edges. Agonists in green box, partial agonists in blue box, and antagonist in red box (Data source: The BioGRID interaction database Version 3.4.1.5.9 (https://thebiogrid.org)).

The identification of genes and molecular pathways involved in its pathogenesis will be of great value in predicting disease outcome and establishing targeted therapeutic approaches (3,4).

Bronchial asthma is characterized by chronic inflammation of the airways, mucus hypersecretion and bronchial hyperresponsiveness (BHR) to various stimuli, resulting in intermittent airflow obstruction that is often reversible either spontaneously or with treatment (5-7). The β_2-adrenergic receptor (β_2-AR), a member of G protein coupled receptor family, is abundantly expressed in the respiratory tract (in bronchial smooth muscle cells, airway epithelial cells, mononuclear cells, and presynaptic cholinergic nerve terminals) (8). It mediates various physiological airway responses, including bronchodilation, vasodilatation and improvement of mucociliary clearance, as well as various anti-inflammatory actions (e.g., mast cell stabilization, cytokine production, reduction of protein extravasation in the airways) (9, 10). Inhaled β_2-AR agonists are therefore used as first line bronchodilators in asthma (11, 12) (Figure 1).

The β_2-AR gene (*ADRB2*) (MIM#109690) is located on chromosome 5q31-32 (13). Numerous single nucleotide polymorphisms (SNPs) exist in the amino terminal region of *ADRB2*, the most common of which are rs1042713 (c.46A>G) and rs1042714 (c.79C>G) (14). These SNPs have been found to alter the amino acid sequence of the β_2-AR in the area flanking the receptor ligand site (Arg16Gly and Gln27Glu, respectively) causing receptor down-regulation and desensitization (10). Our research group has demonstrated that these *ADRB2* polymorphisms are associated with a higher risk of myocardial infarction and of chronic obstructive pulmonary disease, as well as an increased bronchodilator response in Egyptian patients (10, 14). It has been reported that these polymorphisms may result in an increased susceptibility of asthma and bronchodilators response heterogeneity (15, 16). However, inconsistent and conflicting results were reported across various ethnic populations in Asia, South America, and Europe (17).

Due to lack of consistent data regarding the influence of *ADRB2* polymorphisms on different asthma-related phenotypes and the unique genetic make-up of Egyptians due to several colonization events, we aimed at studying the association of these two SNPs with asthma risk and severity and with bronchial hyper-responsiveness in a group of Egyptian

asthmatic children and adolescents. We also explored the potential structural and the functional impact of the aforementioned variants by *in silico* computational tools.

3. MATERIALS AND METHODS

3.1. Study participants

This study was conducted in two groups: asthma individuals: 75 patients diagnosed with bronchial asthma, and control group: 100 age-matched healthy controls. Asthmatic patients (3-15-years-old) were recruited from the Pediatrics and the Allergy and Immunology clinics of the Suez Canal University Hospital, Ismailia, Egypt, from March 2017 to August 2017. Asthmatic patients were diagnosed according to the Global Initiative for Asthma (GINA). Comorbidities, levels of asthma symptom control over the last 4 weeks, disease severity, therapeutic level and response, and adherence to treatment were assessed based on GINA guidelines (18). Chest radiographs were acquired to exclude other causes of airflow limitation. Asthmatic patients with chronic comorbidities, with manifestations of acute respiratory infection within 3 weeks, on systemic steroids within 2 weeks, or non-compliant to treatment were excluded from the study. Control subjects were healthy children who accompanied their sibs to the Pediatrics Clinic and had no previous history of atopy or chronic airway disease. The study was approved by the Medical Research Ethics Committee of the Suez Canal University Faculty of Medicine (Ismailia, Egypt). Written informed consents were obtained from parents/guardians of the participants.

3.2. Pulmonary function tests

Spirometry was performed in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (19). Basal pulmonary parameters, including forced expiratory volume in first second (FEV_1), forced vital capacity (FVC), and peaked expiratory flow rate (PEFR) were determined. A bronchial airway provocation test using methacholine at different dose gradients was performed to assess airway hyper-responsiveness of patients (7). For each subject, the methacholine dose causing a 20% decline in FEV_1 (in mg/mL) was recorded. The positive cutoff value of *Methacholine challenge test* is defined as a PC_{20} < 8 mg/ml (20).

3.3. Anthropometeric measurements

Body mass index (BMI) was calculated as kg/m^2 and transformed into z scores and percentiles adjusted for age and gender (21, 22). Sexual maturity rating was assessed by a single specialist following Tanner staging (23).

3.4. Biochemical analysis

Whole venous blood samples were collected in EDTA vacutainers. Serum total IgE was measured by enzyme-linked immunosorbant assay (ELISA), whereas, absolute eosinophil count (AEC) was counted by Coulter counter. Absolute esinophilic count <0.1. x 10^3/µl and total IgE concentrations <90 IU/ml were considered normal (24).

3.5. Allelic discrimination of *ADRB2* variants

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard® Genomic DNA Purification Kit (Promega Co., USA), quantified by absorbance at 260/280 nm using NanoDrop-1000 spectrophotometer (NanoDrop Tech., Wilmington, USA), and stored at -20°C. Genotypes of the *ADRB2* rs1042713 and rs1042714 SNPs were assayed using Real-Time polymerase chain reaction allelic discrimination TaqMan assays (Applied Biosystems, assay ID C_2084764_20 and C_2084765_20). Alleles for rs1042713 are G and A (Gly_{16} and Arg_{16}, respectively) and for rs1042714 are C and G (Gln_{27} and Glu_{27}, respectively). PCR reaction was carried out in a 25-µl reaction volume containing 20 ng sample DNA, 12.5 µl TaqMan Universal PCR Master Mix (containing AmpErase UNG, AmpliTaq Gold enzyme, dNTPs, and reaction buffer) and 1.2.5 µl of TaqMan SNP genotyping assay Mix. Positive and negative controls were run for each plate. PCR amplification was carried out in StepOne™ Real-Time PCR System (Applied Biosystems, USA) according to the following conditions: two initial holds (50°C for 2 min and 95°C for 10 min) followed by a 40-cycle two-step PCR (95°C denaturation for 15 s and annealing/extension 60°C for 1 min). 10% of the randomly selected samples were re-genotyped in separate runs to exclude the possibility of false genotype calls with a 100% concordance rate.

3.6. *In silico* data analysis

The workflow adopted in this study followed that of a prior publication by this research group (22). In brief, genomic sequence data was retrieved from NCBI. Functional and structural analysis of the promoter region was performed via Eukaryotic Promoter Database. Genome annotation for *ADRB2* transcripts was performed using ECgene and ensemble software. Several databases were used for protein analysis (peptide full sequence identification, secondary structure prediction conserved domains and essential domains identification) including Ensemble, Protein Data Bank, UNiProt/SwissProt, and Protter. Functional annotation was performed using pFam, Prosite, and eMotif. Gene and protein expression in the respiratory tract was identified using GEO. Subcellular localization was determined using

Compartment program. Protein-protein interaction data was retrieved using STRING database version 10. *ADRB2* variations were obtained from Ensembl. Functional prediction of mutation consequence was determined by polymorphism phenotyping (PolyPhen) version 2.0. Finally, the haplotype block structure of *ADRB2* was compared in different populations using HapMap project.

3.7. Statistical analysis

Statistical analysis was carried out using the *"Statistical Package for the Social Sciences (SPSS) for windows"* software, version 23. The Hardy-Weinberg equilibrium (HWE) was calculated Online (http://www.oege.org/software/hwe-mr-calc.shtml) and tested by χ^2 test to compare the expected versus observed distribution of genotypes. The allele frequency and carriage rate was calculated as previously described (22). Haplotype analysis was performed using Haploview software version 4.0. Odds ratios (OR) with a 95% confidence interval (CI) were calculated to determine the effect of each genotype according to the genetic association models. Adjustment of the effect of potential confounders was performed by binary logistic regression analysis. A two-tailed *P*-value of 0.05 was considered statistically significant.

4. RESULTS

4.1. Characteristics of the study participants

Demographic, clinical, and laboratory characteristics of the study participants are shown in Table 1. Asthmatic patients had a significantly higher frequency of positive family history of bronchial asthma ($P = 0.037$).

4.2. Molecular analysis of *ADRB2* SNPs

Genotype frequencies of the *ADRB2* SNPs in both asthma patients and controls were in agreement with frequencies expected by HWE ($P > 0.05$), Table 2. The G allele of the rs1042713 SNP (Gly_{16}) and the A allele of the rs1042714 SNP (Gln_{27}) were more frequent in the study population. However, no significant differences in genotype frequencies or allele frequencies were observed for either SNPs when comparing asthmatic patients and controls under all genetic association models, Table 3.

Haplotype analysis of the study population demonstrated the presence of four haplotype allele combinations. The frequencies of Gly_{16}/Gln_{27}, Gly_{16}/Glu_{27}, Arg_{16}/Gln_{27}, and Arg_{16}/Glu_{27} haplotypes were 27.6 %, 34.9 %, 35.9 % and 1.7 % respectively, Table 4. Homozygote carriers of the Gly_{16}/Glu_{27} haplotype were less likely to develop asthma ($P < 0.05$), Table 5.

4.3. *ADRB2* SNPs and disease characteristics

When asthma patents were stratified according to disease characteristics and laboratory findings, no significant association was found with any of the *ADRB2* genotypes, Table 6. However, asthma patients with the Arg_{16}/Gln_{27} haplotype had higher post-bronchodilator FEV1 (65.3 ± 12) indicative of a better response to therapy, as compared to non-carriers of the same haplotype (58.9 ± 11) ($P < 0.05$). Moreover, patients with the Gly16/Gln27 haplotype had less frequent dyspnea and sputum formation ($P = 0.011$ and 0.019, respectively), Table 7.

4.4. Structural genomic analysis of *ADRB2*

The gene encoding β_2-AR (*ADRB2*) is located on chromosome 5q31-q32 from position 148825245 to position 148828687 (3443 nucleotide long) (*homo sapiens* assembly; GRCh38.p2:CM000667.2). It is intronless; consisting of only one exon. The gene has a single transcript on the forward strand (ENST00000305988) 2041 nucleotides long, including the 5'-and 3'-untranslated regions (UTR). The protein-coding region spans 1242 nucleotides within the full-length transcript; these encode the 413 amino acid residues forming the receptor (Met-1 to Leu-413). Promoter analysis revealed the presence of GC-boxes at positions -484, -428, -367, and -120.

4.5. Genetic variants of *ADRB2*

ADRB2 is highly polymorphic, particularly in the coding region. Most of the variants are benign missense mutations. We studied two common missense mutations in the sequence coding for the N terminal extracellular domain of the protein: c.46G>A (G16R) and c.79C>G (Q27E) at 5:148826877 and 5:148826910, respectively, Figure 2. Online computational tools (Polyphen and SIFT) predicted Gly16Arg and Gln27Glu variants to be benign with a score of 0.043 (sensitivity: 0.94; specificity: 0.83), and 0.009 (sensitivity: 0.96; specificity: 0.77), respectively. However, analysis of nearby miRNA binding sites in *ADRB2* illustrated complementary sites for miR-26a, miR-26b, miR-1297, and miR-590-3p at the 5' end of the gene.

4.6. Structural and functional analysis of β_2-AR

The β_2-AR is a single polypeptide chain consisting of 413 amino acid residues with a molecular weight of 46.5 KDa. It is composed of an extracellular amino terminus, seven transmembrane-spanning domains, 3 intracellular and 3 extracellular loops, and an intracellular carboxyl terminus. The amino acid sequence and predicted membrane topography for the human β_2-AR is presented in Figure 3. After formation of the polypeptide chain, the receptor undergoes three types of post-translational modifications that alter

Table 1. Baseline characteristics of the study groups

Variables		Asthma (n=75)	Control (n=100)	*p*	OR (95% CI)
Demographic data					
(Mean age (y		3.05 ± 9.7	3.09 ± 9.8	0.870	
Age categories	5y≤	(56.0) 42	(55.0) 55	0.983	1.00
	10y≤	(34.7) 26	(36.0) 36		0.94 (0.49-1.80)
	15y≤	(9.3) 7	(9.0) 9		1.01 (0.35-2.95)
Sex	Female	(44.0) 33	(58.0) 58	0.878	1.0
	Male	(56.0) 42	(42.0) 42		1.08 (0.59-1.98)
Residence	Urban	(62.7) 47	(61.0)61	0.876	1.00
	Rural	(37.3) 28	(39.0) 39		0.93 (0.50-1.72)
BMI percentile	85th>	(58.7) 44	(55.0) 55	0.881	1.0
	95th>	(30.7) 23	(34.0) 34		0.84 (0.43-1.63)
	95th≤	(10.7) 8	(11.0) 11		0.90 (0.33-2.45)
Pubertal status	Females	(53.3) 40	(53.0) 53	0.965	1.00
	Males	(46.7) 35	(47.0) 47		0.98 (0.54-1.79)
FH of asthma	Negative	(56.0) 42	(72.0) 72	**0.037**	1.00
	Positive	(44.0) 33	(28.0) 28		2.02 (1.07-3.79)
Disease characteristics					
Risk factor	Allergic	(93.3) 70			
	Non allergic	(94.6) 71			
Onset	Early onset	(49.3) 37			
	Late onset	(50.7) 38			
(Mean duration (y		2.04 ± 5.5			
Symptoms	Cough	(97.3) 73			
	Wheeze	(74.7) 56			
	Dyspnea	(53.3) 40			
	Sputum	(57.3) 43			
	Tightness	(61.3) 46			
Severity	Mild	(45.3) 34			
	Moderate	(32.0) 24			
	Severe	(22.7) 17			
Laboratory data					
(Total IgE (IU/mL		(25-126) 80			
(AEC (x106/L		(34-422) 122			
Pulmonary function test					
FVC		6.95 ± 77.5			
Pre-FEV1		7.40 ± 76.9			
Post-FEV1		8.23 ± 55.3			
Post-PEFR		±11.70 75.1			
BHR	Normal	(44.0) 33			
	Borderline	(9.3) 7			
	Mild	(37.3) 28			
	Moderate/severe	(9.3) 7			

n: number of subjects; BMI: body mass index; FH: family history; AEC: absolute eosinophil count; FVC: forced vital capacity; FEV1: forced expiratory volume in first second; PEFR: peaked expiratory flow rate; BHR: bronchial hyperresponsiveness. Values are shown as number (%) and mean ± standard deviation. Odds ratio (OR) was calculated .at a 95% confidence interval (CI). P-value (p) <0.05 was considered significant

Table 2. Genotype and allele frequencies of *ADRB2* polymorphisms

Variables	**Asthma** (n=75)	**Control** (n=100)	**χ2**	***P***	**OR** (CI 95%)
(Genotype frequency (rs1042713					
GG	(36.0) 27	(36.0) 36	0.989	**0.022**	1.0
GA	(53.3) 40	(54.0) 54			0.9 (0.51-1.88)
AA	(10.7) 8	(10.0) 10			1.1 (0.37-3.06)
Allele frequency					
G	(62.7) 94	(63.0) 126	0.949	**0.004**	1.0
A	(37.3) 56	(37.0) 74			1.0 (0.65-1.57)
P HWE	0.226	0.113			
(Genotype frequency (rs1042714					
CC	(38.7) 29	(40.0) 40	3.438	0.179	1.0
CG	(54.6) 41	(45.0) 45			1.2 (0.66-2.37)
GG	(6.7) 5	(15.0) 15			0.4 (0.15-1.40)
Allele frequency					
C	(66.0) 99	(62.5) 125	0.455	0.499	1.0
G	(44.0) 51	(37.5) 75			0.8 (0.55-1.33)
p HWE	0.059	0.689			

n: number of subjects; p HWE: p value of Hardy-Weinberg equilibrium. Values are shown as number (%). Chi square (c2) for trend was used. Odds ratio (OR) was calculated at a 95% confidence interval (CI). P-value (p) <0.05 was considered significant. The allele frequency within each group was determined as the number of occurrences of an .individual allele divided by the total number of alleles

Table 3. Association between ADRB2 polymorphisms and asthma risk based on genetic models

Genetic model	**rs1042713**	**OR (95% CI)**	**rs1042714**	**OR (CI 95%)**
Homozygote comparison	AA *vs.* GG	1.1 (0.37-3.06)	GG *vs.* CC	0.4 (0.15-1.40)
Heterozygote comparison	GA *vs.* GG	0.9 (0.51-1.88)	CG *vs.* CC	1.2 (0.66-2.37)
Dominant model	GA+AA *vs.* GG	1.0 (0.53-1.86)	CG+GG *vs.* CC	1.0 (0.57-1.95)
Co-dominant model	GA *vs.* GG+AA	0.9 (0.53-1.77)	CG *vs.* CC+GG	1.4 (0.80-2.68)
Recessive model	AA *vs.* GG+GA	1.1 (0.40-2.86)	GG *vs.* CC+CG	0.4 (0.14-1.16)
Allelic model	A *vs.* G	1.0 (0.65-1.57)	G *vs.* C	0.8 (0.55-1.33)

.(Odds ratio (OR) was calculated at a 95% confidence interval (CI

Table 4. *ADRB2* rs1042713/ rs1042714 haplotype allele frequencies

Haplotype rs1042713/ rs1042714	**Alleles**	**Total**	**Asthma** (n=75)	**Control** (n=100)	**χ2**	***p***	**OR** (95% CI)
Gly_{16}/Gln_{27}	GC	83 (27.6)	39 (29.3)	44 (26.2)	1.100	0.294	1.37 (0.75-2.51)
Gly_{16}/Glu_{27}	GG	105 (34.9)	46 (34.6)	59 (35.1)	0.097	0.755	1.10 (0.59-2.03)
Arg_{16}/Gln_{27}	AC	108 (35.9)	45 (33.8)	63 (37.5)	0.163	0.686	0.88 (0.47-1.62)
Arg_{16}/Glu_{27}	AG	5 (1.7)	3 (2.3)	2 (1.2)	0.618	0.432	2.04 (0.33-12.5)

n: number of subjects. Values are shown as number (%). Chi square (c2) test was used. Odds ratio (OR) was calculated at a 95% confidence interval (CI). P-value (p) <0.05 was considered significant. OR for alleles was calculated as .presence versus absence of this particular allele

Table 5. *ADRB2* rs1042713/ rs1042714 haplotype copy number

Haplotype Copy Number rs1042713/ rs1042714	Asthma (n=75)	Control (n=100)	χ2	*p*	Crude OR (95% CI)
Gly_{16}/Gln_{27}					
0 copy	36 (48.0)	56 (56.0)	1.185	0.553	1.0
1 copy	32 (82.1)	35 (35.0)			1.4 (0.75-2.68)
2 copies	7 (17.9)	9 (9.0)			1.2 (0.41-3.53)
Gly_{16}/Glu_{27}					
0 copy	29 (38.7)	41 (41.0)	8.602	**0.014**	1.0
1 copy	44 (58.7)	44 (44.0)			1.4 (0.75-2.66)
2 copies	2 (2.7)	15 (15.0)			0.18 (0.04-0.08)
Arg_{16}/Gln_{27}					
0 copy	30 (40.0)	37 (37.0)	0.403	0.818	1.0
1 copy	37 (49.3)	54 (54.0)			0.8 (0.44-1.59)
2 copies	8 (10.7)	9 (9.0)			1.1 (0.37-3.18)
Arg_{16}/Glu_{27}					
0 copy	72 (96.0)	98 (98.0)	0.618	0.432	1.0
1 copy	0 (0.0)	0 (0.0)			NA
2 copies	3 (4.0)	2 (2.0)			2.0 (0.33-12.5)

n: number of subjects. Values are shown as number (%). Chi square (c2) test was used. Odds ratio (OR) was calculated at a 95% confidence interval (CI). P-value (p) <0.05 was considered significant. OR for alleles was calculated as .presence versus absence of this particular allele

Table 6. Clinical characteristics of asthma patients according to *ADRB2* genotypes

Clinical characteristics	rs1042713 (Gly16Arg) genotypes (n=75)			*p*	rs1042714 (Gln27Glu) genotypes (n=75)			*p*
	GG (Gly_{16}/Gly_{16}) 27 (36.1)	GA (Gly_{16}/Arg_{16}) 40 (53.3)	AA (Arg_{16}/Arg_{16}) 8 (10.6)		CC (Gln_{27}/Gln_{27}) 29 (38.6)	CG (Gln_{27}/Glu_{27}) 41 (54.7)	GG (Glu_{27}/Glu_{27}) 5 (6.7)	
Disease onset								
Early onset (<3y)	9 (33.3)	24 (60.0)	4 (50.0)	0.101	14 (48.3)	19 (46.3)	4 (80.0)	0.360
Late onset (>3y)	18 (66.7)	16 (40.0)	4 (50.0)		15 (51.7)	22 (53.7)	1 (20.0)	
Asthma duration (y)	5.4 ± 2.2	6 ± 2.9	6.2 ± 2.1	0.121	6.2 ± 2.7	6.3 ± 2.6	5.8 ± 3.5	0.723
Symptoms								
Cough	27 (100.0)	39 (97.5)	7 (87.5)	0.155	27 (93.1)	41 (100.0)	5 (100.0)	0.196
Wheezes	22 (81.5)	28 (70.0)	6 (75.0)	0.570	21 (72.4)	32 (78.0)	3 (60.0)	0.639
Dyspnea	10 (37.0)	25 (62.5)	5 (62.5)	0.105	14 (48.3)	23 (56.1)	3 (60.0)	0.774
Sputum	11 (40.7)	28 (70.0)	4 (50.0)	0.054	14 (48.3)	26 (63.4)	3 (60.0)	0.448
Tightness	15 (55.6)	24 (60.0)	7 (87.5)	0.257	19 (65.5)	24 (58.5)	3 (60.0)	0.838
Disease severity								
Intermittent	12 (44.4)	17 (42.5)	5 (62.5)	0.649	17 (58.6)	16 (39.0)	1 (20.0)	0.341
Mild persistent	7 (25.9)	15 (37.5)	2 (25.0)		8 (27.6)	14 (34.1)	2 (40.0)	
Moderate persistent	8 (29.6)	8 (20.0)	1 (12.5)		4 (13.8)	11 (26.8)	2 (40.0)	
Laboratory data								
Total IgE (IU/mL)	81.4 ± 92.1	86.0 ± 91.3	50.3 ± 40.2	0.710	77.5 ± 92.3	78.7 ±85.9	117 ± 79.0	0.280
AEC (x106/L)	249 ± 295	165 ± 247	102 ± 119	0.553	182 ± 256	194 ± 270	178 ± 201	0.826
Pulmonary function test								
FVC	78.2 ± 8.08	78.1 ± 7.34	80.0 ± 8.88	0.818	80.4 ± 7.3	76.8 ± 8.0	79.0 ±2.5	0.160
Pre-FEV1	76.9 ± 8.53	77.0 ± 8.49	78.7 ± 8.46	0.860	79.1 ±7.7	76.4 ± 8.6	71.6 ±8.1	0.127
Post-FEV1	59.2 ± 11.92	64.2 ± 12.5	66.8 ± 11.6	0.167	64.8 ± 13.4	62.4 ± 11.6	53.6 ±8.9	0.169
PEFR	76.4 ± 12.8	75.1 ± 11.1	80.7 ± 10.1	0.461	78.6 ± 11.6	75.2 ± 11.7	69.8 ±8.7	0.217
Positive BHR	15 (55.6)	17 (42.5)	3 (37.5)	0.495	13 (44.8)	18 (43.9)	4 (80.0)	0.302

n: number of patients; y: years;AEC: absolute eosinophil count; FVC: forced vital capacity; FEV1: forced expiratory volume in first second; PEFR: peaked expiratory flow rate; BHR: bronchial hyperresponsiveness. Values are shown as number (%) and mean ± standard deviation. Chi square test was used for categorical variables. One way ANOVA or Kruskal-Wallis tests were used for quantitative data followed by post-hoc Dunnett t (2-sided) test. P-value (p) <0.05 .was considered significant

Table 7. Clinical characteristics of asthma patients according to *ADRB2* haplotypes

Clinical characteristics	Gly_{16}/Gln_{27}		*P*	Gly_{16}/Glu_{27}		*p*	Arg_{16}/Gln_{27}		*p*	Arg_{16}/Glu_{27}		*p*
	Negative 36 (48)	**Positive 39 (52)**		**Negative 29 (39)**	**Positive 46 (61)**		**Negative 30 (40)**	**Positive 45 (60)**		**Negative 72 (96)**	**Positive 3 (4)**	
Disease onset												
(Early onset (<3y	22 (61.1)	15 (38.5)	0.066	14 (48.3)	23 (50.0)	0.884	12 (40)	25 (55.6)	0.240	34 (47.2)	3 (100)	0.115
(Late onset (>3y	14 (38.9)	24 (61.5)		15 (51.7)	23 (50.0)		18 (60)	20 (44.4)		38 (52.8)	0 (0.0)	
(Asthma duration (y	5.8 ± 2.0	5.2 ± 2.0	0.185	5.4 ± 2.1	5.6 ± 2.0	0.691	5.0 ± 1.5	5.8 ± 2.2	0.065	5.5 ± 20	5.6 ± 2.0	0.909
Symptoms												
Cough	35 (97.2)	38 (97.4)	0.954	27 (93.1)	46 (100.0)	0.071	30 (100)	43 (95.6)	0.514	70 (97.2)	3 (100)	0.770
Wheezes	24 (66.7)	32 (82.1)	0.184	21 (72.4)	35 (76.1)	0.788	24 (80.0)	32 (71.1)	0.430	54 (750)	2 (66.7)	0.745
Dyspnea	25 (69.4)	15 (38.5)	0.011	14 (48.3)	26 (56.5)	0.635	13 (43.3)	27 (60.0)	0.167	37 (51.4)	3 (100)	0.243
Sputum	26 (72.2)	17 (43.6)	0.019	14 (48.3)	29 (63.0)	0.238	13 (43.3)	30 (66.7)	0.058	41 (56.9)	2 (66.7)	0.739
Tightness	24 (66.7)	22 (56.4)	0.477	19 (65.5)	27 (58.7)	0.631	17 (56.7)	29 (64.4)	0.629	44 (61.1)	2 (66.7)	0.846
Disease severity												
Intermittent	15 (41.7)	19 (48.7)	0.815	17 (58.6)	17 (37.0)	0.152	12 (40.0)	22 (48.9)	0.458	34 (47.2)	0 (0.0)	0.253
Mild persistent	12 (33.3)	12 (30.8)		8 (27.6)	16 (34.8)		9 (30.0)	15 (33.3)		22 (3.6)	2 (66.7)	
Moderate persistent	9 (25.0)	8 (20.5)		4 (13.8)	13 (28.3)		9 (30.0)	8 (17.8)		16 (22.2)	1 (33.3)	
Laboratory investigations												
(Total IgE (IU/mL	77.6±72	83.8±100	0.758	77.5±92	82.9±85	0.798	89.3±92	75.2±84	0.496	77.5±86	160±68	0.107
(AEC (x106/L	163±223	212±287	0.409	182±256	193±262	0.868	227±287	163±236	0.296	195±261	33.3±7.6	0.290
Pulmonary function tests												
FVC	77.1±7.8	79.5±7.4	0.174	80.4±7.3	77.0±7.6	0.068	78.2±7.6	78.4±7.7	0.899	78.4±7.8	77.6±1.5	0.872
Pre-FEV1	75.5±8.7	78.7±7.8	0.101	79.1±7.7	75.9±8.6	0.105	76.3±8.5	77.7±8.3	0.477	77.4±8.3	70.6±7.0	0.172
Post-FEV1	62.5±12	62.9±12	0.892	64.8±13	61.4±11	0.254	58.9±11	65.3±12	0.027	63.0±12	55.3±8.3	0.293
PEFR	74±11.1	78±11.8	0.117	78.6±11	74.6±11	0.148	75.5±12	76.6±11	0.703	76.5±11	67.6±4.0	0.197
Positive BHR	16 (44.4)	19 (48.7)	0.818	13 (44.8)	22 (47.8)	0.817	17 (56.7)	18 (40.0)	0.167	33 (45.8)	2 (66.7)	0.596

Number of patients=75.y: years; AEC: absolute eosinophil count; FVC: forced vital capacity; FEV1: forced expiratory volume in first second; PEFR: peaked expiratory flow rate; BHR: bronchial hyperresponsiveness. Values are shown as number (%) and mean ± standard deviation. Chi square test was used for categorical variables. .Student-t and Mann-Whitney U tests were used for comparison. P-value (p) <0.05 was considered significant

Table 8. Structural and functional of the topological domains of the β_2-adrenergic receptor

Topology	Position	Length	PTM	Site	Function	Mutagenesis	Consequence
N terminus	1-34	34	Glycosylation	Asn-6, -15			
Transmembrane 1	35-58	24					
Intracellular loop1	59-71	13					
Transmembrane 2	72-95	24		Asp-79	Proper receptor folding & transmembrane assembly of the receptor	Asp-79	Affects binding to catecholamine & uncoupling with Gs protein
Extracellular loop 1	96-106	11	Disulfide bond	Cys-106	Generating the thermodynamically stable conformation of the receptor required for agonist or antagonist binding		Cys-106 substitution could reduce binding affinities 14- to 1400-fold for agonist and 4- to 16-fold for antagonist
Transmembrane 3	107-129	23		Asp113, Thr-118	Agonist or antagonist binding site		
Intracellular loop 2	130-150	21	Phosphotyrosine	Tyr-141	Supersensitization of the receptor	Tyr-141	Abolishes insulin-induced tyrosine phosphorylation & insulin-induced receptor supersensitization
Transmembrane 4	151-174	24					
Extracellular loop 2	175-196	22	Disulfide bond	Cys-184, -190, -191	Generating the thermodynamically stable conformation of the receptor required for agonist or antagonist binding		Cys-184, -190, -191 substitution could reduce binding affinities 14- to 1400-fold for agonist and 4- to 16-fold for antagonist
Transmembrane 5	197-220	24		193-207	Agonist and antagonist binding		
Intracellular loop 3	221-274	54	Phosphoserine by PKA	Ser-246	Mediates agonist-promoted desensitization, internalization, & degradation of the receptor		
Transmembrane 6	275-298	24	Phosphoserine by PKA	Ser-261, -262	Mediates agonist-promoted desensitization, internalization, & degradation of the receptor		
				286-293	Agonist and antagonist binding		
Extracellular loop 3	299-305	7					
Transmembrane 7	306-329	24		312-316	Agonist and antagonist binding		
C terminus	330-413	84	Palmitoylation	Cys-341	Anchoring to the plasma membrane, reduce accessibility of Ser-345 and -346. Agonist stimulation promotes depalmitoylation and further allows Serine .phosphorylation	Cys-341	Uncoupled receptor
			Phosphoserine by PKA	Ser-345, -346	Mediates agonist-promoted desensitization, internalization, & degradation of the receptor		Delayed agonist-promoted desensitization
			Phosphoserine by BARK	Ser--355, -356	Mediates agonist-promoted desensitization, internalization, & degradation of the receptor		
			Hydroxyproline	Pro-382, 395			
				410-413	PDZ-binding: (1) anchoring receptor proteins in the membrane to cytoskeletal components, (2) when endocytosed to prevent degradation in lysosomes & promote recycling to the plasma membrane		

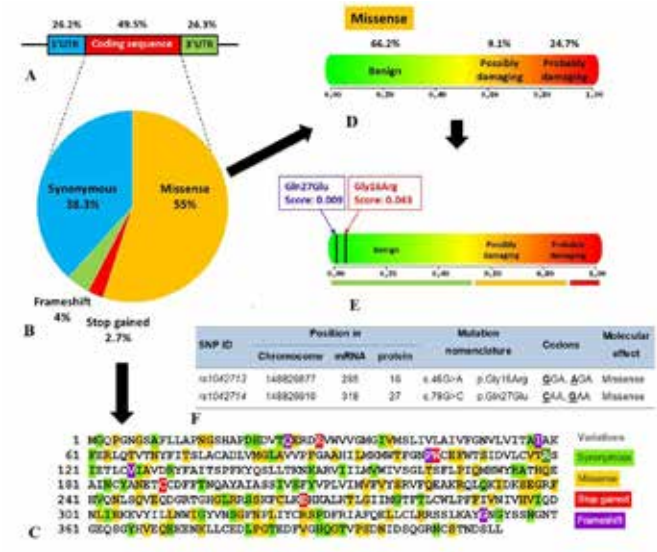

Figure 2. Types and distribution of *ADRB2* polymorphisms. (a) Distribution of variants in *ADRB2*. (b) Types of coding sequence polymorphisms. (c) cDNA sequence with highlighted variations. Positions of coding sequence polymorphisms along β_2-AR sequence are (Uniprot P07550). Single letter amino acids are used. Colored residues highlight variations; light green for synonymous mutations; yellow for missense and start loss; violet for frameshift mutations; and red for gained stop codon (Data was retrieved from Ensemble Annotation release 79 (ENSP00000305372) and consensus CDS protein set (CCDS) database). (d) Predicted functional effects of overall missense coding variants of the whole *ADRB* gene by PolyPhen web-based program. (e) Predicted functional impact of Gly16Arg and Gln27Glu polymorphisms using PolyPhen server. The Gly16Arg and Gln27Glu variants were predicted to be benign with a score of 0.043 (sensitivity: 0.94; specificity: 0.83), and 0.009 (sensitivity: 0.96; specificity: 0.77), respectively. (f) The site of the studied single nucleotide polymorphisms allocated in the protein at the amino-terminal extracellular domain. The first SNP G16R; amino acid substitution from glycine to arginine at codon 16 (Gly16Arg; *rs1042713*) caused by a change of guanine to adenine (G > A) at nucleotide 46. The second SNP Q27E; substitution from glutamine to glutamic acid at codon 27 (Gln27Glu; *rs1042714*) due to the change of cytosine to guanine (C > G) at nucleotide 79.

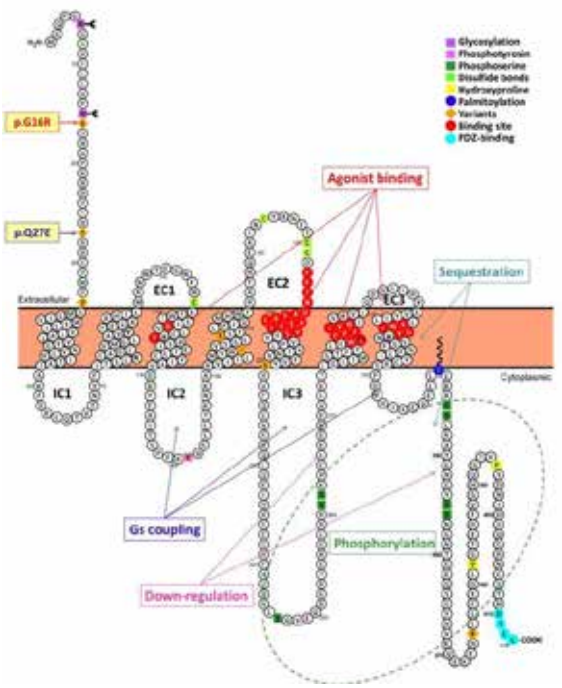

Figure 3. Membrane topography for the human β_2-adrenergic receptor protein. The primary amino acid sequence of β_2-AR is shown. Single letter amino acid nomenclature is used. It is composed of an extracellular amino terminus, seven transmembrane helical regions of 20-25 amino acids each, 3 intracellular and 3 extracellular loops, and an intracellular carboxyl terminus. Important regions and residues are indicated. Location of the studied polymorphisms is shown at the N terminal of the extracellular protein domain. Agonist binding to β_2-AR occurs in the extracellular pockets between the "third and fourth" and "fifth and sixth" transmembrane domains.

protein structure and molecular weight: glycosylation, palmitoylation, and disulfide bond formation, Table 8. The N-linked glycosylation at the amino acids 6 and 15 in the extracellular space, is responsible for membrane insertion and agonist-induced receptor trafficking. Palmitoylation occurs at the cysteine residue of amino acid 341, and is responsible for the reversible anchoring of the carboxy-terminus to the membrane. Palmitoylation has been found to stimulate agonist-induced adenylyl cyclase activity and affects β_2-AR phosphorylation and desensitization. The region between the seventh transmembrane-spanning domain and the palmitoylated cysteine is sometimes denoted as the fourth intracellular loop. Disulfide bond formation has been found to occur at cysteine residues 106 ↔ 191 and 184 ↔ 190. These bonds stabilize the β_2-AR ligand-binding pockets. β_2-AR also contains phosphorylation sites in the third intracellular loop and proximal cytoplasmic tail. Phosphorylation of these sites directly interferes with receptor coupling to stimulatory G proteins and triggers the agonist-promoted desensitization, internalization, and degradation of the receptor (Figure 4).

4.7. Functional enrichment analysis for β_2-AR

Pathway enrichment analysis in the KEGG pathways identified eight pathways: calcium signaling (hsa04020), cGMP-KG signaling (hsa04022), cAMP signaling (hsa04024), neuroactive ligand-receptor interaction (hsa04080), adrenergic signaling in cardiomyocytes (hsa04261), regulation of lipolysis in adipocytes (hsa04923), renin secretion (hsa04924), and salivary secretion (hsa04970). The β_2-AR transmembrane protein is predicted to be located in various components (plasma membrane, lysosomes, endosomes, nucleus, and extracellular matrix). Activated receptors are internalized into endosomes prior to their degradation in lysosomes (UniProt.org), Figure 5. The gene ontology enrichment analysis identified several GO terms for ADRB2. Main molecular functions were adenylate cyclase binding, beta2-adrenergic receptor activity, epinephrine and norepinephrine binding, and potassium channel regulator activity. Protein-protein interaction network is depicted in Figure 5 revealed physical and functional associations with other proteins, and demonstrated some enriched biological processes which are related to asthma pathogenesis; activation of transmembrane receptor protein tyrosine kinase activity, cell-cell signaling, G-protein coupled receptor signaling pathway, negative regulation of smooth muscle contraction, positive regulation of mitogen activated protein kinase (MAPK) cascade, positive regulation of mini excitatory postsynaptic potential, positive regulation of protein ubiquitination, regulation of sodium ion transport, and response to psychosocial stress and cold.

5. DISCUSSION

Though bronchodilators are the main drug of choice for the management of bronchial asthma, multiple clinical trials have showed that 70% of asthmatic patients receiving β_2-AR agonists partially lose their drug-induced protection (25,

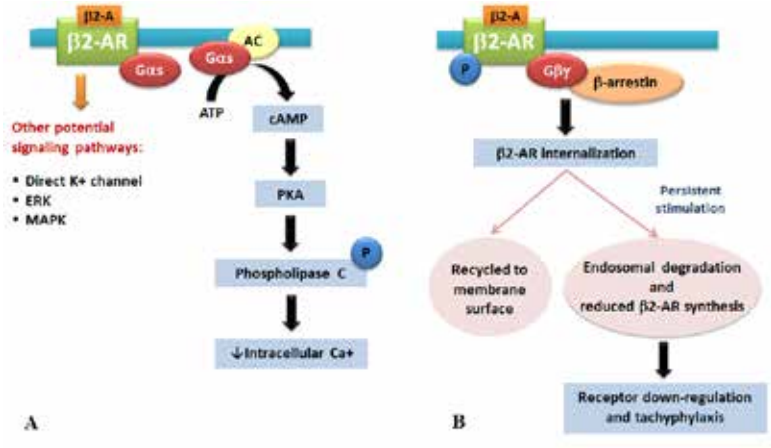

Figure 4. Schematic diagram of conventional β2-AR signaling pathway and receptor trafficking upon activation by β2AR-agonists. A, Binding of β2-AR agonists (β2-A) to β2-AR results in binding to the α-subunit of stimulatory G protein (Gαs), which leads to activation of adenylyl cyclase (AC) and a consequent increase in cAMP. cAMP activates protein kinase A (PKA), which induces phosphorylation (P) of various downstream proteins, including phospholipase C and β2-AR itself. Phopholipase C, together with other potential various mechanisms, e.g. direct K channels, reduces intracellular calcium (Ca2+) thereby causing bronchodilatation. B, Phosphorylated β2-AR, however, is uncoupled from Gαs and binds to the β and α-subunits of inhibitory G protein (Gβα). This terminates the receptor activation signal and stimulates binding to β-arrestin, causing receptor internalization. Receptors are recycled back to the membrane upon prompt removal of the agonist. However, with persistent receptor activation, receptors are down-regulated through degradation of internalized receptors and reduced synthesis of new receptors.

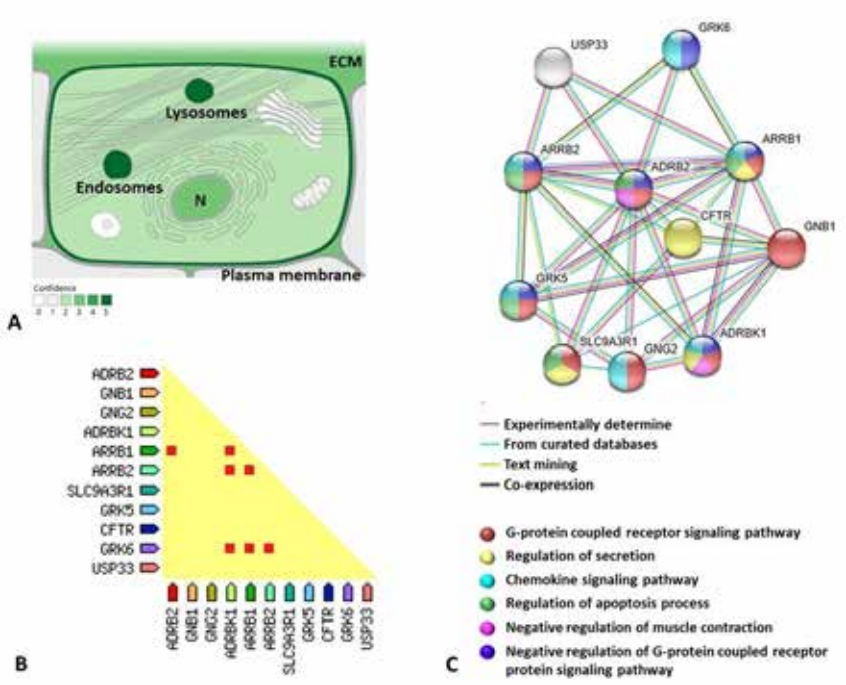

Figure 5. Functional annotation and enrichment analysis of human β2-AR. A, Subcellular localization of β2-AR protein. ECM; extracellular matrix, N; nucleus. The β2-AR transmembrane protein is localized in various components (lysosomes, plasma membrane, endosomes, and extracellular matrix). Activated receptors are internalized into endosomes prior to their degradation in lysosomes. The confidence of each association is noted by the grade of green color with the highest confidence shown by darker color. Image were derived from Compartments: Subcellular localization database, depending on automatic text mining of the biomedical literature and sequence-based predictions (Data source: Compartment database). B, Protein-protein interaction (PPI) network. STRING version 10.5. was used to explore known and predicted direct physical and indirect functional associations. The network is composed of 11 nodes and 34 edges, with average node degree of 6.18 and average local clustering coefficient of 0.709 (PPI enrichment p-value = 9.9.e-05). Functional enrichment biological process is represented by node colors. C, Co-expression analysis in Homo Sapiens. Triangle matrix show proteins whose genes are observed to be correlated in expression across a large number of experiments. ADRB2: Adrenoceptor beta 2, surface; Beta-adrenergic receptors, GNB1: Guanine nucleotide binding protein (G protein), beta polypeptide 1, GNG2: Guanine nucleotide binding protein (G protein), gamma 2, ADRBK1: Adrenergic, beta, receptor kinase 1, ARRB1: Arrestin, beta 1, ARRB2: Arrestin, beta 2, SLC9A3R1: Solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3 regulator 1, GRK5: G protein-coupled receptor kinase 5, CFTR: Cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7), GRK6: G protein-coupled receptor kinase 6, USP33: Ubiquitin specific peptidase 33.

26). Human *ADRB2* is located in a region that has been linked to allergic phenotypes and BHR (27, 28). SNPs in the *ADRB2* gene have been linked to pathogenesis of and therapeutic responses in bronchial asthma (29).

In the current study, we investigated the relationship of *ADRB2* gene variants at codons 16 and 27 with susceptibility and clinical outcomes in asthmatic children and adolescents. These two polymorphisms are located within the amino-terminal extracellular domain of the receptor near the ligand-binding site (15). They are proposed to alter the functional properties of the receptor and its behavior after agonist exposure (30). Both Arg16Gly and Gln27Glu polymorphisms have been linked with a range of disorders, including chronic obstructive pulmonary disease (11), cystic fibrosis (31), congestive heart failure (32), coronary heart disease (14), hypertension (33), rheumatoid arthritis (34), Graves' disease (35), and atopic dermatitis (36), thus highlighting the putative role of these SNPs in immune system-related diseases.

Our results revealed that the distribution of genotype frequencies at position 16 and 27 did not deviate from HWE among patients and controls. The minor allele frequency (MAF) at Arg16Gly variant was identical (0.37 for Arg_{16}) in controls and patients, while at Gln27Glu the frequency of Glu_{27} was 0.37 in controls and 0.44 in the patient group. Overall and stratified analysis revealed no evidence of an association of Arg16Gly and Gln27Glu genotypes with asthma risk or severity under any genetic association models. In addition, both variants did not show associations with clinical features or treatment response. Consistently, previous studies reported no significant differences in genotype and allele frequencies of the two variants with bronchial asthma (30, 37-39). Subsequent meta-analysis studies did not detect association between *ADRB2* polymorphisms and risk of asthma across various ethnic groups (40, 41). In comparison with other various populations and other studies conducted in different regions of Egypt (Table 9), our findings were strikingly consistent with most studied populations and with other studies conducted in the same region, which

Table 9. Comparison of allele frequencies of the rs1042713 and rs1042714 polymorphisms in asthmatic patients in diverse populations

Population	rs1042713		*p* *vs.* current study	rs1042714		*p* *vs.* current study
	G allele	A allele		C allele	G allele	
Egyptian population						
Current study	0.63 (126)	0.37 (74)		0.63 (125)	0.37 (75)	
Hussein *et al.*, 2017 (10)	0.61 (66)	0.39 (42)	0.770	0.63 (68)	0.37 (40)	1.000
Toraih *et al.*, 2014 (13)	0.63 (94)	0.37 (56)	1.000	0.55 (82)	0.45 (68)	0.250
Karam *et al.*, 2013 (42)	0.37 (82)	0.63 (138)	**<0.001**	0.74 (164)	0.26 (56)	0.094
Hamdy *et al.*, 2002 (43)	0.43 (103)	0.57 (137)	**0.004**	0.76 (182)	0.24 (58)	**0.045**
1000 Genome project						
ALL	0.52 (2626)	0.47 (2382)	0.134	0.79 (3985)	0.20 (1023)	**0.008**
AFR	0.48 (634)	0.52 (688)	0.032	0.86 (1142)	0.13 (180)	**<0.001**
AMR	0.54 (377)	0.45 (317)	0.226	0.75 (526)	0.24 (168)	0.050
EAS	0.45 (455)	0.54 (553)	0.012	0.92 (934)	0.08 (74)	**<0.001**
EUR	0.61 (618)	0.38 (388)	0.840	0.59 (594)	0.41 (412)	0.562
SAS	0.55 (542)	0.44 (436)	0.286	0.80 (789)	0.19 (189)	**0.005**
gnomAD exomes						
ALL	0.57 (142444)	0.42 (103492)	0.434	0.68 (168082)	0.31 (78116)	0.397
NFE	0.62 (69972)	0.37 (41472)	0.956	0.57 (63973)	0.42 (47689)	0.434
gnomAD genomes						
ALL	0.57 (17683)	0.42 (13197)	0.434	0.67 (20731)	0.33 (10203)	0.553
NFE	0.62 (9326)	0.37 (5638)	0.956	0.56 (8523)	0.43 (6455)	0.354
HapMap project						
TSI	0.66 (117)	0.33 (59)	0.588	NA	NA	NA
NHLBI Exome Sequencing						
African-American	0.51 (2259)	0.48 (2147)	0.105	0.81 (3597)	0.18 (809)	**0.002**
European-American	0.62 (5351)	0.37 (3249)	0.956	0.58 (4987)	0.42 (3613)	0.469

Allele frequency (count) is shown for each polymorphism. AFR: African; AMR: American; EAS: East Asian; EUR: European; SAS: South Asian; NFE: Non-Finnish European; TSI: Toscans in Italy (Data source: Ensembl.org). P-value (p) <0.05 was considered significant

correlated the study variants with other disorders (10, 14), but differ from that of other regions of Egypt (42, 43) (Table 9). As we speculated previously (44), the heterogeneous genetic profile noted in the current region could be attributed to the historical and geographical background of Suez Canal area where the current research has been executed. This area which links the three continents was controlled by a succession of powerful empires which could affect the genetic profile signatures of the common variants. Additionally, the different study design and type of patients will contribute to the apparent genetic heterogeneity.

It is becoming a necessity that populations with diverse ancestral histories and ethnic backgrounds be included in genetic association studies. Minor allele frequencies of common SNPs in a genomic region greatly differ among study populations. Populations of African descent were formed prior to Asian or European populations, thus are supposedly more prone to genetic variations and diversity. Additionally, this heterogeneity could be also a reflection of the diversity of the mutation pattern among Arabs, as most of the Egyptian population is now of Arab origin. Thus haplotype analysis of more SNPs in the gene and linkage disequilibrium patterns should be undertaken rather than single polymorphism analysis.

Therefore, we performed a haplotype analysis which revealed that the frequencies of Gly_{16}/Gln_{27}, Gly_{16}/Glu_{27}, Arg_{16}/Gln_{27}, and $Arg16/Glu_{27}$ haplotypes in the current study population are 27.6%, 34.9%, 35.9% and 1.7% respectively. Haplotype distributions showed that carrying two copies of the Gly_{16}/Glu_{27} haplotype conferred protection against the development of asthma. A possible explanation is that the Glu_{27} variant is more resistant to down-regulation as has been demonstrated in a previous *in vitro study*, and it is consequently possible that increasing numbers of β_2-ARs are produced (45), while the Gln_{27} allele has been shown to be associated with higher IgE profile (46, 47). Moreover, it has been demonstrated that the Gly_{16} receptor has a higher alveolar-capillary membrane conductance and a better ability to clear fluid from the lungs compared to the Arg_{16} allele (48).

Regarding the clinical outcomes of asthma patients, the Gly_{16}/Gln_{27} haplotype was associated with a significantly lower frequency of dyspnea and sputum production, while carriers of the Arg_{16}/Gln_{27} haplotype exhibited a better response to treatment in

the form of a higher post-bronchodilator FEV1. The results of recent genetic association studies are quite paradoxical. Lee *et al.* (49) reported that children with the Gly_{16}/Glu_{27} haplotypes had a lower risk of wheezing illness with a clear dose-response relationship. The study by Matheson *et al.* (50) showed that the Arg_{16}/Gln_{27} haplotype is associated with severe respiratory symptoms and frequent exacerbations. In contrast, other clinical studies have supported the possibility that the Gly_{16} form of the receptor is associated with markers of more severe asthma (51, 52), while other studies reported the association of the Glu_{27} allele with asthma severity in Asian patients (53).

The precise impact of the Arg16Gly and Gln27Glu polymorphisms on the pathophysiology of bronchial asthma remains unclear. Some studies have demonstrated that these SNPs do not affect the binding capacity of catecholamines to the receptors, the adenylate cyclase activity of the receptor, receptor synthesis rates or agonist-promoted internalization (54). Other studies, however, suggested that these SNPs play a role in the down-regulation phenomenon and functional desensitization of the receptor due to alterations in receptor degradation after the internalization step (55-57) (Figure 4). Further functional studies are required to clarify this issue. In addition, exploring other genetic determinants for BHR is necessary for selecting and adjusting the appropriate medications.

6. ACKNOWLEDGEMENTS

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Abbreviations: β_2-AR: β_2-adrenergic receptor; *ADRB2*, human β2 adrenergic receptor gene; AEC, absolute eosinophil count; ATS/ERS, American Thoracic Society/European Respiratory Society; BHR,

bronchial hyperresponsiveness; BMI, Body mass index; FEV1, forced expiratory volume in first second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; KEGG, Kyoto encyclopedia of genes and genomes; MAF, minor allele frequency; MAPK, mitogen activated protein kinase; PEFR, peaked expiratory flow rate; PTMs, post-translational modification; SNP, single nucleotide polymorphism.

Key Words: Beta$_2$-Adrenergic Receptor Gene, Single Nucleotide Polymorphism, Haplotype, Bronchial Asthma, Egyptian, Review

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