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BETA-2 ADRENERGIC RECEPTOR GENE POLYMORPHISM IN EGYPTIAN ASTHMATIC PATIENTS

AYMAN A. YOUSEF*, OSAMA I. MOHAMMAD*, SHUZAN A. MOHAMMED**,

NADIA L.A ELKHAWAGA***

**Department of Chest Diseases, **Medical Biochemistry Department, Faculty of Medicine,*

*Benha University, Egypt, *Benha Chest Hospital, Egypt.*

Abstract:

Background: Diverse factors, including gene polymorphism, influence the development of asthma predisposing an individual to atopy and airway hyper-responsiveness.

Objective: To explore the contribution of beta-2 adrenergic receptor (β2-AR) gene polymorphisms (Arg16Gly and Gln27Glu) to the risk and severity of adult bronchial asthma in Egypt.

Subjects and Methods: This study was included 75 Egyptian subjects divided into: Group I: 50 bronchial asthma patients subdivided into mild, moderate and severe asthma. Group II: 25 apparently healthy controls. Full history taking, physical examination, plain chest x-ray, pulmonary function tests, and laboratory investigations were performed. Serum IgE was estimated by ELISA. Detection of Arg16Gly and Gln27Glu polymorphisms was performed by PCR-based RFLP using fast-digest NcoI and BbvI restriction enzymes, respectively.

Results: Non-significant differences in Arg16Gly genotype and allele frequencies were found in asthmatics versus controls. There was significant increased Gln/Gln genotype and Gln allele but significant decreased Gln/Glu genotype of Gln27Glu in asthmatics versus controls. Combined haplotypes showed significant increased Arg16Arg-Gln27Gln but significant decreased Gly16Gly-Glu27Gln in asthmatics versus controls. As regard severity, there were significant increased Gln/Gln and 16Arg/Arg-27Gln/Gln in severe asthma (risk factors) while Glu/Glu was significantly decreased (protective). There was also significant increased 16Arg/Gly-27Glu/Glu haplotype in mild asthma and significant increased 16Gly/Gly-27Glu/Gln haplotype in moderate asthma (both protect against severity).

Conclusion: The isolated β2-AR Gln27Glu polymorphism and the combined Gln27Glu and Arg16Gly haplotypes but not the isolated Arg16Gly polymorphism might contribute to asthma risk and grades of severity.

Key Words: Bronchial asthma, β2-AR gene polymorphism.

Introduction:

Bronchial asthma (BA) is a chronic inflammatory illness of airways with clinical, physiological and pathological characteristics. The main characteristics are shortness of breath, wheezing, obstruction, airway inflammation, and atopy. Genetically, BA is a multifactorial illness in which various genes interact with each other and with the environment [(1)].

The prevalence of asthma in Egypt varies; it has been 9.4% in Cairo [(2)], 7.7% in Nile Delta region [(3)] and 6.2% in Assiut district [(4)]. Many predisposing factors for BA do exist including genetic variation, atopy, obesity, sex, and environmental factors; allergens (house-dust mite, animal fur, and fungus), viral infections, occupational sensitizers, smoking, air pollution and eating habits. Additionally, ethnicity, which reflects wide genetic variations, social and economic diversity, affects exposure to allergens and asthma risk [(1)].

An influential factor researched for in BA is the beta-2 adrenergic receptor (β2-AR) gene[(5)]. The β2-AR protein product is a functional G-coupled protein expressed in vascular endothelium of lungs, alveolar walls, and ganglions of cholinergic nerves. It enhances bronchodilation via catecholamines [(6)]. The β2-AR gene is located on chromosomal site 5q31-q32 and has many gene variations. Four of these variations cause non-synonymous changes in the amino acid sequence (Arg16Gly, Gln27Glu, Val34Met and Thr164Ile) [(7)]. The most frequent harmful variations in the β2-AR gene are those at sites; +46 and +79 [Arg16Gly (rs1042713) and Gln27Glu (rs1042714)] located near the receptor's ligand-binding site [(8)]. Therefore the aim of this study was to explore the contribution of β2-AR gene polymorphisms (Arg16Gly and Gln27Glu) to the risk and severity of adult BA in Egypt.

Subjects and Methods:

This cross-sectional prospective study was performed in cooperation between the Chest Department and the Molecular Biology Unit, Faculty of Medicine, Benha University. The study was performed on 75 subjects; 50 BA patients (group I) and 25 apparently healthy controls (Group II). Ethical approval was obtained from the Ethical Committee of the Faculty of Medicine, Benha University. Each subject signed a written informed consent before being enrolled in this study.

All patients were of allergic asthma. Allergy classification was defined by co-occurrence of asthma with atopic dermatitis, increased serum IgE serum or eosinophils >4% in peripheral blood in absence of parasitic infestations [(1)]. Subgrouping by severity into mild, moderate and severe asthma occurred according to GINA guidelines [(1)]. Patients with other forms of chest diseases, DM or hypertension or any other chronic disease and malignancy or those who received additional medications other than for BA, were excluded.

Methods:

Full history taking, physical examination (general &local), postero-anterior plain chest x-ray, pulmonary function tests, and laboratory investigations were done.

Pulmonary function tests (spirometry):

The procedure was performed using Schiller Spirovit SP-10 (USA) [(9)]. The best of three trials was taken. If FEV1/FVC <70%, reversibility test was done using a dose of 200µg salbutamol via pressurized metered dose inhaler, the best of three additional trials after 15 min was recorded. Post-bronchodilator FEV1/FVC and post-bronchodilator FEV1 were calculated.

Laboratory investigations:

Sample collection

1. A venous blood sample (3ml) was taken from each subject and divided into 2 parts. The first part (1ml) was left to clot for serum separation that was kept at -20°C for total IgE estimation. The second part (2ml) was put on EDTA of which 1ml was used for CBC and the other was kept at -20°C for genotyping.

2. Three stool specimens for exclusion of parasitic infestations

The following laboratory investigations were done:

a. Complete blood picture performed using hematology autoanalyzer Sysmex XS-1000i (Sysmex, Japan).

b. Total IgE antibodies estimation in 20µl serum by total IgE ELISA Kit (Omega Diagnostics, Germany)[10]. The minimum detectable concentration of total IgE was 0.9 IU/ml. The optical density was read at 450/620 nm by TECAN Infinite F50 ELIZA Reader (Singapore) with Magellan Tracker software (Tecan Trading AG, Switzerland).

c. Parasitological stool examinations to exclude any parasitic infestation.

d. Detection of β2-AR gene single nucleotide polymorphisms (SNPs); Arg16Gly and Gln27Glu by PCR-based restriction fragment length polymorphism (PCR-based RFLP) assay in the following steps:

I. Genomic blood DNA extraction:

Genomic DNA was extracted from 200µl blood sample; using Quick-DNA Miniprep Kit (The Epigenetics Company, Zymo Research, CA, USA) according to manufacturer's instructions. The extracted DNA concentration was measured by Nanodrop Spectrophotometer 2000 (Thermo-Fisher Scientific, Wilmington, USA). Readings were taken at wave lengths 260 and 280 nm [11]. The ratio of optical density (OD) at 260 nm and 280 nm provides an estimate of DNA purity. Pure preparations of DNA have OD260/OD280 of 1.7-2.0.

II. Amplification of Genomic DNA by PCR:

Genomic DNA amplification was done in 50µl reaction/sample. Primer sequences for β2-AR were FP: 5'-GCCTTCTTGCTGGCACCCCAT-3' and RP: 5'-CAGACGCTCGAACTTGGCCATG-3' [12]. PCR amplification mixture contained 25µl Dream Taq Green PCR Master Mix (2X), 2.5µl FP (0.5 uM), 2.5µl RP (0.5 uM), 5µl DNA extract and 15µl nuclease-free H_2O. The thermal cycling program included initial denaturation (5min at 94°C) then 35 cycles of denaturation (40sec at 94°C), annealing (1min at 62°C) and extension (40sec at 72°C) and final extension (10min at 72°C). Amplification was done in Veriti™ Thermal Cycler (Applied Biosystems). PCR products (10µl) and 100 base pair ladder marker (M) (5µL) were resolved in 2.5% agarose gel with 0.3ug/ml ethidium bromide. PCR product was visualized by UV transilluminator (254 nm) and imaged with a digital camera 8 mega pixel. The image was analyzed by computer software (Alpha InoTech Gel Documentation System). The PCR product was seen at 168 bp fragment (figure 1).

(M: DNA marker)

III. RFLP analysis:

1. Digestion of amplified products:

RFLP was done for Arg16Gly and Gln27Glu by fast-digest NcoI and fast-digest BbvI restriction enzymes, respectively. The enzymes were supplied by Thermo Fisher Scientific. In 30µl total reaction

volume, a separate reaction was done for each enzyme with the same conditions for both: 10µl of PCR products + 1µl enzyme (1 unit) + 2µl buffer (10X) + 17µl nuclease-free water. The digestion mixtures were incubated at 37°C for 2 hours then incubated 10min at 65°C for inactivation.

2. Analysis of digested PCR products:

Post-digestion PCR product (10µl) and 100bp ladder (5µl) were separated on 2.5% agarose gel with 0.3ug/ml ethidium bromide and visualized as previously discussed. For Arg16Gly; the Arg (A) allele gave two fragments (146 bp and 22 bp), while the Gly (G) allele gave 3 fragments (128 bp, 22 bp and 18 bp). Small bands (22 bp and 18 bp) were lost in the gel (figure 2). For Gln27Glu; the Gln (C) allele gave 2 fragments (105 bp and 63 bp), while the Glu (G) allele gave 168 bp (figure 3).

Hardy-Weinberg equilibrium (HWE) was calculated by the Online Encyclopedia for Genetic Epidemiology software (http://www.oege.org/software/hardy-weinberg.html) comparing the observed and expected genotype frequencies. The controls were in HWE (Arg16Gly; $X^2=0.23$, $p>0.05$ and Gln27Glu; $X^2=0.99$, $p>0.05$), but the patients were not in HWE (Arg16Gly; $X^2=11.51$, $p<0.05$ and Gln27Glu; $X^2=18.29$, $p<0.05$).

Statistical analysis:

Data were analyzed by the statistical package for social science (SPSS) IBM version 20. Quantitative data were expressed as mean ± standard deviation (mean±SD). Student's t-test was used to compare the mean of quantitative data of two groups. ANOVA (F) test was used to compare the mean of quantitative data of more than two groups. Inter-group comparison of the distribution for qualitative data was performed by using chi square test (X^2). $p<0.05$ was significant and $p<0.01$ was high significant.

Results:

In the present study, there were statistically significant higher eosinophil count and IgE level in asthmatics compared to controls (p=0.00 for both) (Table 1).

This study showed non-significant difference in β2-AR Arg16Gly genotype or allele distribution in BA compared to controls (p=0.185 for genotype, p=0.298 for allele). However, there was significant difference in the Gln27Glu polymorphism of β2-AR in BA compared to controls (p=0.006). There was significant increased Gln/Gln (CC) (OR=2.86, 95%CI=1.41-5.78) but significant decreased Gln/Glu (CG) (OR=0.44, 95%CI=0.24-0.79). Also, there was significant increased Gln allele (C allele) in asthmatics versus controls (p=0.002, OR=3.09, 95%CI=1.5-6.38) (Table 2).

As regards the combined haplotype distribution of both SNPs, there was significant increased Arg16Arg-Gln27Gln (p=0.022, OR=5.41, 95%CI=1.14-25.81) but significant decreased Gly16Gly-Glu27Gln (p=0.021, OR=0.22, 95%CI=0.06-0.86) in asthmatics compared to controls (Table 3).

In the current study, there was non-significant difference in the β2-AR Arg16Gly genotype distribution among asthmas as regards severity (p=0.30), however there was statistical significant difference in β2-AR Gln27Glu polymorphism (p=0.01). The Gln/Gln (CC) significantly increased (OR=3.55, 95%CI=1.01-12.6) predisposing for severity while Glu/Glu (GG) genotype was significantly decreased in severe asthma (OR=0.17, 95%CI=0.03-0.89) protecting against severity (Table 4).

There was also significant increased 16Arg/Gly-27Glu/Glu haplotype in mild asthma

(OR=2.3, 95%CI=1.65-3.2) and 16Gly/Gly-27Glu/Gln haplotype in moderate asthma (OR=3.29, 95%CI=2.1-5.1). Both haplotypes protect against severe asthma. There was also significant increased 16Arg/Arg-27Gln/Gln haplotype in severe asthma (OR=1.4, 95%CI=1.7-1.81) to act as a risk factor for severity (Table 5).

Table (1): Statistical comparison between bronchial asthma and control groups as regarding age, sex, BMI, eosinophils, IGE and pulmonary function tests

Variables	BA (n.=50)	Controls (n.=25)	test	p
	Mean±SD or n.(%)			
Age (years)	46.8±7.21	62±6	t=3.5	0.07
Sex (♂/♀)	19(38)/31(62)	11(44)/14(56)	X^2=0.25	0.62
Body mass index (kg/m²)	32.3±6.36	25.1±4.7	t=1.5	0.22
Eosinophil count (x10³ c/mm³)	6.4±1.7	4.0±0.0	t=86.08	0.00**
IGE (IU/ml)	181.8±68	42.16±28.06	t=17.4	0.00**
FEV1/FVC	66.08±6.07	-	-	-
FEV1% predicted	55.2±18.75	-	-	-
FEV1 post	31.86±5.51	-	-	-

BA: bronchial asthma, IGE: immunoglobulin E, FEV1: forced expiratory volume in the first second, FVC: forced vital capacity, post: post-bronchodilator change, n.: number, X^2: Chi square test, t: student t test, **: high significant

Table (2): Isolated β2-AR genotype and allele distributions in bronchial asthma versus controls

Genotype or allele	BA (n.=50)	Controls (n.=25)	X^2	p	OR (95% CI)
Arg16Gly genotype	n.(%)				
Arg/Arg (AA)	19(38)	5(20)	3.38	0.185	1.88 (0.80-4.41)
Arg/Gly (AG)	13(26)	11(44)			0.6 (0.32-1.12)
Gly/Gly (GG)	18(36)	9(36)			1.00 (0.51-1.95)
Arg16Gly allele	n.(%)				
Arg (A)	51(51)	21(42)	1.08	0.298	1.437 (0.73-2.85)
Gly (G)	49(49)	29(58)			
Gln27Glu genotype	n.(%)				
Gln/Gln (CC)	35(70)	8(32)	10.37	0.006**	2.86 (1.41-5.78)
Gln/Glu (CG)	7(14)	10(40)			0.44 (0.24-0.79)
Glu/Glu (GG)	8(16)	7(28)			0.64 (0.33-1.25)
Gln27Glu allele	n.(%)				
Gln (C)	77(77)	26(52)	9.68	0.002**	3.09 (1.5-6.38)
Glu (G)	23(23)	24(48)			

BA: bronchial asthma, n.: number, X^2: Chi square test, **: high significant, OR: odd ratio, CI: confidence interval

Table (3): β2-AR haplotype distribution in bronchial asthma versus controls.

Combined Haplotype	BA (n.=50)	Controls (n.=25)	X^2	p	OR (95%CI)
	n.(%)				
Arg16Arg-Gln27Gln	16 (32)	2(8)	5.26	0.022*	5.41 (1.14-25.81)
Gly16Gly-Gln27Gln	11(22)	2(8)	2.28	0.131	3.24 (0.66-15.94)
Arg16Gly-Gln27Gln	7(14)	4(16)	0.053	0.82	0.86 (0.23-3.25)
Gly16Gly-Glu27Gln	4(8)	7(28)	5.33	0.021*	0.22 (0.06-0.86)
Arg16Arg-Glu27Gln	3(6)	1(4)	0.13	0.72	1.53(0.15-15.53)
Gly16Gly-Glu27Glu	3(6)	0(0)	-	-	-
Arg16Gly-Glu27Glu	3(6)	5(20)	3.43	0.064	0.26 (0.06-1.17)
Arg16Gly-Glu27Gln	3(6)	2(8)	0.11	0.74	0.73 (0.11-4.7)
Arg16Arg-Glu27Glu	0	2(8)	-	-	-

BA: bronchial asthma, n.: number, X^2: Chi square test, *: significant, OR: odd ratio, CI: confidence interval

Table (4): Association of isolated β2-AR polymorphisms with asthma severity.

	Mild BA (n.=6)		Moderate BA (n.=18)		Severe BA (n.=26)			
	n. (%)	OR (95%CI)	n. (%)	OR (95%CI)	n. (%)	OR (95%CI)	X^2	p
Arg16Gly genotype								
Arg/Arg (AA)	1 (16.7)	0.89 (0.73-1.07)	5 (27.8)	0.79 (0.53-1.18)	13 (50)	1.84 (0.89-3.8)	4.85	0.30
Arg/Gly (AG)	3 (50)	1.2 (0.87-1.63)	5 (27.8)	1.05 (0.65-1.72)	5 (19.2)	0.79 (0.45-1.39)		
Gly/Gly (GG)	2 (33.3)	0.98 (0.8-1.21)	8 (44.4)	1.24 (0.77-1.99)	8 (30.8)	0.7 (0.4-1.24)		
Gln27Gln genotype								
Gln/Gln (CC)	1 (16.7)	0.5 (0.27-1.13)	12 (66.7)	1.1 (0.32-3.77)	21 (80.8)	3.55 (1.01-12.6)	13.2	0.01*
Gln/Glu (CG)	2 (33.3)	5 (0.69-36.4)	1 (11.1)	3.15 (0.34-29.3)	3 (11.5)	0.91 (0.17-5.03)		
Glu/Glu (GG)	3 (50)	0.19 (0.03-1.14)	5 (22.2)	2.08 (0.51-8.47)	2 (7.7)	0.17 (0.03-0.89)		

BA: bronchial asthma, n.: number, OR: odd ratio, CI: confidence interval, X^2: Chi square test, *: significant

Table (5): Association of β2-AR haplotypes of with asthma severity.

Combined haplotypes	Mild BA (n.=6)		Moderate BA (n.=18)		Severe BA (n.=26)	
	n.(%)	OR(95%CI)	n.(%)	OR(95%CI)	n.(%)	OR(95%CI)
16Arg/Arg-27Gln/Gln	0(0.0)	-	5(27.8)	0.34(0.2-2.6)	11(42.3)	1.4(1.7-1.81)*
16Gly/Gly-27Gln/Gln	1(16.7)	0.68(0.1-6.5)	4(22.2)	1.02(0.3-4.1)	6(23.1)	1.14(0.3-4.4)
16Arg/Gly-27Gln/Gln	0(0.0)	-	3(16.7)	1.4(0.3-7.1)	4(15.4)	1.27(0.3-6.4)
16Gly/Gly-27Glu/Gln	0(0.0)	-	4(22.2)	3.29(2.1-5.1)*	0(0.0)	-
16Arg/Arg-27Glu/Gln	1(16.7)	0.52(0.04-6.2)	0(0.0)	-	2(7.7)	1.92(0.2-22.6)
16Gly/Gly-27Glu/Glu	1(16.7)	0.52(0.04-6.2)	0(0.0)	-	2(7.7)	1.92(0.2-22.6)
16Arg/Gly-27Glu/Glu	2(33.3)	2.3(1.65-3.2)*	1(5.6)	0.9(0.1-10.5)	0(0.0)	-
16Arg/Gly-27Glu/Gln	1(16.7)	3.1(0.5-19)	1(5.6)	0.9(0.1-10.5)	1(3.8)	0.44(0.04-5.1)
16Arg/Arg-27Glu/Glu	0(0.0)	-	0(0.0)	-	0(0.0)	-

BA: bronchial asthma, n.: number, OR: odd ratio, CI: confidence interval, *: significant
Chi square test was used and significant values are written in bald

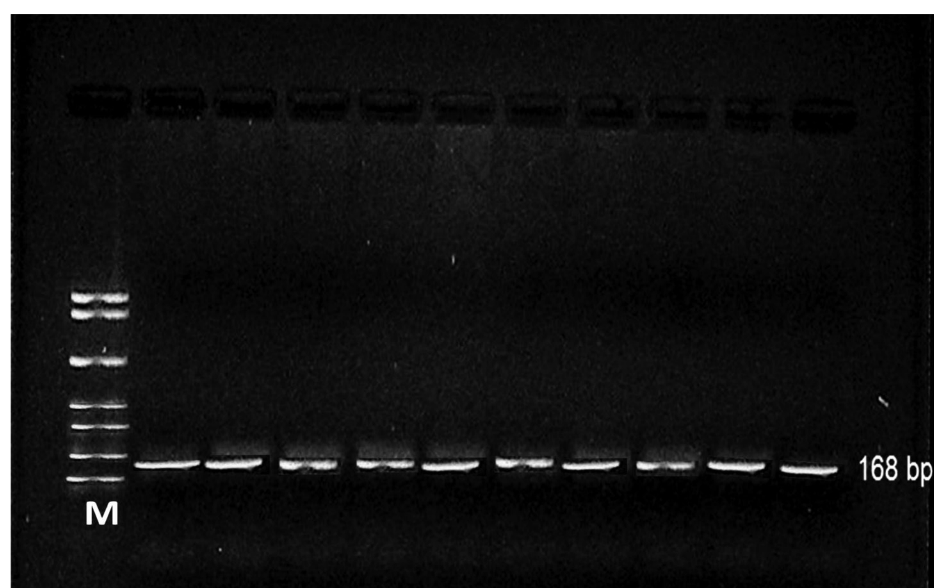

Figure (1): PCR products of amplified β2-AR gene before digestion .

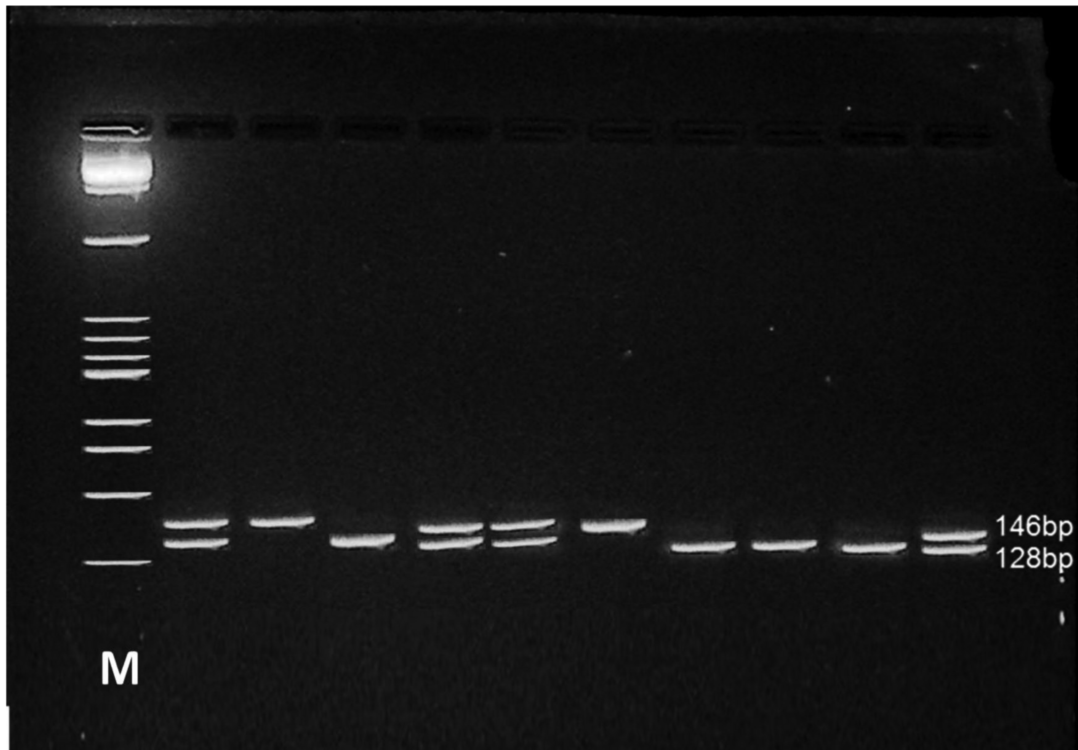

Figure (2): PCR products of amplified β2-AR gene after digestion by fast-digest NcoI (Arg16Gly). (M: DNA marker)

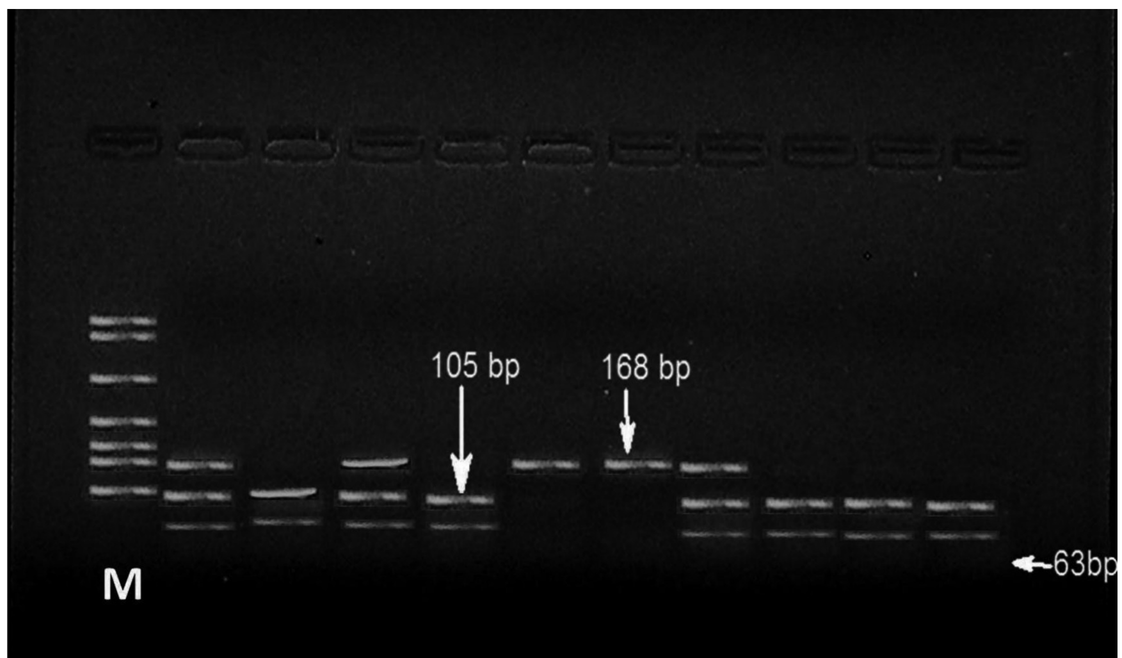

Figure (3): PCR products of amplified β2-AR gene after digestion by fast-digest BbvI (Gln27Glu) (M: DNA marker)

Discussion:

β2-AR SNPs have been searched for and were related to varied expression, function and regulation of β2 receptor. These genetically-based variations may account for some of the diversity in β2-AR agonist response among asthmatics [13]. In the present study, there was no statistical significant difference in the distribution of β2-AR Arg16Gly genotype frequency in asthmatics versus controls. Similar result was found by Bandaru et al.[13], Kaymak et al.[6] and Khan et al. [15], they suggested that the Arg16Gly may not form a risk variant to asthma development nor severity. In addition, Shah et al. [16] reported that Arg16Gly does not determine the occurrence of asthma. However, our finding disagreed with Berenguer et al. [17] who found that heterozygote Arg/Gly genotype increased the risk of asthma by 3.5 folds and Larocca et al. [12] who suggested that Arg/Arg was protective against asthma. In contrast, Alghobashy et al. [18] found increased Arg/Gly genotype (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, $p<0.001$) in asthmatics compared to controls. Concerning allele frequency, our results showed non-significant allele distribution (51% for A allele and 49% for G allele in asthmatics) in asthmatics versus controls. These results were similar to Weir et al. [19] who showed that the allelic frequency of Arg16Gly in the African American was 50% in asthmatics. However our finding disagreed with Green et al. [20] who indicated that down-regulation of β2-AR receptors occurs in those with Gly16 allele in response to circulating catecholamines. The Arg16 allele, which demonstrates resistance to down-regulation, might therefore be expressed at greater levels than the Gly16 allele within airways. Accordingly, individuals with Gly/Gly genotype might be more sensitive to stimuli resulting in bronchoconstriction, and therefore have more reactive airways than individuals with Arg16 allele. Wang and his collegues [21] suggested in their systematic review and meta-analysis that β2-AR Arg16Gly polymorphism was not associated with response to asthma treatment with inhaled corticosteroids plus long-acting beta2-agonists.

As regard Gln27Glu polymorphism, this study showed a statistically significant increased Gln/Gln (risk factor) and a statistically significant decreased Gln/Glu (protective factor) in asthmatics versus controls. Our study was matched with de Paiva et al. [22] who found that Gln/Gln contributed to the risk of asthma (OR=2.12, 95%CI=1.22-3.71) and that Glu/Glu protected against asthma (OR=0.084, 95%CI=0.04-0.17). Moreover, Xie et al. [23] concluded that Gln27Glu polymorphism might be a contributor to asthma susceptibility for adults. On the other hand, Hall et al. [24] concluded that there were no differences in Gln27Glu genotype frequencies. The conflicting results might be due to the diverse ethnic origin of studied populations. In the current study, there was statistical significant increase in Gln (C) allele in asthmatics versus controls with 3.09 folds increased risk. Similar result was obtained by Chiang et al. [25] with increased C allele in asthmatics ($p=0.009$). Also, Leite et al. [26] found that the association of the Glu27 (G) allele with overweight asthmatic adolescents contributing to the development of obesity and asthma. Against our results, Guo et al. [27] reported that none of the Arg16Gly and Gln27Glu was associated with asthma risk in children. Also, Larocca et al. [12] found no differences in β2-AR polymorphism at codon 27 in a study performed on adult asthmatics.

In the present study, there was significant increase in Arg16Arg-Gln27Gln haplotype ($p=0.022$) but significant decrease in Gly16Gly-Glu27Gln

haplotype (p=0.021) in asthmas versus controls. Our result agreed with de Paiva et al. [22] who found similar results in asthmatics versus controls; Arg16Arg-Gln27Gln (OR=5.108, CI=1.82 to 16.37) and Gly16Gly-Gln27Glu (OR=0.1036, CI=0.02 to 0.39). These results were matched with Woszczek et al. [28] who revealed the Arg16Arg and Gln27Gln genotypes were related to asthma susceptibility. Supporting their finding, increased serum IgE levels have been found in Arg16 and Gln27 homozygous genotype patients. However, Khan et al. [15] found no significant association between haplotypes of Arg16Gly and Glu27Gln and asthma (p>0.05). Also, Shah et al. [16] reported that Gly16Gly-Glu27Gln haplotype was associated with increased risk of asthma and Alghobashy et al. [18] found significant increased Arg16Gly-Gln27Gln and Arg16Gly-Gln27Glu haplotypes in their asthmatic Egyptian children studied.

In this study, there was no statistical significant difference in Arg16Gly genotype distribution among asthmatics as regards severity. Against our result, Salama et al. [29] found an association between Arg16Gly genotypes and severe asthma when compared to mild/moderate asthma. Moreover, Scichilone et al. [30] found that β2-AR Arg/Arg polymorphism was associated with the severity of allergic asthma. However, Gly/Gly genotype was present with lower frequencies in severe compared to mild/moderate asthma. We did not agree with Alghobashy et al. [18] who reported that the predisposition of Arg16 homozygous or heterozygous with asthma severity.

In the current study, severe asthma showed significantly increased Gln/Gln (CC) predisposing for severity, while Glu/Glu (GG) genotype significantly decreased protecting against severity. Our finding agreed with de Paiva et al. [22] who reported that Gln/Gln genotype was a risk factor for severe asthma (OR=2.798, CI=1.099 to 6.674) but disagreed with them in the finding that the Gln27Glu genotype was a protective against severity. Also, we agreed with Alghobashy et al. [18] who reported that the Glu27 homozygous or heterozygous was correlated with asthma severity. In a study done by Dewar et al. [31], Gln27Glu genotype was not associated with fatal or near fatal asthma, although it was more prevalent in moderate than in mild asthma.

In our study, there was significant increased 16Arg/Arg-27Gln/Gln haplotype in severe asthma to act as a risk factor for severity, a finding that agreed with Drysdale et al. [32]. However, they reported 16Gly/Gly-27Glu/Glu haplotype was increased in mild asthma to be protective against severity. Our finding did not agree with theirs as we found significant increased 16Arg/Gly-27Glu/Glu haplotype in mild asthma and significant increased 16Gly/Gly-27Glu/Gln haplotype in moderate asthma. Both haplotypes protected against severe asthma.

Conclusion:

The isolated β2-AR Gln27Glu polymorphism and the combined Gln27Glu and Arg16Gly haplotypes but not the isolated Arg16Gly polymorphism might contribute to asthma risk and grades of severity.

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تعدد الأنماط الوراثية لجين مستقبلات الأدرينالين بيتا2- في المصريين الذين يعانون من الربو الشعبي

أيمن عبد الرحمن يوسف*، أسامة محمد إبراهيم*، شوزان علي محمد، نادية لطفي عبد العظيم الخواجة*****

***قسم الأمراض الصدرية، ** قسم الكيمياء الحيوية الطبية، كلية الطب جامعة بنها- مصر**

***** مستشفى الأمراض الصدرية بنها- مصر**

الخلفية: تؤثر عوامل مختلفة ، بما في ذلك تعدد أشكال الجينات ، على تطور الربو الشعبي عن تزيد استعداد الفرد للحساسية و الاستجابة المفرطة للقنوات الهوائية.

الهدف: هدفت هذه الدراسة إلى تحديد النمط الوراثي و تكرار الأليل للاختلاف أحدي النيوكليوتيدات عند الموضعين ١٦ (أرجنين/جليسين) و ٢٧ (جلوتامين/جلوتاماك) في جين مستقبلات الأدرينالين بيتا-٢، و ربط هذه البيانات بالربو الشعبي كعوامل خطورة للإصابة بالمرض و التأثير في شدته.

الطريقة: أجريت هذه الدراسة على ٧٥ فرد مصري مقسمين إلى مجموعتين، الأولى: ٥٠ مريض ربو شعبي ينقسمون حسب شدة المرض إلى ربو خفيف ومتوسط وشديد، و الثانية: خمس و عشرون من الأصحاء. تم أخذ التاريخ الطبي الكامل، و الفحص الطبي الشامل و الموضعي، و أشعة إكس العادية على الصدر «خلفي - أمامي»، بالإضافة إلى عمل اختبارات التنفس الوظيفية، و كذا بعض الاختبارات المعملية متضمنة قياس الأجسام المضادة المناعية (IgE) بتقنية الإنزيم المُمْتز المناعي، كما تم عمل تفاعل البلمرة المتسلسل للنيوكليتيدات محل الدراسة متبوعًا بالهضم بالإنزيمات القاطعة سريعة الهضم وهي " إن كو - ١ و بي بي في - ١" على الترتيب.

النتائج: لم تجد الدراسة أي نتائج ذات أهمية إحصائية فيما يخص النمط الجيني و الأليل عند الموضع ١٦ في حالات الربو مقارنة بالمجموعة الضابطة، في حين توصلت الدراسة إلى زيادات ذات دلالة إحصائية فيما يتعلق بالنمط الجيني و الأليل عند الموضع ٢٧ في حالات الربو مقارنة بالمجموعة الضابطة. كما زاد النمط الجيني أرجنين١٦أرجنين-جلوتيمين٢٧جلوتامين المركب من كلا الموضعين بينما قلَ النمط الجيني المركب جليسين١٦جليسين-جلوتامين٢٧جلوتامك في حالات الربو مقارنة بالمجموعة الضابطة.

و عند تناولنا لشدة المرض، أظهرت الدراسة زيادة النمط الجيني جلوتامين٢٧ جلوتامين و النمط الجيني أرجنين١٦أرجنين-جلوتامين٢٧جلوتامين المركب في الربو الشديد حيث بدا كلاهما كعامل محفز لشدة المرض، في حين تقلص النمط الجيني جلوتامك٢٧جلوتامك مما أهله لأن يكون عامل حماية من شدة المرض. كما زاد النمط الجيني المركب أرجنين١٦جليسين-جلوتامك٢٧جلوتامك في الربو الخفيف، و زاد النمط الجيني المركب جليسين١٦جليسين-جلوتامين٢٧جلوتامك المركب في الربو المتوسط مما جعلهما عاملا حماية من شدة الربو.

الخُلاصة: خلُصت الدراسة إلى أن الاختلاف الجيني المنعزل عند الموضع ٢٧، و كذا النمط الجيني المركب من الاختلاف عند الموضعين ١٦ و ٢٧ معًا في جين مستقبلات الأدرينالين بيتا- ٢ قد يسهما في الإصابة بالربو الشُعبي و التأثير في شدة المرض، في حين لا يسهم الاختلاف الجيني المنعزل عند الموضع ١٦ في ذلك.