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ORIGINAL ARTICLE

The Effect of Adrenergic β2 Receptor Thr164Ile Gene Polymorphism on Asthma Risk, Severity and Response to B2 Agonists in Egyptian Children

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ABSTRACT

***Background**: Asthma is a global health problem affecting millions of adults and children. Pathogenesis of asthma is multifactorial and the genetic component is of particular importance.* ***Objectives**: To assess the role of ADRB2 Thr164Ile gene polymorphism in asthma risk, severity and response to β2 agonist therapy in Egyptian children.* ***Methodology**: The study enrolled 50 asthmatic and 50 control children. Pulmonary function tests and serum levels of IgE of asthmatic children were measured. The Thr164Ile genotypes were detected for all study subjects by Amplification Refractory Mutation System- Polymerase Chain Reaction (ARMS-PCR).* ***Results**: Serum IgE levels were significantly higher on comparing mild to moderate and severe cases (P=0.002& 0.02, respectively). The Ile/Ile genotype of Thr164Ile SNP was significantly present in asthmatic subjects (P=0.039). The Thr164Ile SNP was associated with lowered response to β2 agonist inhalation (P<0.001) but there was no association between the studied SNP and asthma severity.* ***Conclusion**: The Thr164Ile SNP can be linked to asthma risk and lowered response to β2 agonist treatment but not to asthma severity in asthmatic children.*

INTRODUCTION

Asthma is a chronic inflammatory airway disorder characterized by reversible airway obstruction, hyperresponsiveness and remodeling of the airway wall. It is prevalent worldwide affecting millions of children and adults, with increasing numbers in developed and developing countries [1,2,3]. Pathogenesis of asthma is heterogenous, involving various risk factors that can exacerbate disease attacks as pollution, exercise, chemicals and microbes. The genetic component is crucial in the pathogenesis of asthma and identification of asthma susceptibility genes is important in understanding the disease [4,5].

The β2 adrenergic receptors (AR) are expressed numerously on the bronchial smooth muscle cells; they mediate Broncho protection, bronchodilatation and mucociliary action [6]. The β2 agonists are therefore used as the first line relievers of asthma exacerbations to activate the β2 AR [7]. The *ADRB2* gene is located on chromosome 5q31-q32 and its genetic variants are associated with airway hyperresponsiveness and change in responses to treatment [8].

Several single nucleotide polymorphisms (SNPs) in the *ADRB2* gene have been described. These polymorphisms may change the expression and function of the receptor; moreover, they can affect the receptor response to β2 agonists and increase asthma risk [9]. The C >T base exchange at the 491 position in *ADRB2* gene results in the replacement of the amino acid Threonine with Isoleucine at 164th position in the *ADRB2* receptor gene. This SNP can lower the affinity of the receptor to β2 agonists [10]. Few studies have been conducted to reveal the impact of the Thr164Ile SNP on the pathogenesis and treatment of asthma.

In view of the above, our aim was to assess the potential role of the *ADRB2* gene Thr164Ile SNP in asthma risk, severity and response to β2 agonists in Egyptian asthmatic children.

METHODOLOGY

Study design and subjects:

The current case control study was conducted in the Microbiology & Immunology as well as the Pediatrics Departments in collaboration with The Scientific and Medical Research Center, Faculty of Medicine, Zagazig University over the period from 2016 to 2018. The study enrolled 100 children, whose ages ranged from 6 to 12 years, classified into two groups; a patients' group of 50 children (25 males & 25 females) recruited from the Inpatient Wards and Outpatient Clinics of the Pediatrics Department and a control group of other 50 (25 males & 25 females) apparently healthy children matched with patients for age and sex.

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Inclusion and exclusion criteria:

The study included children diagnosed with asthma according to the Global Initiative for Asthma (GINA) [11], with ages ≥ 6 years to ensure the child could understand and perform the pulmonary function tests. Children below 6 years old, those with other allergic conditions, chronic diseases that may affect the pulmonary function tests, children with a restrictive pattern in spirometry or those with body mass index (BMI)> 40 were excluded [12,13].

Ethical considerations:

An informed consent was obtained from parents of each participant after explaining the nature as well as the purpose of this work. The study was approved by the Institutional Reviewer Board (IRB) of Faculty of Medicine, Zagazig University.

Clinical evaluation:

Patients were subjected to thorough medical history taking, general and local chest examinations, chest X-rays (postero-anterior and lateral views), arterial blood gases (ABGs) analyses, and calculation of BMI (BMI= weight (kg)/height (m2)).

The severity of asthma was assessed according to GINA 2016 guidelines and cases were classified into mild, moderate or severe asthma [11]. Pulmonary function tests were performed for all patients, including Forced Expiratory Volume in the first second (FEV1), Forced Vital Capacity (FVC) and FEV1/FVC ratio using a spirometer (D-97024 Hochberg, Germany). Three assessments were done for each patient, of which the highest value was recorded [14].

Asthmatic children were instructed to stop systemic bronchodilators and corticosteroids for 72 hours, as well as the short acting β2 agonists for the last 12 hours before testing. To detect the response to short acting bronchodilators; a single dose of salbutamol (0.15 mg/kg) was nebulized, and the pulmonary functions were assessed before and 15 minutes after its administration [15]. A response greater than 12% change in FEV1 is consistent with asthma [11].

Laboratory studies:

Assessment of IgE serum levels:

Samples of 1-2 ml venous blood were collected from patients on gel containing serum collecting tubes, incubated in room temperature, centrifuged at 2000 rpm to separate sera which were stored at -20^0 C till being used. Serum levels of IgE were measured using enzyme linked immuno-sorbent assay (ELISA) (Immunospec Corporation, Ref: E29-006, Netherlands), performed according to the manufacturer's protocol.

Analysis of *ADRB2* Thr164Ile (C 491 T; rs1800888) gene polymorphism by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR)

DNA extraction

Genomic DNA was extracted from 2 ml samples of whole blood collected on EDTA from all study subjects. Extraction was performed using a commercial kit (G-spin™, Intron Biotechnology, South Korea) in accordance with the manufacturer's instructions. DNA was stored at -20°C till the time of use.

Genotyping of ADRβ2 Thr164Ile (C 491 T; rs1800888) gene polymorphism

The Thr164Ile gene polymorphism genotyping was performed by the ARMS-PCR technique. The target for amplification was a 662 bp region in the *ADRB2* gene. Two complementary reactions were used for each allele using the primers; antisense (forward) 5´CACAGCAGTTTATTTTCTTT3´, sense (reverse –C allele specific)5 ´TGGATTGTGTCAGGCCTTAC3´and sense (reverse –T allele specific) 5´TGGATTGTGTCAGGCCTTA T3´ (Biovision, Egypt). Both reactions for each sample were carried out in 20µl volumes containing 1µl of sense primer either (C-specific or T-specific), 1µl of antisense primer, 10µl of PCR master mix solution (QIAGEN, Germany), 3µl of nuclease free water and 5µl of extracted DNA were added. A Step one ™ Thermo Fisher Scientific thermal cycler (Germany) was adjusted for reaction conditions of initial denaturation at 94ºC for 4 min, followed by 35 cycles of 30s at 94ºC, 20s at 61ºC and 30s at 72°C, and the final extension step for 6 min at 72ºC. The amplified products were visualized under UV light by gel electrophoresis using 2% agarose gel stained with ethidium bromide [10].

Statistical analysis:

The results of the quantitative variables were expressed as means ± standard deviation (SD). Student's t-test was used to ascertain the significance of differences between the values of two means involving independent samples, while Student's paired t-test was used to ascertain the significance of differences between the values of two means involving paired samples. Qualitative data were compared by the chi-squared-test (X2). Genotype frequencies in cases and controls were tested for Hardy–Weinberg equilibrium, and any deviation between the observed and expected frequencies was tested for significance using the chi-squared-test. A difference was considered significant if P was ≤ 0.05. All data were analyzed using SPSS version 18.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

RESULTS

This study enrolled 50 asthmatics (25 males & 25 females) as well as 50 apparently healthy children (25 males & 25 females), matched with patients for age and sex, as a control group (P= 0.49 &1; respectively). Ages of patients and controls ranged from 6-12 years (mean±SD= 8.10±2.52 & 8.44±2.40; respectively).

Our patients were exposed to various risk factors predisposing to asthma, where the highest percentage of children had upper respiratory tract infections (34/50,

68%), followed by a family history of asthma or other allergies (26/50, 52%), asthma attacks induced by exercise (25/50, 50%), then comes the exposure to smoke (18/50, 36%). Regarding severity of asthma, the studied cases were classified according to GINA into mild (29/50, 58%), moderate (16/50, 32%) and severe asthma (5/50, 10%).

The mean values of total serum IgE levels showed a statistically significant difference on comparing mild to moderate (P=0.002) and mild to severe asthma patients (P=0.02), while comparing mean levels of serum IgE between moderate and severe cases showed no statistically significant difference (P=0.78) (table 1).

Table 1: Relation between total serum IgE levels and severity of asthma

	Total serum IgE (mean±SD IU/ml)	F	P
Mild asthma	215.22±353.55	4.894	**P1**= 0.002*
Moderate asthma	635.18±512.80		**P2**= 0.78
Severe asthma	719.72±787.42		**P3**= 0.02*

*Significant value
P1--à comparing mild to moderate asthma
P2--à comparing moderate to severe asthma
P3--à comparing mild to severe asthma

The genotype distribution of the Thr164Ile (C491T, rs1800888) polymorphism was assessed in the studied groups by ARMS-PCR, where the homozygous wild Thr/Thr (CC) genotype was equally distributed among patients and controls (OR;95%CI=1 (0.34-2.91), P=1), the heterozygous Thr/Ile (CT) genotype showed a statistically significant occurrence among the control group compared to patients (OR;95%CI=0.44(0.2-0.99), P=0.045), while the homozygous mutant Ile/Ile (TT) genotype was significantly detected in asthmatic compared to control children (OR;95%CI=2.37(1.04-5.44), P=0.039) (table 2).

Table 2: Distribution of Thr164Ile genotypes among the studied groups

	Patients N=50 (%)	Control N=50 (%)	X^2	OR (95%CI)	P
Thr/Thr (CC genotype)	8 (16)	8 (16)	0.0	1 (0.34-2.91)	1
Thr/Ile (CT genotype)	18 (36)	28 (56)	4.026	0.44(0.2-0.99)	0.045*
Ile/Ile (TT genotype)	24 (48)	14 (28)	4.244	2.37(1.04-5.44)	0.039*

*Significant

Comparing the frequency of occurrence of the Thr164Ile genotypes among patients with different asthma severity showed no statistically significant difference between them (table 3). In addition, the mean values of total serum IgE levels in children carrying different Thr164Ile genotypes were compared and no statistically significant differences were found between those with Thr/Thr (P=0.9), Thr/Ile (P=0.3), or the Ile/Ile genotype (P=0.2).

Table 3: Relation between Thr164Ile genotypes and severity of asthma

	Mild asthma N=29 (%)	Moderate asthma N=16 (%)	Severe asthma N=5 (%)	X^2	P
Thr/Thr (CC genotype)	4 (13.8)	2 (12.5)	2 (40)	2.394	0.302
Thr/Ile (CT genotype)	9 (31)	9 (56.25)	0 (0)	5.971	0.051
Ile/Ile (TT genotype)	16 (55.2)	5 (31.25)	3 (60)	2.685	0.261

The comparison between the mean values of pre and post nebulized β2 agonist FEV1% among patients with different Thr164Ile genotypes, showed statistically significant differences among them. Asthmatic children carrying the homozygous wild Thr/Thr genotype showed the highest mean difference between pre and post nebulized values (16.57%, P=0.001), followed by those with the heterozygous Thr/Ile genotype (14.56%, P<0.001) and the homozygous mutant Ile/Ile genotype showed the least mean difference (13.71%, P<0.001) (table 4).

Table 4: Relation between Thr164Ile genotype distribution and response to β2 agonist inhalation in asthmatic children

	Pre neubilized B2 agonist FEV1 % (mean ± SD)	Post neubilized B2 agonist FEV1% (mean ± SD)	Paired sample t. test	Mean difference (%)	P
Thr/Thr (CC genotype)	71.11±12.66	87.68±5.40	-5.635-	16.57	0.001*
Thr/Ile (CT genotype)	77.3±9.54	91.86±11.68	-7.825-	14.56	<0.001*
Ile/Ile (TT genotype)	79.45±13.06	93.17±9.06	-8.766-	13.71	<0.001*

*Significant

DISCUSSION

Asthma is a global public health problem that affects millions of children worldwide, the disease has multifactorial pathogenesis with a strong effect of the genetic component [3,16]. Several genes have been implicated as risk factors for asthma susceptibility including the *ADRB2* gene which encodes the β2 ARs [17]. The β2 ARs are expressed on pulmonary vascular endothelium, alveolar walls, airway smooth muscle cells and presynaptic cholinergic nerve terminals, mediating bronchodilatation and protection of airways [6,18]. Therefore, genetic variations in the *ADRB2* gene can affect the β2 AR function which may increase the risk of asthma or change the response to treatment [8].

The present study included 50 asthmatics as well as 50 control children matched for age and sex. Analysis of the risk factors of asthma, showed that upper respiratory tract infections was the most common risk factor, followed by the presence of a positive family history to allergy, exercise and exposure to smoke. These findings are consistent with the distribution of asthma risk factors in different studies including a study performed on Egyptian children [19,20,21].

Asthma is mostly of atopic nature in children and is associated with high levels of IgE production in response to exposure to allergens. The strong relation between IgE levels and asthma prevalence can help us in determining disease severity using serum IgE levels [22,23]. We found the mean values of total serum IgE to be significantly higher in moderate and severe compared to mild asthma cases, while no significant difference was found between serum levels of moderate and severe cases. These results agreed with the studies of Kovac et al and Ahmed et al, who found that asthma severity is reflected by serum IgE levels in children [24,25]. Nevertheless, Rosario et al, did not correlate serum IgE levels with asthma severity[26]. Different findings could be explained by race differences and environmental conditions leading to exposure to various allergens [25].

Studies concerned with asthma risk described different SNPs in the coding region of the *ADRB2* gene, one of them is the Thr164Ile SNP that occurs at the binding pocket of the receptor which lies in the fourth membrane spanning domain where the substitution of threonine by isoleucine can alter the receptor function as it leads to ineffective binding of Salbutamol to the mutated receptor [9,27]. Analysis of the Thr164Ile genotype distribution among our patients revealed that the mutant Ile/Ile (TT) genotype was significantly found in asthmatic children compared to the control group with about two fold increased risk of asthma among carriers of Ile/Ile (TT) genotype of the Thr164Ile polymorphism, while the Thr/Ile (CT) genotype was significantly detected in control subjects and the wild type Thr/Thr (CC) genotype was equally distributed among the studied groups. These results are inconsistent with other studies conducted in China, where they found no association between the Thr164Ile SNP and asthma risk [28,29]. This discrepancy can be attributed to ethnic differences and limited number of studies conducted on this SNP.

Despite the frequency of the presence of Thr164Ile SNP in asthmatic patients, we could not find an association between the distribution of the studied SNP among patients and the severity asthma, in the same context, Hall et al could not detect any effect of Thr164Ile SNP on asthma symptoms [30]. Moreover, studies on other SNPs in the β2 AR genes revealed no association with asthma severity [31]. However, Sood et al found their studied SNP to be associated with increased asthma exacerbations [12]. Additionally, the serum IgE levels were not correlated to the frequency of Thr164Ile SNP among our asthmatic children. On the other hand, Giubergia et al found IgE levels to be associated with the AR gene polymorphism [32].

The β2 agonist inhalation therapy, including salbutamol, are the primary treatment options of acute asthma for their rapid onset of action, high bronchodilator effects and few side effects [33]. However, some patients show suboptimal response to inhalation therapy which can increase asthma morbidity [10]. Varying responses to salbutamol as a β2 agonist therapy were assessed by measuring the pulmonary function tests for our patients before and after inhalation treatment. The mean values of the FEV1% showed a statistically significant difference on comparing the pre to the post nebulized treatment values of patients carrying different Thr164Ile genotypes. The mean difference between the pre and post nebulized treatment values were the highest among carriers of the Thr/Thr

(CC), followed by Thr/Ile (CT), then the Ile/Ile (TT) genotype which showed the least mean difference value. These data suggest that carriers of the mutant Ile/Ile (TT) genotype showed less response to β2 agonist inhalation therapy than other patients. Our findings are in accordance with Bandaru and his coworkers, who noticed a higher frequency of Ile/Ile genotype carriers among non-responders to β2 agonist inhalation [10]. Nevertheless, Wang et al. could not find an association between their studied SNP and the response to asthma treatment [34].

The β2 agonists act by binding to β2 AR activating adenyl cyclase and increasing cAMP levels which leads to a signal transduction pathway activation resulting in relaxation of airway smooth muscles [35]. Genetic polymorphisms in the *ADRB2* gene have significant effect on the receptor function. The Thr164Ile SNP can cause ineffective binding of the drug to the receptor leading to 4 times decreased legend affinity and about 50% decreased adenyl cyclase activation[36].

CONCLUSION

The current study reported a significant occurrence of the mutant Ile/Ile genotype of the Thr164Ile SNP in asthmatic children, suggesting its potential role in asthma risk. The Thr164Ile SNP can be a main determinant of decreased response to salbutamol inhalation therapy. The severity of asthma is reflected on increased IgE serum levels in our patients, but is not related to the studied polymorphism.

Recommendations

We recommend performing further studies on more samples to confirm the role of the Thr164Ile SNP in asthmatic children.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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