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Study of Forkhead Box p3 Gene Polymorphism in Asthmatic Children

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ABSTRACT

Background: One of the heterogenous chronic diseases affecting the lungs is asthma. Etiology of asthma could be attributed to altered functions of forkhead box p3 Gene (FOXP3) through epigenetic mechanism and genetic polymorphism.

Objective: To assess the frequency of two FOXP3 polymorphism rs3761548 and rs2232365 among asthmatic children in comparison with healthy children.

Patients and methods: A total of 202 Egyptian children were recruited and divided into 2 separate groups, 1st one of asthmatic contained 101 children with asthma and a control group of 101 non-asthmatic apparently healthy children.

Results: We found a statistically significant difference in FVC, FEV1 concerning degree of asthma. The lowest values were reported among sever asthmatic cases. The genotype analysis of patients and controls revealed non-statistical significant difference was found in patients when compared to control concerning foxp3 polymorphism for 2 different single nucleotide polymorphisms (SNPs) rs 3761548 and rs 2232365. The AC genotype of rs3761548 and the GG genotype of rs2232365 polymorphisms were the most frequent genotypes among the studied children. The rs3761548 C allele carriers could be more susceptible to have asthma since it could be considered a bronchial asthma allele risk factor.

Conclusion: We investigated the association between FOXP3 polymorphism in (rs 3761548 and rs2232365) and occurrence of asthma in Egyptian children. The AC genotype of rs3761548 and the GG genotype of rs2232365 polymorphisms were the most frequent genotypes among the studied children. The rs3761548 C allele carriers could be more susceptible to have asthma since it could be considered a bronchial asthma allele risk factor.

Keywords: Asthmatic Children, Forkhead box p3 Gene (FOXP3).

INTRODUCTION

Regarding asthma, which is a chronic inflammatory illness, whereas Th1 and regulatory T cells may protect against asthma, auto-reactive Th2 cells promote the disease. CD4+ T lymphocytes and eosinophils entered the bronchial mucosa, resulting in asthma etiology. Instead of infection fighting Th1 cells, CD4+ T cells develop into pro-inflammatory Th2 cells by allergen provoked dendritic cells**.** Some key Th2 type cytokines, including Granulocyte monocyte-Colony stimulating factor (Gm-CSF) IL3, IL4, and IL5, are secreted during allergic asthma and may play a role in the development of the condition **(1).**

Asthma is mostly caused by pathogens such as bacteria, viruses and parasites activating Treg cells. These cells are classified into thymus-derived foxp3 Treg cells, which express foxp3 and peripheral Treg cells. Then there are Th1 cells that do not express Foxp3 **(2).**

In CD4 CD25 Treg cells, Foxp3 is a transcription factor that may be associated with T cell suppression activation. It is natural anti-inflammatory released by Treg either thymus or peripheral type in hyper immune condition attributing to bronchial asthma **(1).**

We aimed in this work to assess the frequency of two FOXP3 polymorphism rs3761548 and rs2232365 among asthmatic children in comparison with healthy children.

PATIENTS AND METHODS

This study was done as a case control trial that contained 101 asthmatic children who were categorized to 91 asthmatic children taking steroid and 10 not taking steroid, they were five to fifteen years old. The control group contained 101 healthy children with almost same age and sex.

Asthmatic children were recruited from Pulmonology, Allergic Disease Unit and Clinical Pathology Department in the period from October 2019 to March 2020. We confirmed diagnoses of asthma according to Global Initiative for Asthma Management and Prevention guidelines **(3).**

We collected the healthy subjects from clinics of general outpatients at Children Hospital of Zagazig University.

Patients with cardiovascular diseases, malignant tumors, autoimmune diseases, acquired immunodeficiency, patients treated with B-blockers, patients who had mental disorders, patients who were on or monoamine oxidase inhibitors or Ace inhibitors, children aged less than 5 years, severe uncontrolled asthma, and children who were under immunotherapy as it alters cytokine profile have been excluded from the study.

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Ethical consent:

We took approval from Institutional Review Board (IRB) at Zagazig University and also, we obtained written consent from children's parents. We performed this study with respect to (Declaration of Helsinki), ethics code of World Medical Association regarding human studies.

All studied groups underwent the following

1. History taking:

All participants completed a questionnaire with reference to global initiative for asthma guidelines [(3)], about previous symptoms (cough at night, wheezes, recurrent chest tightness, dyspnea, disturbance of sleep rhythm and recurrent chest infection), history of allergy, family history and medications.

2. Clinical examination: General and chest examination was done to all subjects, severity of the asthma episodes was assessed with reference to the Global Initiative Guidelines for Asthma[(3)].

3. Spirometry pulmonary function tests:

Forced Expiratory Volume in one second (FEV_1), Forced Vital Capacity (FVC), and FEV_1/FVC ratio.

4- Laboratory investigations:

- CBC: blood eosinophils and neutrophils.
- Total serum IgE.

Foxp3 gene genotyping through the use of polymerase chain reaction with sequence-specific primers PCR-SSP.

Under aseptic circumstances, two millilitres of peripheral venous blood samples were taken for DNA extraction from each patient. To avoid contamination, the tips and pipettes used for DNA extraction were DNAase and RNAse-free tubes supplied from Promega (Mandison, USA).

Primers utilized in genotyping SNPS in the FOXP3 gene [(4)]:

Position	Method	Primer Sequences	Allele Pheno-types
−3279 A > C (rs3761548)	PCR-SSP	Forward: CTGGCTCTCTCCCCAACTGA Forward: TGGCTCTCTCCCCAACTGC Common Reverse: ACAGAGCCCATCATCAGACTCTCTA	A: 334 bp C: 333 bp
−924 A > G (rs2232365)		Forward: CCCAGCTCAAGAGACCCCA Reverse: GGGCTAGTGAGGAGGCTATTGTAAC Forward: CCAGCTCAAGAGACCCCG Reverse: GCTATTGTAACAGTCCTGGCAAGTG	A: 442 bp G: 427 bp
FoxP3 gene	qRT- PCR	Forward: CAGCTGCCCACACTGCCCCTAG Reverse: CATTTGCCAGCAGTGGGTAG	
GAPDH gene		Forward: CCAGGTGGTCTCCTCTGACTTCAACA Reverse: AGGGTCTCTCTCTTCCTCTTGTGCTCT	

Data interpretation:

Single nucleotide polymorphisms in FOXP3 gene were selected for genotyping. Regarding rs3761548, variations of FOXP3 gene at positions -3279 A>C were assessed in asthmatic children and contrasted with control group. A 3% agarose gel stained with ethidium bromide obtained by PCR- SSP showing PCR product for this polymorphism. The amplified products were visualized under an ultraviolet illuminator as follows: (a) The homozygous CC genotypes appeared as single band in both female and male groups. (b) The heterozygous AC genotypes appeared as two bands only in female groups as FOXP3 is located on the X-chromosome. (c) The homozygous AA genotypes appeared as single band in both female and male groups.

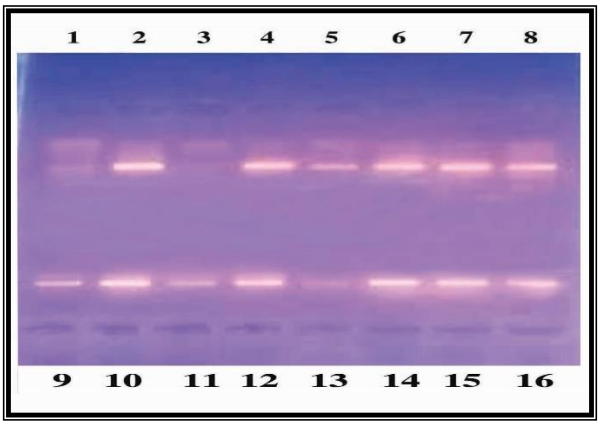

Figure (1): PCR-based SSP analysis of -3279 A>C (rs3761548) polymorphism in asthmatic children. DNA ladder, CC genotype: Lanes 3,4,13,14, AC genotype: Lanes 1, 2, 5,6,7,8,9,10,11,12,15,16

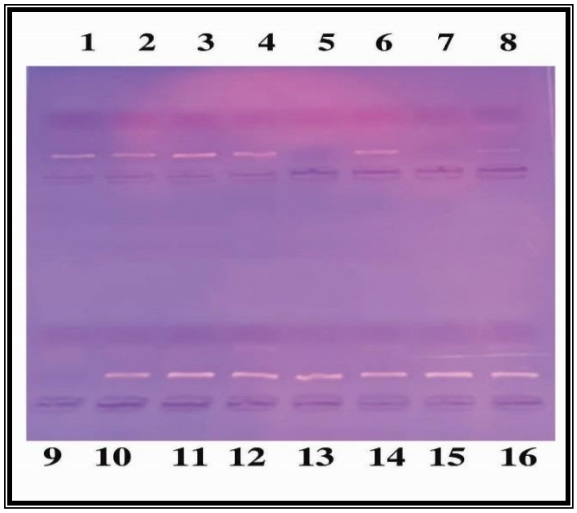

Figure (2): PCR-based SSP analysis of -3279 A>C (rs3761548) polymorphism in controls.
DNA ladder, CC genotype: Lanes 5,6,7,8,9,10, **AC genotype:** Lanes 1, 2, 3, 4,11,12,13,14,15,16

Regarding rs2232365, variations of FOXP3 gene at positions -924 A>G were assessed in asthmatic children and contrasted with control group. A 3% agarose gel stained with ethidium bromide obtained by PCR- SSP showing PCR product for this polymorphism. The amplified products were visualized under an ultraviolet illuminator as follows: (a) The genotypes homozygous AA were showed in form of single band in both female and male groups. (b) The genotypes homozygous AG were showed in form of 2 bands only in female groups as FOXP3 is located on the X-chromosome. (c) The genotypes homozygous GG were shown in form of single band in both female and male groups.

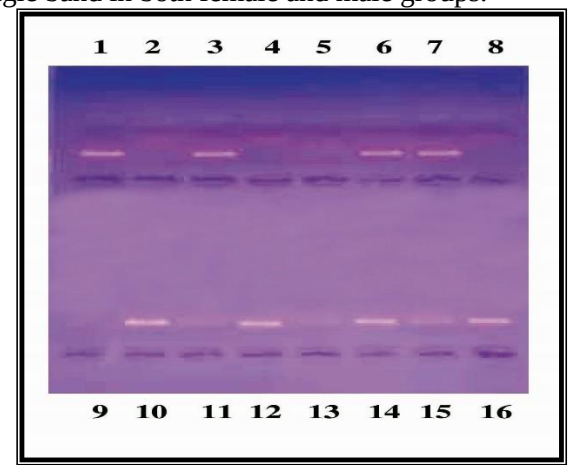

Figure (3): PCR-based SSP analysis -924 A>G (rs2232365) of polymorphism in asthmatic children. DNA ladder, GG genotype: Lanes 5,6,9,10,11,12,13,14, AG genotype: Lanes 15,16, AA genotype: Lane 1,2,3,4,7,8

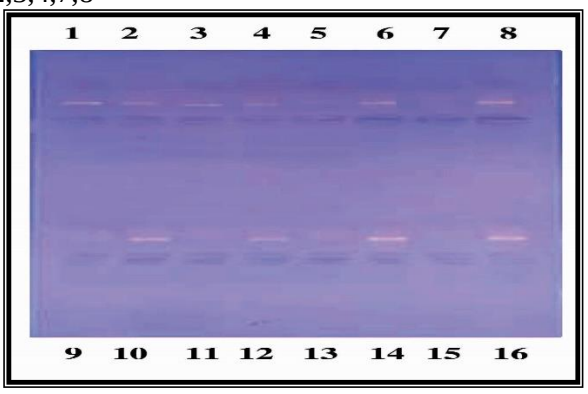

Figure (4): PCR-based SSP analysis -924 A>G (rs2232365) of polymorphism in controls. DNA ladder, GG genotype: Lanes 5,6,7,8, 9,10,11,12,13,14,15,16, AG genotype: Lanes 1,2,3,4.

Statistical analysis

The IBM SPSS software programme version 20.0 was used to examine the data that were entered into the computer, IBM Corporation, Armonk, New York. Number and percent were used to describe qualitative data, which were compared by chi^2 test. In order to determine if the distribution was normal, the Kolmogorov-Smirnov test was utilised. The range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR) were used to characterise quantitative data, which were compared by independent t-test or one-way ANOVA test, if the number of groups was 2 or 3 respectively. Odd ratio (OR) was used to calculate the ratio of the odds and Hardy-Weinberg to explore and find its equilibrium with Hardy-Weinberg equation. P value < 0.05 was considered significant.

RESULTS

Gender and age:

There was no statistically significant difference found between both groups according to age and gender (**Table 1).**

Table (1): Comparison between the two studied groups concerning gender and age

Variables	Group A (n = 101)		Group B (n = 101)		Test of Sig.	p
	No.	%	No.	%		
Gender						
• Male	50	49.5	52	51.5	χ^2= 0.079	0.778
• Female	51	50.5	49	48.5		
Age (years)						
• Min. – Max.	5.0 – 13.0		5.0 – 14.0		t= 1.362	0.175
• Mean ± SD.	7.30±2.02		7.71 ±2.31			
• Median (IQR)	7.0 (6.0 – 8.0)		7.0 (6.0 – 8.0)			

n: Number of subjects in the group; Group A: Asthmatic children; Group B:Controls ; χ^2: Chi square test; t: Student t-test; p: p value for comparing between the studied group

Asthma severity, symptoms control:

In asthmatic cases, mild and moderate persistent asthma were more frequent among our patients (49.5%, 48.5%) and 2% only were severe persistent. According to level of symptoms control, most cases were partially controlled (45.5%) and only (22.8%) were not controlled **(Table 2).**

Table (2): Cases distribution concerning the severity degree of asthma with asthma control and frequency of hospital admission (n=101)

Variables	No.	%
Degree of asthma		
• Mild persistent	50	49.5
• Moderate persistent	49	48.5
• Severe persistent	2	2.0
Level of symptoms control		
• Controlled	32	31.7
• Partially controlled	46	45.5
• Not controlled	23	22.8
Hospital admission		
• No	27	26.7
• Yes	74	73.3
• Once	37	50.0
• recurrent	37	50.0

Inflammatory biomarkers characteristics and pulmonary function tests:

A significant decrease was found in FVC% and FEV1 % among asthmatic children and severe persistent cases were the most affected **(Table 3).**

Table (3): Descriptive analysis of pulmonary function tests regarding degree of asthma severity

Variables	Degree of asthma			F	p
	Mild persistent (n =50)	Moderate persistent (n = 49)	Severe persistent (n = 2)		
FVC (%)					
• Mean ± SD.	89.40 ± 12.73	78.27 ± 16.71	71.50 ± 7.78	7.709*	0.001*
• Median	93.50	82.0	71.50		
FEV1 (%)					
• Mean ± SD.	94.41 ± 12.17	81.94 ± 18.53	70.50 ± 3.54	9.214*	<0.001*
• Median	96.0	90.0	70.50		

FEV1: Forced expiratory volume, n: Number of subjects in the group; FVC: Forced vital capacity;
F: F for ANOVA test; p: p value for comparison between different categories *: Statistically significant

In most asthmatic cases, serum IgE level was highly elevated. Eosinophils were elevated. Whereas neutrophils were slightly elevated **(Table 4).**

Table (4): Descriptive analysis of laboratory tests in asthmatic children (n = 101)

Variables	Mean ± SD.	Median (IQR)
Ig E (IU/ml)	225.9± 19.4	180.0 (100.0 – 300.0)
TLC (thousands/cmm)	9.59±2.55	8.90 (6.80 – 11.30)
Eosinophil's (%)	0.69±0.19	0.30 (0.20 – 0.90)
Neutrophils (%)	4.64±0.60	4.10 (3.0 – 5.30)

FOXP3 gene polymorphisms characteristics in the studied groups:

There was no statistically significant difference regarding different genotypes of FOXP3 polymorphism (rs3761548) and (rs2232365) between asthmatic and healthy controls **(Table 5).**

Table (5): Comparison between the two groups concerning FOXP3 polymorphisms

FOXP3 polymorphism	Group A (n = 101)		Group B (n = 101)		χ^2	p
	No.	%	No.	%		
rs3761548						
• AA	12	11.9	12	11.9		
• AC	58	57.4	67	66.3	2.176	0.337
• CC	31	30.7	22	21.8		
HWE	0.055#		0.001#			
rs2232365						
• AA	21	20.8	21	20.8		
• AG	26	25.7	28	27.7	0.112	0.946
• GG	54	53.5	52	51.5		
HWE	<0.001#		<0.001#			

n: Number of group; Group A: Asthmatic children; Group B:Controls ;
χ2: Chi square test; p: p value for comparing between the studied groups;
HWE: p value for Hardy-Weinberg Equilibrium was assessed in single nucleotide polymorphisms and deviated here

The AC of (rs3761548) and the GG (rs2232365) polymorphisms were the most frequent genotype among the studied children. C allele of (rs3761548) and G allele of (rs2232365) were insignificantly more frequent among asthmatic cases compared to control group **(Table 6).**

Table (6): Comparison between the two groups concerning FOXP3 polymorphisms and frequency of A, C, G alleles

FOXP3 polymorphism	Group A (n = 101)		Group B® (n = 101)		χ^2	p	OR (95% C.I)
	No.	%	No.	%			
rs3761548							
• AA	12	11.9	12	11.9	0.000	1.000	1.000 (0.426 – 2.345)
• AC	58	57.4	67	66.3	1.700	0.192	0.685 (0.387 – 1.211)
• CC	31	30.7	22	21.8	2.072	0.150	1.590 (0.844 – 2.998)
HWE	0.055		0.001#				
Allele							
• A	82	40.6	91	45.0	0.819	0.366	0.834 (0.562 – 1.237)
• C	120	59.4	111	55.0	0.819	0.366	1.200 (0.809 – 1.780)
rs2232365							
• AA	21	20.8	21	20.8	0.000	1.000	1.000 (0.507 – 1.973)
• AG	26	25.7	28	27.7	0.101	0.751	0.904 (0.485 – 1.686)
• GG	54	53.5	52	51.5	0.079	0.778	1.083 (0.623 – 1.88)
HWE	<0.001#		<0.001#				
Allele							
• A	68	33.7	70	34.7	0.044	0.834	0.957 (0.634 – 1.444)
• G	134	66.3	132	65.3	0.044	0.834	1.045 (0.693 – 1.577)

n: Number of group; Group A: Asthmatic children; Group B:Controls ; χ2: Chi square test; p: p value for comparing between the studied groups ; # HWE : p value for Hardy-Weinberg Equilibrium was assessed in single nucleotide polymorphisms and deviated here ; OR: Odds ratio; CI; Confidence interval; UL: Upper Limit; LL: Lower limit; ®: reference group.

DISCUSSION

Asthma is characterized by chronic airway inflammation, and it is a diverse disease. Respiratory symptoms such as wheezes, chest tightness and cough that change over time and in intensity, as well as fluctuating expiratory airflow limitation, are used to diagnose it [(5)].

In addition, $FOXP3^+$ Tregs have the strongest inhibitory function and most comprehensive inhibitory targets of any immune cell type. Activated $FOXP3^+$ Tregs suppress autoreactive T cell activation, inhibit the development of autoimmune and allergy diseases, exercise anti-inflammatory effects, and preserve immunological tolerance. As a result of the downregulation of FOXP3, Tregs may be unable to control infection and tumors [(6)].

Although FOXP3 is a key transcription factor in Treg activation, it may not be enough to explain all Treg functions. To explain the genes produced by Tregs, their functional stability, and cell lineage maintenance, an additional mechanism is required. A new understanding of the relationship between genes and the environment is provided by epigenetic regulation, which could explain this occurrence [(7)].

FOXP3 expression in nTregs can be initiated and maintained by epigenetic inheritance. DNA methylation, histone changes, and posttranscriptional alterations influence FOXP3 expression. In order for FOXP3 to remain stable, epigenetic control and methylation are essential. Modifications to FOXP3's DNA methylation can influence Treg differentiation and immunological response. Finding out whether upstream FOXP3 enhancers are methylated can help with disease diagnosis and subtype classification [(8)].

In the current study we did not find any statistical between cases and healthy group concerning age and sex. The mean age of the asthmatic group in our study was 7.30 ± 2.02 years and control group was 7.71±2.31 years. This agreed with **Syed *et al.*** [(9)] in their prospective case control study at Zagazig University Hospital studying asthmatic children. They found mean age of the asthmatic group was 6.8 ± 2.5 years.

In our study, we found that (43.6%) of patients had family history of asthma. This agreed with **Gawad *et al.*** [(1)] who found that, in 55% in their study had positive family history, and with **Hassane *et al.*** [(10)] who study Egyptian outpatients with asthma and found higher rates of asthma incidence correlated with positive family history. Also, **Magdy *et al.*** [(11)] stated that the positive history in family is a great risk factor.

In the current study, smoke exposure percentage represented (98%) of the studied asthma patients. These results were in accordance with **Vargas *et al.*** [(12)] as they reported that cases admitted to the emergency department was attributed to exposure to the environmental tobacco smoke. **Hassane *et al.*** [(10)] agreed with these results since they performed in Egypt a case-control trial involved 43 asthma patients admitted to outpatient clinics and matched 21 apparently healthy children as controls, they reported that higher exposure to environmental smoke correlated with higher rates of admission with asthma.

In our study, a less common risk factor in asthmatic children was consanguinity (35.6%). This goes hand by hand with **El Mouzan *et al.*** [(13)] who found in their study that consanguinity is not correlated with risk of asthma in children.

FEV1 and FVC showed statistical significant difference concerning asthma severity. And this is compatible with GINA classification of asthma severity[(3)]**.** In the mild asthmatic children FVC ranged from 33 to 99 with mean 89.4±12.73, FEV1 mean 94.41±12.17. In moderate cases it ranged from 30 to 104 with mean 78.27±16.71, FEV1 mean 81.94±18.53 and in severe asthmatic it was from 66 to 77 with mean 71.5±7.78, FEV1 mean 70.5±3.54. This agreed with **Güngen *et al.*** [(14)] who found mean FVC was 86.6 ± 18.2 and FEV1 was 78.2 ± 19.5.

We also found that, high level of eosinophil count with mean 0.69±0.79% in asthmatic groups. **Goossen** [(15)] found that the mean of eosinophil count was 0.2% in healthy children.

There was high level of mean IgE in asthmatic groups 225.9 ± 189.4 IU/ml. The total IgE reference ranges from 0 to 148 kU/L in healthy individuals [(16)]**. Kim and Yoo** [(17)] in their study reported that asthmatic children with higher asthma severity have higher serum concentration of both total IgE (>288.0kIU/L) and specific IgE to Dermatophagoides pteronyssinus (>44.1 kIUA/L).

Some previous studies described the association between FOXP3 protein expression in asthmatic patients [(1, 18, 19)]. Other studies were on the role of FOXO3 gene polymorphism in asthmatic patients [(20,21)]. As these studies did not cover the role of rs3761548 and rs2232365 FOXP3 gene polymorphisms in asthmatic patients, so we tried to link their results by our findings and conclude the causative relation between rs3761548 and rs2232365 FOXP3 gene polymorphisms and FOXP3 protein in asthmatic patients.

In our study, we investigated two of the FOXP3 polymorphisms; rs3761548 and rs2232365 among asthmatic children in comparison to healthy controls. Our study showed that among the all studied children, each polymorphism had three genotypes. For rs3761548 polymorphism, there were three genotypes AA, CC and AC. The most frequent genotype was the heterozygous form AC followed by the homozygous form CC then AA. For rs2232365 polymorphism, there were three genotypes AA, GG and AG. The commonest genotype was GG followed by AG and AA. The genotype distribution of rs3761548 and rs2232365 were not significantly different between asthmatic patients and the control.

Candidate gene and SNPS were evaluated in this study. Our case-control association study for the

FOXP3 variants showed that rs3761548 does not seem to affect the risk for bronchial asthma. Whereas the rs2232365 G allele may confer a susceptibility to asthma for their carriers. The frequency of G allele and C allele was higher in asthmatic compared to healthy children. Allele C was higher among asthmatic but with no statically difference but allele G had nearly equal frequency in both groups. Our results showed no significant association between different genotypes and bronchial asthma in the studied population**.** The AC genotype of rs3761548 and the GG rs2232365 genotype of polymorphisms were the most frequent genotype among the studied children. Only the frequency of C allele was detected in (59.4%) in the asthmatic children compared to controls (55%). G allele had nearly the same frequency in both groups (66.3%) and (65.3%) respectively.

This is in agreement with **Elrifai *et al.*** [(20)] who studied the relationship between a single nucleotide polymorphism of the FOXO3 gene and bronchial asthma, as well as the severity of asthma in children in Egypt. It was a cross-sectional case-control study that involved 75 asthmatic children aged 2 to 12 and 75 healthy controls of a similar age and gender. PCR-RFLP technology was used to genotype the FOXO3 gene polymorphism. The heterozygous type CT had the highest prevalence in both the case and control groups. There were 12 and 16 percent of cases and controls with mutant type TT, respectively, and 37.2 and 46.7 percent of cases and controls with mutant type CC, while 37.3 and 22.6 percent of asthmatic patients had CC genotypes, and 62.8 and 53.3 percent of asthmatic patients had C genotypes, respectively. Regarding the diverse genotypes of the FOXO3 gene polymorphism, no statistically significant differences was found between asthmatic patients and controls (p=0.161).

Similarly, another study stated that the CC genotype represented the highest prevalence (50.7%) in controls while the TT genotype had the least (12.7%) and the C allele was more prevalent (69%) than the mutant T allele. The similarities in the frequency of the gene distribution in controls in our study and the previous two studies in addition to the discrepancy in the distribution in asthmatic patients especially for the mutant TT genotype suggests that there may be no association between FOXO3 gene polymorphism and asthma in the Egyptian population [(21)].

The study suggests SNP −3279 –AA genotype and, −2383-TT genotype in association with certain haplotypes pose a risk for allergy development. There was no correlation between different genotypes and serum levels of various cytokines. FOXP3 polymorphisms rs3761548 and rs3761549 and its haplotype pose greater risk of allergic asthma [(22)]**.** This is in agreement with **Karagiannidis *et al.*** [(23)] who found no significant difference in FOXP3 level in both healthy and asthmatics. This also agreed with **Zhang *et al.*** [(24)] who found no association with any specific haplotype in rs2232365 and rs3761548 and bronchial asthma in the studied population.

This is in contradict with **Hori *et al.*** [(25)] who stated that FOXP3 was highly expressed in $CD4^+CD25^+$ Treg cells in asthmatics and they explained their finding that FOXP3 acts as anti-inflammatory agent in asthma. In their opinion, decreased Foxp3 expression causes immune disease by subverting the suppressive function of Treg cells and converting Treg cells into effector cells.

FOXP3 protein expression within Treg-cells is dramatically reduced in asthmatic patients, according to a number of studies [(18-20)]. Treg cells may fail to control Th proliferation and cytokines in people with this condition [(26)].

In a recent study, it was found that asthmatic patients had more FOXP3 expression than healthy individuals, and that both groups had a Treg cell–suppressive capacity [(20)].

Hassannia *et al.* [(4)] aimed to investigate single nucleotide polymorphisms (SNPs) of Forkhead Box Protein 3 (FOXP3) genes in Iranian patients with allergic rhinitis (AR). It was shown that patients with AR have variations at the -3279 A>C and -924 A>G sites of FOXP3. As part of a case-control investigation, PCR-SSP was used to genotype 155 AR patients and 163 non-allergic controls. They found that patients with FOXP3 -3279 A allele had a considerably higher frequency of this haplotype than controls. A risk allele in this case would be the -3279 A allele.

Thes contradictory results could be explained by methodological discrepancies between studies, by using various Treg sub-types, or by using PBMCs, which indicate a systemic response that may be impacted by the environment, among other possibilities. Alternatively, the increased frequency of Treg cells in asthmatic patients, particularly those with atopic asthma, may reflect a counter-regulatory mechanism that is still insufficient to control allergic inflammation.

CONCLUSION

From the previous results, we reported no association between FOXP3 polymorphism in (rs 3761548 and rs2232365) and occurrence of asthma in Egyptian children. The AC genotype of rs3761548 and the GG genotype of rs2232365 polymorphisms were the most frequent genotypes among the studied children. The rs3761548 C allele may confer a susceptibility to asthma for their carriers being a risk allele.

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Author contribution: Authors contributed equally in the study.

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