# Relation between Substitution of Threonine for Isoleucine at B2 Adrenergic Receptor on Bronchial Asthma in Children

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## ABSTRACT

**Introduction:** Pediatric asthma is a complex disorder involving immunologic, genetic, environmental and other factors. **Objective:** To evaluate the role of substitution of threonine for isoleucine at codon 164 on bronchial asthma susceptibility, severity and response to short- and long- term acting $\beta_2$-adrenergic receptor agonists in children. **Patients and Methods:** This study was a prospective case control study, which was done in Pediatric Department of Zagazig University Hospital from Pulmonology Clinic in the period from February 2016 to February 2018. 100 children were included, 50 of them had asthma with bronchodilator and their ages ranged from 5 to 12 years (25 males and 25 females) with the mean age of 6.8 ± 2.5 years. In addition, 50 healthy age and sex matched worked as control children. All studied groups were subjected to full history taking, clinical examination, pulmonary function tests, total serum IgE and identification of adrenergic $\beta_2$ receptor (ADR$\beta_2$) substitution of threonine for isoleucine at codon 164 polymorphism. **Results:** In this study, there was a significant association between homozygous isoleucine and increase incidence of asthma, this mean that the gene gives harmful effect when it is in a homozygous form. However, there was no statistically significant difference between asthma severity and gene polymorphism (CC, CT and TT). **Conclusion:** In the present study ADRβ Thr164Ile polymorphism is reported as an important variant at salbutamol refractoriness in sever asthmatics. In addition, the polymorphism form is susceptible variant to develop asthma risk.

**Keywords:** Threonine for Isoleucine, Adrenergic Receptor and bronchial, asthma**,** ADR$\beta_2$.

## INTRODUCTION

Asthma is a complex inflammatory disorder that has multifactorial inheritance mediated by a variety of environmental triggers. According to WHO, it affects about 300 million people worldwide and approximately 250000 patients die from asthma annually [(1)]. Several factors influence development of asthma including gene (that predispose individual to atopy and airway hyper-responsiveness), obesity, sex and environmental causes like house dust mites, animal fur and fungi. There is also viral infections, tobacco smoke, air pollution and eating habits. Additionally some immunological characteristics, such as immune system maturation and the number of exposure to infectious agents at first year of life. These factors affect the risk of developing asthma [(2)]. A number of single nucleotide polymorphism have been reported in ADR$\beta_2$. Four of these results in amino acid substitutions at amino acids16, 27, 34 and164, whereas the silent mutations are located at amino acids 84, 175, 351 and 43. Inhaled $\beta_2$ agonists have been established as first line in treatment of asthma as it has excellent bronchodilator effect, low side effects and wide range therapeutic range [(3)].

β2 agonist form an effective and well tolerated bronchodilator in patients with asthma [(4, 5)]. In studies using site-directed mutagenesis and recombinant expression, the amino acid substitution in the receptor alter function of receptor leading to pathology in body. The replacement of threonine with isoleucine at 164th position in $\beta_2$ adrenergic receptor leads to decrease of ligand-receptor interactions and decline of coupling of $\beta_2$AR to adenylyl cyclase are often observed.

The aim of this work was to evaluate the role of substitution of threonine for isoleucine at codon 164 on bronchial asthma susceptibility, severity and response to short- and long-term acting $\beta_2$ adrenergic receptor agonists in children.

## PATIENT AND METHODS

This study was a prospective case control study, which was done in Pediatric Department of Zagazig University Hospital from Pulmonology Clinic in the period from Feb 2016 to Feb 2018. It was done on 100 children, 50 of them had asthma with bronchodilator and their ages ranged from 5 to 12 years (25 males and 25 females) with the mean age of 6.8 ±2.5 years and 50 healthy age and sex matched control children.

**Inclusion criteria:** Children having asthma according to Global Initiative for Asthma Management and Prevention, 2016. Ensure that the child could understand and perform the pulmonary function maneuvers efficiently.

**Exclusion criteria:** Other allergic disorders. Children with any chronic disease other than asthma that may affect pulmonary function. Children with restrictive pattern in spirometry.

**Ethical and Patients' approval:** Written Informed consent was taken from the patients' parents to participate in the study. **Approval for performing the study was obtained from Pediatrics, Microbiology**





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**and Immunology Departments, Zagazig University Hospitals after taking Institutional Review Board (IRB) approval.** The work has been carried out in accordance with the code of ethics of the world medical association (Decleration of Helsinki) for studies involving humans.

**Methods:**

Our patients were subjected to full history taking and clinical examination, pulmonary function tests, total serum IgE and identification of ADR$\beta_2$ substitution of threonine for isoleucine at codon 164 polymorphism. Cases of intermittent asthma treated with selective beta 2 agonist as salbutamol in inhalation forms as 100 mcg/metered inhalation, the daily dose is one puff of the metered aerosol, 3 times daily.

**Anthropometric measurements:**

Weight, height, body mass index (BMI). For the diagnosis of overweight, if the BMI is at least the 85$^{th}$ percentile but less than the 95% for the age and sex:

$$BMI = Weight (Kg) / Height (m^2)$$

**Assessment of asthma severity according to the Guidelines of Global Initiative for Asthma Management and Prevention (2016):**

Classification of asthma severity into intermittent, mild persistent, moderate persistent and severe persistent.

**Performance of spirometry:** Forced vital capacity (FVC), forced expiratory volume in first second (FEV1) and FEV1/FVC were measured by a spirometer (D-97024 Hochberg, Germany).

Every subject underwent three assessments and the highest value of them was recorded.

**Laboratory investigations:**

Assessment of total serum IgE. Identification of ADRβ2 Thr164 Ile polymorphism using ARMS- PCR method. Serum IgE level (1-2 ml of venous blood was collected on gel containing serum collecting tubes, incubated in room temperature and centrifuged at 2000x g samples were stored at -20$^{o}$C).

***Statistical Analysis***

The data were coded, entered and processed in computer using SPSS (version 18). The results were represented in tabular and diagrammatic forms then interpreted. Mean, standard deviation, range, frequency, and percentage were use as descriptive statistics. The following test was done: Chi-Square test was used to test the association variables for categorical data. Student's t-test was used to assess the statistical significance of the difference between two population means in a study involving independent samples. Student's paired t-test was used to assess the statistical significance of the difference between two population means in a study involving paired samples. ANOVA (F test) for normal quantitative variables, to compare between more than two groups and Post Hoc test (LSD) for pairwise comparisons. r→Pearson's Product correlation coefficient to evaluate the linear association between 2 quantitative variables (one is the independent var. X and the other is the dependent var., Y). Value of "r" ranges from -1 to 1. P value was considered significant as the following: P > 0.05: Non-significant, P ≤ 0.05: Significant.

**RESULTS**

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

| | | | Patients (No.=50) | Control (No.=50) | t. test | P. value |
|---|---|---|---|---|---|---|
| **Age (years)** | **Mean ± SD** | | 8.10 ± 2.52 | 8.44 ± 2.40 | 0.689 | 0.492 |
| **Sex** | **Male** | **No.** | 25 | 25 | **$X^2$** 0.000 | 1.000 |
| | | **%** | 50.0% | 50.0% | | |
| | **Female** | **No.** | 25 | 25 | | |
| | | **%** | 50.0% | 50.0% | | |

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

**Table (2):** Distribution of common risk factors among patients

| Risk factors | Positive. | | Negative. | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Smoke exposure. | 18 | 36 | 32 | 64 |
| Urinary tract infection (URTI) | 34 | 68 | 16 | 32 |
| Exercise-induced asthma. | 25 | 50 | 25 | 50 |
| Family history of asthma or atopy | 26 | 52 | 24 | 48 |

This table showed the distribution of common risk factors among the studied groups. URTI showed the highest incidence of risk factors (68%), followed by exercise-induced asthma and positive family history which exerted the same frequency among patients (52%), while smoke exposure was the least frequently occurring risk factor among patients (36%) as shown in table (2).

**Table (3):** Comparison between grades of asthma severity regarding Total IgE level

| | | Mild asthma | Moderate asthma | Severe asthma | F | P. value | |
|---|---|---|---|---|---|---|---|
| Total IgE level | **Mean ± SD (IU/ml)** | 215.22 ± 53.55 | 719.72 ± 87.42 | 635.18 ± 12.80 | 4.894 | 0.012 | P1=0.005<br>P2=0.762<br>P3=0.04 |

P1---→ between mild asthma and moderate asthma P2--→between moderate asthma and severe asthma
P3---→ between mild asthma and severe asthma

There was a statistically significant difference between mild asthma and moderate asthma regarding total IgE level (P. value= 0.005). There was no statistically significant difference between moderate asthma and severe asthma regarding total IgE level (P. value= 0.762). There was statistically significant difference between mild asthma and severe asthma regarding total IgE level (P. value= 0.04) as shown in table (3).

**Table (4):** Comparison between patients and control regarding gene polymorphism

| | | | Patients | Control | $X^2$ | P. value |
|---|---|---|---|---|---|---|
| Homozygous THREONINE | **Thr/Thr Homozygous** | No. | 8 | 8 | .0 | 1 |
| | | % | 16.0% | 16.0% | | |
| Heterozygous THR/ISOLEUCINE | **Thr/Ile Heterozygous** | No. | 18 | 28 | 4.026 | .045 |
| | | % | 36.0% | 56.0% | | |
| Homozygous ISOLEUCINE | **Ile/Ile Homozygous** | No. | 24 | 14 | 4.244 | .039 |
| | | % | 48.0% | 28.0% | | |

There was no statistically significant difference in the prevalence of homozygous (Thr/Thr) genotype between patients and controls. However, the prevalence of heterozygous (Thr/Ile) was significantly higher in the control group and the prevalence of homozygous (Ile/Ile) was significantly higher in the patients group (Table 4).

**Table (5):** Description of asthma severity among patients regarding gene polymorphism

| | | | Mild asthma | Moderate asthma | Severe asthma | X2 | P. value |
|---|---|---|---|---|---|---|---|
| Homozygous THREONINE | **Thr /Thr** | **No.** | 4 | 2 | 2 | 2.394 | .302 |
| | | **%** | 13.8% | 12.5% | 40.0% | | |
| Heterozygous THR/ISOLEUCINE | **Thr/Ile** | **No.** | 9 | 9 | 0 | 5.971 | .051 |
| | | **%** | 31.0% | 56.3% | .0% | | |
| homozygous ISOLEUCINE | **Ile /Ile** | **No.** | 16 | 5 | 3 | 2.685 | .261 |
| | | **%** | 55.2% | 31.3% | 60.0% | | |

There was no statistically significant difference between asthma severities regarding the gene polymorphism (Table 5).

**Table (6):** Comparison between pre-nebulized $\beta_2$ agonist FEV1 % and post-nebulized $\beta_2$ agonist FEV1% among asthmatic children regarding gene polymorphism

| | Pre nebulized B2 agonist FEV1 % Mean +SD | post nebulized B2 agonist FEV1% Mean +SD | Paired sample t. test | P. value | Mean difference % |
|---|---|---|---|---|---|
| Homozygous THREONINE | 71.11± 12.66 | 87.68 ± 5.40 | -5.635- | 0.001 | 16.57 |
| Heterozygous THRONINE/ ISOLEUCINE | 77.3 ± 9.54 | 91.86 ± 11.68 | -7.825- | 0.001 | 14.56 |
| Homozygous ISOLEUCINE | 79.45 ± 13.06 | 93.17 ± 9.06 | -8.766- | 0.00 | 13.71 |

Table (6) showed that FEV1 values before and after nebulized $\beta_2$ agonist and the percentage of difference. Patients having homozygous threonine were significantly responsive to $\beta_2$ agonist (16.57%), followed by heterozygous Thr/Ile (14.56%), while patients having homozygous isoleucine respond to $\beta_2$ agonist (13.71%) as shown in table (6).

## DISCUSSION

This study showed that, there was no statistical significant difference between cases and controls regarding age and sex. This agrees with **Hoshino *et al.*** [6] who done his study on Forty-two healthy controls and 20 patients with asthma. He found that there were no significant differences in age and gender were observed between the two groups.

This study showed that, positive family history was found in 52% of patients while no family history was found in 48% of patients. This agrees with **Hassane *et al.*** [7] who made case-control study that

included 43 Egyptian outpatients with asthma. 21 apparently matched healthy children were included as controls. He found increase in asthma occurrence among children with family history. This result is in accordance with **Magdy *et al.*** [8] who stated that positive family history of asthma was a risk factor for asthma where family history of asthma is common.

In our study, smoke exposure was found to be a risk factor among patients (36%). This means that exposure to tobacco smoke is one of the most consistent risk factors in the development and exacerbation of asthma. This result is in accordance with **Vargas *et al.*** [9] who found that environmental tobacco smoke exposure has been associated with the increased use of the Emergency Department for acute asthma care.

This study showed that there was a statistically significant increase in total IgE level among moderate asthma than mild asthma (P. value = 0.005). There was no statistically significant difference between moderate asthma and severe asthma regarding total IgE level (P. value = 0.762). There was a statistically significant increase in total IgE level among severe asthma than mild asthma (P. value = 0.04). There was statistically significant positive correlation between total IgE level and eosinophilic count. This agrees with **Inoue *et al.*** [10] where twenty-eight children with asthma (BA) and 27 children without asthma (as control group) aged 6–16 years were included. The asthmatic group also had a significantly higher eosinophil count [298.5 ± 571.1 vs 87.9 ± 263.0/μl ($p < 0.001$)] and total IgE level [328.6 ± 1201.8 vs 20.3 ± 282.0 IU/ml ($p < 0.001$)].

Regarding risk factors distribution among asthmatic patients in the current study, URT infection was considered the highest incident risk factor in the studied group (68%), followed by exercise-induced asthma and positive family history, which represented the same percentage (52%) then smoke exposure (36%). **Subbarao *et al.*** [11] confirms our results regarding distribution of risk factors of asthma.

In our study, there was a significant association between homozygous isoleucine and increase incidence of asthma, this mean that the gene gives harmful effect when it is in a homozygous form. However, there was no statistically significant difference between asthma severity and gene polymorphism (CC, CT and TT). This finding disagrees with **Bandaru *et al.*** [12] who found no association between the genetic polymorphism in that locus and increased incidence of bronchial asthma.

Our study showed different FEV1 values before and after nebulized $\beta_2$ agonist. Patients who are homozygous threonine are significantly responsive to $\beta_2$ agonist (16.5%), followed by heterozygous threonine/ isoleucine (14.56%), while patients having homozygous isoleucine response to $\beta_2$ agonist (13.7%). This finding agrees with **Maxwell *et al.*** [13] who found the substitution of T allele in place of C allele at 491 positions in ADR$\beta_2$ leads to mutation (Thr164lle) in the receptor, which displays decreased agonist binding and depressed coupling of $\beta_2$AR to adenylyl cyclase, so there is significant association of the polymorphism and salbutamol refractoriness.

Polymorphism at Ile 164 receptor was documented to be a reason for suboptimal response to salbutamol.

## CONCLUSION

In the present study, ADRβ Thr164Ile polymorphism reported as an important variant at salbutamol refractoriness in sever asthmatics. In addition, the polymorphism form susceptible variant to develop asthma risk.

## RECOMMENDATIONS

To combine more than one locus of ADR$\beta_2$ in the same study, in order to be able to understand haplotype-genotype interaction. To conduct further pharmacogenomics studies on $\beta_2$ adrenergic receptor on Egyptian asthmatic children to reach a more genotype-guided practice and to compare more than one drug category in relation to SNPs on large sample size.

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