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Association of IL-13 rs20541, FOXP3 rs3761548 genes polymorphisms and serum level of IL-13 with allergic asthma in Egyptian patients

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Abstract

The interleukin 13 (IL-13) gene single nucleotide polymorphisms (SNPs) are frequently linked to increased vulnerability to allergic asthma. Forkhead box protein P3 (FOXP3) is an important molecule in the formation of regulatory T cells (Treg). The genetic variants that alter FOXP3 function may have a role in the development of asthma and other allergic disorders. We aimed to determine the association of IL-13 rs20541, FOXP3 rs3761548 genes SNPs and serum levels of IL-13 with allergic asthma patients. In this case–control study, 41 Egyptian patients with allergic asthma were included. Age and gender matched. 41 normal volunteers were considered the controls. All subjects were examined for IL-13 rs20541 and FOXP3 rs3761548 SNPs by the polymerase chain reaction /restriction fragment length polymorphism technique. The serum level of IL-13 was assessed by the enzyme linked immunosorbent assay (ELISA). AA genotype at IL-13 rs20541 SNP was statistically significantly different between the studied groups (p= 0.042). Also, a statistically significant difference was detected when compared AA genotype to GG genotype as AA genotype was three times at risk for asthma (p1=0.031) (OR=3.95) and A allele increased the risk of asthma by about 3 times (OR=3.2). AA genotype at FOXP3 rs3761548 SNP was statistically significantly different between the studied groups (p=0.013). Also, a statistically significant difference was detected when compared AA genotype to CC genotype as AA genotype was 7 times at risk for asthma (p1=0.003) (OR=7.04) and A allele increased the risk of asthma by about 3 times (p<0.001) (OR=3.07). The serum level of IL-13 was statistically significant different between both groups (p<0.001). We can conclude that IL-13 could be a useful tool for predicting allergic asthma. Patients with AA genotype of IL-13 rs20541 and AA genotype of FOXP3 rs3761548 have a higher risk for developing allergic asthma.

Keywords: Allergic asthma, IL-13, FOXP3, Polymorphism.

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Introduction

Allergic asthma is an atopic inflammatory disease that occurs due to allergen exposure in a sensitized individual. Asthma affects 1%–18% of people worldwide, as stated by the Global Initiative for Asthma (GINA) 2020 study, and its prevalence has been rising.[1] The prevalence in Egypt is approximately 6.7% of the total

population.[2] Chronic airway inflammation, a history of respiratory complaints such as wheezing, shortness of breath, chest tightness, and coughing that fluctuate in duration and severity, as well as fluctuating expiratory air flow limitation, are characteristics of asthma.[3] Immunological anomalies that arise in both adults and children are the cause of asthma. It frequently coexists with bronchial hyperresponsiveness, airway blockage, and formation of IgE antibodies in response to inhaled allergens.[4] Despite new and improved pharmacologic choices, many patients with allergic diseases like asthma still experience uncontrolled symptoms. To help manage these patients, novel biologic factors that focus on important and specific pathophysiologic pathways have been investigated.[5] Recent developments in patient improvement, including the discovery of pertinent biomarkers that predict clinical responsiveness, have facilitated the utility of biologic medicines for the therapy of allergic illnesses.[6] The pathophysiology of asthma is influenced by cytokines and transcription factors, which organize and control these immunological and inflammatory responses.[7] Interleukin-13 (IL-13) is a pleiotropic cytokine generated by activated type 2 T helper (Th2) cells, as well as mast cells, macrophages, dendritic cells, and natural killer cells to a lesser amount. The function of IL-13 is regulation of proinflammatory cytokine expression by macrophages, overexpression of adhesion molecules, promotion of B-cell production of immunoglobulin E (IgE), and influences on mucus synthesis.[8] The release of IL-13 influences the immunoglobulin isotype switch from other antibodies to IgE.[7] IL-13 stimulates airway hyperreactivity (AHR) because it regulates the production of certain substances that change the contraction or relaxation of airway smooth muscle cells such as nitric oxide.[9] Among the polymorphisms most frequently linked to increased vulnerability to allergic asthma are single nucleotide polymorphisms (SNPs) in the IL-13 gene.[10] One of the most important IL -13 polymorphisms is the IL-13+2044 G/A (rs20541), in the fourth exon of the IL -13 gene. Many studies have characterized this polymorphism, however, their exact role in bronchial asthma needs more elucidation, especially in the presence of conflicting data from different ethnicities.[11, 12] Forkhead box protein P3 (FOXP3) is a transcription factor, belongings to the forkhead–winged-helix family of transcription factors.[13] The human FOXP3 gene is found on the X-chromosome (Xp11.23), 1296 bp in size, and has 11 coding exons and 3 noncoding exons. The transcription factor FOXP3 is constitutively expressed in CD4+ CD25+ FOXP3+ regulatory T cells (Tregs). It plays a crucial role in immunological homeostasis and suppresses the Th2 response after allergen exposure. The genetic variants that alter FOXP3 function may have a role in the development of asthma and other allergic disorders.[14] Although FOXP3, a main regulator of T regulatory cells, has an essential role in allergic illnesses like asthma, the impact of its gene variations on disease risk is still unknown.[15] To shed light on these concerns among Egyptian allergic patients, this study aimed to investigate the association of IL-13 rs20541, FOXP3 rs3761548 genes SNP, and serum levels of IL-13 with patients suffering from allergic asthma.

Subjects and Methods

This case-control study was performed at the Department of Medical Microbiology and Immunology, the Allergy and Immunology Unit, the Chest Department' Outpatient Clinics and the Medical Research Center, Faculty of Medicine, Zagazig University Hospitals during the period from March 2022 to December 2022.

This study included 82 subjects. They were classified into two groups: 41 patients in the case group and 41 normal volunteers in the control group. Patients with allergic bronchial asthma more than 18 years old were eligible to be included in the study. However, patients in pediatric age group, patients with intermittent asthma, patients with malignancy and autoimmune disease, and patients in acute asthma exacerbation were excluded from the study.

Subjects selected in the control group did not exhibit any respiratory symptoms and did not have history of allergies or respiratory

illness. Diagnosis of allergic asthmatic patients was confirmed by complete history taking including history of atopy, positive skin prick test at the Allergy and Immunology Unit together with physical examination performed by specialists in the Chest Department's Outpatient Clinics and dynamic spirometric results including forced vital capacity (FVC), forced expiratory volume in 1st second (FEV1) and FEV1/FVC ratio. Assessment of asthma severity was done according to GINA 2022 criteria.[3.]

Skin Prick Test

Skin prick testing was carried out utilizing several Coca's derived antigens from the Allergy and Immunology Unit, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. The following aeroallergens were included: house dust mites, feathers, smoke, wool, corn pollen, cotton, mixed fungi, clover pollen, data palm pollen, and hay dust. Saline 0.9% was utilized as the negative control and histamine dihydrochloride (10 mg/mL) as the positive control. Participants were instructed to cease using corticosteroids for 14 days and antihistamines for 5 days prior to skin testing. By measuring the wheal's largest diameter after 15 to 20 minutes, a positive wheal was one that was 3 mm or greater than the negative control.[16]

Sampling

A venous blood sample (5 ml) was collected from each subject enrolled in the study. Serum was separated from an aliquot of blood and preserved at - 20°C till used for measurement of total IgE and IL-13 by the enzyme linked immunosorbent assay (ELISA). The second aliquot of blood was added to an EDTA tube, mixed thoroughly, and preserved in the refrigerator at 2-8°C until used for the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique for detection of IL-13 and FOXP3 genes polymorphisms.

Measurement of serum IL-13 and total IgE Levels

IL-13 serum level was measured by an ELISA commercial kit (No. EH3266 FINE TEST, 1st Floor, Building A02, Optics Valley Biomedical Industrial Park, Wuhan430075, China) according to the manufacturer's instructions. At a 450 nm wavelength, the optical density (OD) was measured with a microtiter plate reader (Sunrise, Tecan Austria GmbH 5082 Grodig, Austria).

Total IgE serum level was determined utilizing a commercial ELISA kit (IMMUNOSPEC, Canoga Park, CA, 91303, USA), according to the manufacturer's instructions. At a 450 nm wavelength, OD was measured with a microtiter plate reader. (Stat Fax 2100, Awareness Technology, Inc. USA). The concentration of serum IL-13 and total IgE levels were calculated using plotted standard curves.

DNA extraction and PCR genotyping

DNA was extracted using QIAamp Blood Mini Kits (cat. no. 51104, Qiagen, Germany), according to the manufacturer's instructions. Extracted DNA was stored at −20°C until used for genotyping test.

Utilizing the PCR/restriction fragment length polymorphism approach, genotyping for IL-13 rs20541 and FOXP3 rs3761548 was carried out. The sequence of used primers (Invitrogen, Thermo Fisher Scientific, analysis, USA) is listed in (Table 1). Commercial kits (2X TOPsimple™ DyeMIX-nTaq kit, Enzynomics, Korea) was used for amplification. For each genotype, PCR was performed in a PCR thermal cycler (Applied Biosystems, Spain) in a total volume of 20 µl containing 10 µl of 2x Master Mix, 1 µl of each primer, 5 µl of the extracted DNA, and 3µl of nuclease-free water. The PCR thermocycling conditions included an initial denaturation step at 95°C for 5 min, followed by 35 cycles each of denaturation at 95°C for 30 sec, annealing at 60°C for 45 sec, extension at 72°C for 30 sec or 45 sec for IL-13 and FOXP3, respectively, then a final extension at 72°C for 5 min. The restriction enzymes NlaIV and PstI (New England BioLabs, UK) were used to digest the resultant PCR products for IL-13 and FOXP3, respectively. The resulting IL-13 and FOXP3 fragments are mentioned in (Table 1). The fragments were visualized under ultraviolet light

transilluminator using 2.5 % agarose gel (Figures 1,2,3,4).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 26.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to gather, tabulate, and statistically analyze all data. Numbers and percentages were used to describe qualitative data while the mean ± standard deviation (SD) and median (interquartile range, IQR) were used to express quantitative data. To compare between two groups of normally distributed variables, we used independent samples Student's t-test while for non- normally distributed variables, we used Mann Whitney U test. To compare between more than two independent groups of normally distributed variables, we used one way ANOVA test. To compare between more than two independent groups of non-normally distributed variables, we used the Kruskal Wallis test. One way ANOVA test with post hoc test to compare between groups. The Fisher's exact test or the Chi-square test were used to compare the percentage of categorical variables when appropriate. The relationship between the several study variables was evaluated by calculating the Spearman's rank correlation coefficient, the (+) and (-) signs denote direct and inverse correlation, respectively, in addition, values close to 1 denote strong correlation and values close to 0 denote weak correlation. All tests were two sided. *p*-value <0.05 was regarded as statistically significant.

Table 1. Primers utilized for amplification and RFLP digestion pattern of IL-13 rs20541and FOXP3 rs3761548 SNPs.

SNP	Primer sequence	Amplicon size	RFLP pattern
IL-13 G/A rs20541	F 5'-CTTCCGTGAGGACTGAATGAGACGGTC -3' R 5'GCAAATAATGATGCTTTCGAAGTTTCAGTGGA-3'	236 bp	GG: 210 bp, 26 bp GA: 210, 178, 32, 26 bp. AA: 178, 32, 26 bp.
FOXP3 C/A rs3761548	F 5'-GCCCTTGTCTACTCCACGCCTCT-3' R 5'-CAGCCTTCGCCAATACAGAGCC-3'	487 bp	CC: 329 bp, 158 bp AC: 487 bp, 329 bp, 158 bp AA: 487 bp

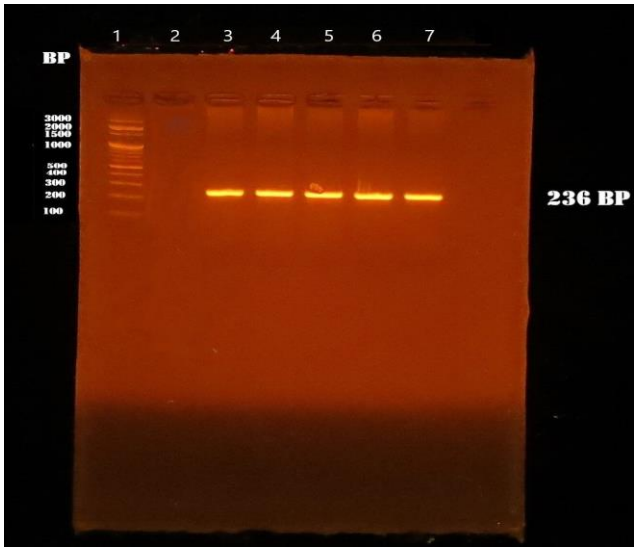

Figure 1. Results of the polymerase chain reaction (PCR) for IL-13 rs20541 gene amplified products. Lane 1: 100 bp DNA ladder. Lane 2: Negative control. Lane 3,4,5,6,7: one band at 236 bp representing the IL-13 rs20541 gene.

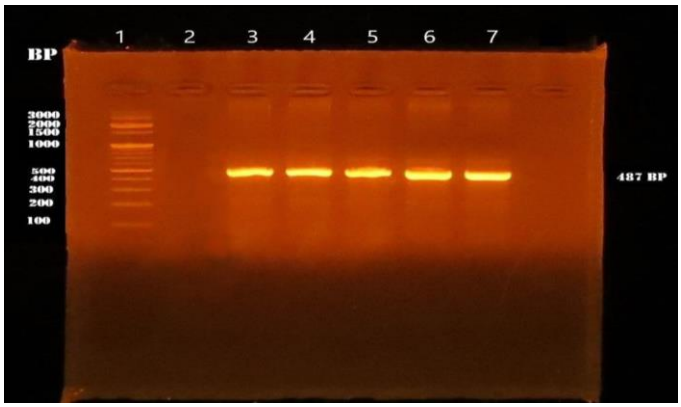

Figure 2. Results of the polymerase chain reaction (PCR) for FOXP3 rs3761548 gene amplified products. Lane 1: 100 bp DNA ladder. Lane 2: Negative control. Lane 3,4,5,6,7: one band at 487 bp representing the FOXP3 rs3761548 gene.

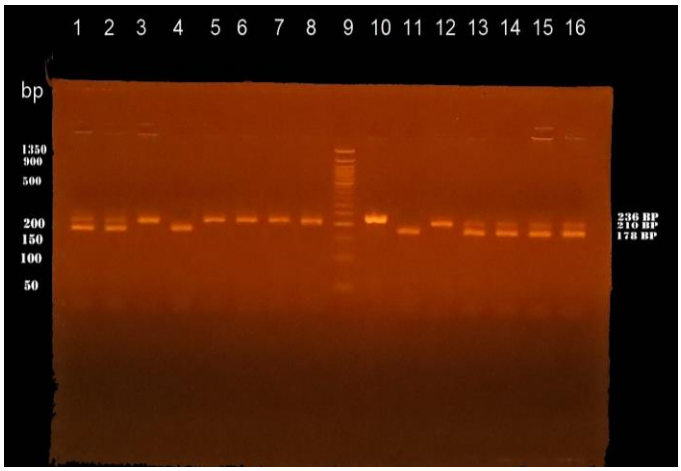

Figure 3. Polymerase chain reaction-restriction fragment length polymorphism (PCR-REFLP) pattern of 236 bp fragments after digestion with NlaIV enzyme to detect rs20541 SNP in IL-13 gene. Lane 9: 50 bp DNA ladder. Lane 1,2,13,14,15,16: four bands (210, 178, 32, 26 bp) represent heterozygous mutant type (GA). Lane 3,5,6,7,8,12: two bands (210, 26 bp) represent wild type (GG). Lane 4,11: three bands (178, 32, 26 bp) represent homozygous mutant type (AA). Lane 10: one band 236 bp represents unrestricted specimen.

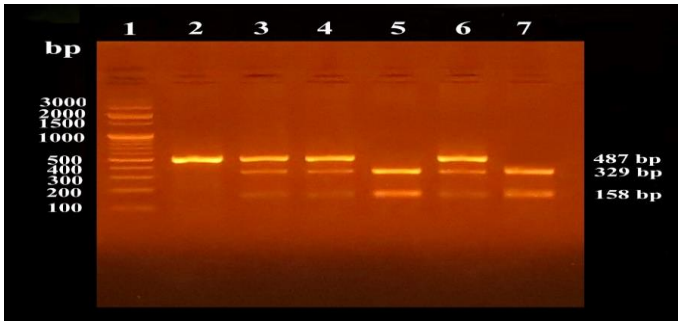

Figure 4. Polymerase chain reaction-restriction fragment length polymorphism (PCR-REFLP) pattern of 487 bp fragments after digestion with PstI enzyme to detect rs3761548 SNP in FOXP3 gene. Lane 1: 100 bp DNA ladder. Lane 2: one band (487 bp) represent homozygous mutant type (AA). Lane 3,4, 6: three bands (487, 329, 158 bp) represent heterozygous mutant type (AC). Lane 5,7: two bands (329, 158 bp) represent wild type (CC).

Results

Demographic characteristics, respiratory function, and asthma severity of the studied groups

This study included 82 subjects, 41 patients in the case group and 41 normal volunteers in the control group. No statistically significant difference in the age and gender between the studied groups. But a statistically significant difference was detected between the studied groups as regards the family history of allergic asthma as 39% of patients had positive family history while only (4.9%) of controls had positive family history (Table 2). A statistically significant difference in the respiratory functions (FEV1 % predicted and FEV1/FVC) was found between the studied groups, with the lowest measures was reported in cases group (p <0.001, Table 2). Concerning asthma severity, most cases (43.9%) had moderate asthma, (36.6%) of them had mild asthma, and only (19.5%) complained of severe asthma (Table 2).

Table 2. Demographic characteristics, respiratory function, and asthma severity of the groups under study.

Variable		Cases group (n=41)		Control group (n=41)		^{t}p value
Age (years) Mean ±SD		38.8±11.49		38.80±10.42		NS
Respiratory function	FEV1 % Predicted Mean ±SD	45.73±15.46		84.32±6.35		<0.001
	FEV1/FVC Mean ±SD	49.46±16.5		85.12±6.20		<0.001
Variable		No	(%)	No	(%)	^{X2}p value
Gender						
Male		21	51.2	22	53.7	NS
Female		20	48.8	19	46.3	
Family history						
Negative		25	61	39	95.1	<0.001
Positive		16	39	2	4.9	
Asthma severity						
Mild		15	36.6			-----
Moderate		18	43.9	-----	-----	
Severe		8	19.5			

(t) Independent sample t test, (X^2) chi-square test $p > 0.05$ is not significant (NS).

Skin prick test

Most of the cases (82.9%) had corn pollen allergy, 78% had clover pollen allergy, more than half of cases (68.3%) were allergic to date palm pollen and 63.4% of cases were sensitive to smoke (Figure 5).

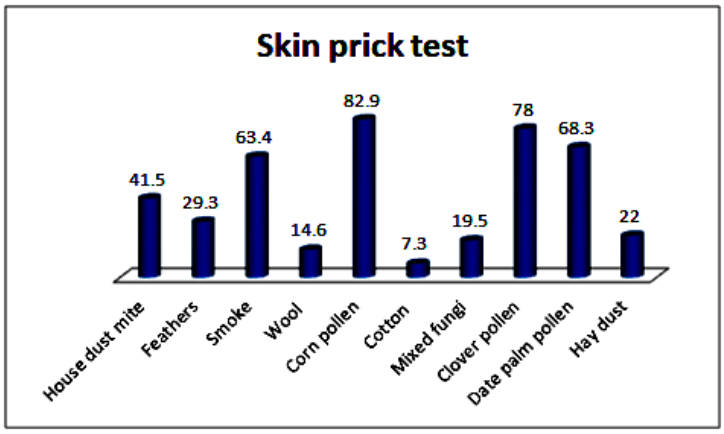

Figure 5. Bar chart illustrating skin prick test results in the cases group.

Serum analysis for total IgE and IL-13

A statistically significant difference in total IgE level was found between the study groups with the highest measures 111.24 IU/mL (107.88-121.63 IU/mL) was reported in cases group (p<0.001). A statistically significant increase in serum level of IL-13 was found within cases group with median 40 pg/mL (36.2-54.5 pg/mL) (Table 3).

Table 3. Serum level total IgE (IU/mL) and IL-13 (pg/mL) of the studied groups.

	Cases group (n=41)	Control group (n=41)	^{z}p
Total IgE			
Mean± SD	131.65±54.15	41.03±18.02	<0.001
Median (IQR)	111.24 (107.88-121.63)	37.5 (26.95-53.1)	
Serum level of IL13			
Mean± SD	95.08±169.64	24.47±4.77	<0.001
Median (IQR)	40 (36.2-54.5)	24.9 (19.6-28.4)	

*$p \leq 0.05$ is significant. Z:-score

Single nucleotide polymorphisms (SNP) analysis

Regarding IL-13 rs20541 polymorphism, a statistically significant difference was found between the studied groups with AA genotype and A allele increases the risk of asthma by about 3 times (Table 4). Also, a statistically significant difference in FOXP3 rs3761548 polymorphism was detected between the groups under study with those have AA genotype are 7 times at risk for asthma and A allele increases the risk of asthma by about 3 times (Table 4).

Table 4. IL-13 rs20541 and FOXP3 rs3761548 polymorphisms between the study groups.

Variable	Cases group (n=41)		Control group (n=41)		Tests		OR (CI)
	No	%	No	%	^{x2}p value	*p* value between groups	
IL-13 rs20541							
AA (n=14)	10	24.4	4	9.8	0.042	*p*1=0.031	3.95 (1.08-14.39)
GA (n=19)	12	29.3	7	17.1		*p*2=0.70	2.7 (0.91-8.09)
GG (n=49)	19	46.3	30	73.2		Ref.	
Alleles							
A (n=47)	32	39.5	15	18.3	0.001		3.2
G (n=117)	50	60.5	67	81.7			(1.6-6.5)
FOXP3 rs3761548							
AA (n=16)	13	31.7	3	7.3	0.013	*p*1=0.003	7.04 (1.73-28.59)
AC (n=24)	12	29.3	12	29.3		*p*2=0.347	1.63 (0.59-4.48)
CC (n=42)	16	39	26	63.4		Ref.	
Alleles							
A (n=56)	38	46.3	18	21.9	<0.001		3.07
C (n=108)	44	53.7	64	78.1			(1.55-6.05)

(X^2) chi-square test (*p*1 AA vs. GG) (*p*2 GA vs. GG), for IL-13 rs20541 (*p*1 AA vs. CC) (*p*2 AC vs. CC), for FOXP3 rs3761548
*$p \leq 0.05$ is significant.

Comparison between different IL-13 rs20541 gene and FOXP3 rs3761548 gene polymorphisms as regard respiratory functions

A statistically significant relation was found between IL-13 rs20541 gene polymorphism and respiratory function in the cases group as AA genotype had worst function (Table 5). Also, a statistically significant relation existed between FOXP3 rs3761548 gene polymorphism and respiratory function as AA genotype had the worst function (Table 5).

Table 5. Comparison of respiratory functions between the different IL-13 rs20541 gene and FOXP3 rs3761548 gene polymorphisms within the cases group.

Variable	IL-13 rs20541			Tests	
	AA (N=10)	GA (N=12)	GG (N=19)	*p* value	Post hoc
FEV1%Predicted					
Mean ±SD Range	33.5±10.13 (18-49)	41.66±13.5 (22-67)	54.73±13.7 (20-70)	<0.001	*p*1=0.148 (NS) *p*2=0.001 *p*3=0.009
FEV1/FVC					
Mean ±SD Range	37.1±13.64 (18-70)	46.16±15.54 (15-68)	58.05±13.96 (21-69)	0.002	*p*1=0.149 (NS) *p*2=0.001 *p*3=0.031
	FOXP3 rs3761548				
	AA (N=13)	AC (N=12)	CC (N=16)		
FEV1%Predicted					
Mean ±SD Range	35.38±10.49 (18-49)	41.58±13.82 (23-69)	57.25±12.72 (25-70)	<0.001	*p*1=0.22 *p*2<0.001 *p*3=0.002
FEV1/FVC					
Mean ±SD Range	39.3±14.38 (15-58)	46.08±15.4 (24-70)	60.25±12.84 (26-75)	0.001	*p*1=0.238 (NS) *p*2=0.001 *p*3=0.012

One way ANOVA test. $p > 0.05$ is not significant (NS). *p*1: AA vs. GA, *p*2: AA vs. GG, *p*3: GA vs GG, for IL-13 rs20541. *p*1: AA vs. AC, *p*2: AA vs. CC, *p*3: AC vs CC for FOXP3 rs3761548.

Comparison of total IgE and serum level of IL-13 between different IL-13 rs20541 gene and FOXP3 rs3761548 gene polymorphisms

A statistically significant relation was detected between IL-13 rs20541 gene polymorphism and each of total IgE and serum level of IL-13 as AA gene showed higher total IgE and serum level of IL-13 median (Table 6). Also, a statistically significant relation was found between FOXP3 rs3761548 polymorphism and each of total IgE and serum level of IL-13 as AA gene showed higher total IgE and serum level of IL-13 median (Table 6).

Table 6. Comparison of total IgE and serum level of IL-13 between different IL-13 rs20541 gene and FOXP3 rs3761548 gene polymorphisms within the cases group.

SNP	Total IgE Median (IQR)	*p* value	Post hoc	Serum level of IL-13 Median (IQR)	*p* value	Post hoc
IL-13 rs20541						
AA (N=10)	118.6 (113.5-199.3)		*p*1=0.187 (NS)	43.05 (39.6-204.8)		*p*1=0.166 (NS)
GA (N=12)	109.3 (108.1-146.6)	0.024	*p*2=0.005	39.7 (34.8-77.9)	0.04	*p*2=0.023
GG (N=19)	110 (105.7-112)		*p*3=0.265 (NS)	39.5 (34.3-42.9)		*p*3=0.453 (NS)
FOXP3 rs3761548						
AA (N=13)	115.5 (111-183.4)		*p*1=0.265 (NS)	42.1 (40-165.7)		*p*1=0.277 (NS)
AC (N=12)	110.07 (108.1-131.8)	0.030	*p*2=0.006	40.93 (36.4-83.5)	0.021	*p*2=0.007
CC (N=16)	108.7 (104.9- 111.8)		*p*3=0.246 (NS)	36.4 (31.3-41.2)		*p*3=0.109 (NS)

Kruskal-Wallis Test. $p > 0.05$ is not significant (NS). *p*1: AA vs. GA, *p*2: AA vs. GG, *p*3: GA vs GG, for IL-13 rs20541. *p*1: AA vs. AC, *p*2: AA vs. CC, *p*3: AC vs CC for FOXP3 rs3761548.

Discussion

One of the most prevalent allergy disorders in the world is allergic asthma. Individuals' susceptibility to certain allergy disorders, such as asthma, might be influenced by genetic diversity. This study examined and compared the group of patients with allergic asthma to gender and age-matched, control subjects. No statistically significant difference was found between both groups concerning age and sex (p=1.000 and p= 0.825, respectively). This is consistent with findings of previous studies.[17,15] In relation to the family history, a statistically significant difference was found between the two study groups, which is consistent with the findings of Hafez et al., 2022.[18] Regarding pulmonary function (FEV1% predicted and FEV1/FVC), there was statistically significant difference between the study groups with the lowest measures was reported in cases group (p <0.001). This agrees with the findings of several previous reports.[19- 21]

In the current work, the cases group skin prick test results showed that the allergic patients had polysensitization, and most cases (82.9%) had corn pollen allergy, 78% of cases had clover pollen allergy, more than half of cases (68.3%) were allergic to date palm pollen and 63.4% of cases were sensitive to smoke. The study by Salama et al., 2022 showed that most of the allergic patients had poly sensitization,[22] but the most frequent aeroallergen was mixed fungi while Dibek & Reha, 2007 showed that house dust mite was the most important aeroallergens.[23] The discrepancy in the result may be attributed to environmental and genetic variation. Concerning total IgE level in the current study, a statistically significant difference was detected between the study groups (p<0.001). Dimitrova et al., 2019, and Hafez et al., 2022 showed similar results.[24,18] However, Gergen et al., 2009 reported that the impact of total IgE was non-uniform across the asthma-related atopic group according to the National Health and Nutrition Examination Survey (NHANES) 2005–2006 study in USA.[25] The larger sample size (N= 7398) of the NHANES allowed a more complete enumeration of asthma at low levels of total IgE.

The Th2 phenotype predominates in allergic people, causing an increase in IL-13 production and class-switching in B cells to produce IgE.[26] In the present study, the serum level of IL-13, was significantly different between both study groups (p<0.001). According to the study by Jebur and Saud, 2020, asthmatics had a higher level of IL-13 than healthy people (p < 0.001),

which is consistent with the current study findings.[27] Conversely, the study by Davoodi et al., 2012 found that the median serum level of IL-13 was 40.0 pg/ml in asthmatic patients and 58.25 pg/ml in healthy controls, but the difference was not significant between both groups.[28] The discrepancies in the results could be related to the design of the studies, the sample size, and the used laboratory techniques.

Several studies indicated that the SNPs in the IL-13 gene were connected to a higher incidence of allergic disorders and an increase in their severity.[17, 29] But meta-analysis studies demonstrated that people of various ethnicities differ in the frequency of various SNPs and their relevance to allergy diseases [30]. In the current study, regarding IL-13 rs20541 polymorphism, 24.4% of patients had genotype AA, 29.3% had genotype GA and 46.3% had genotype GG, while 9.8% of the controls had genotype AA, 17.1% had genotype GA and 73.2% had genotype GG. Allele A was 39.5% in cases and 18.3% in controls. G allele was 60.5% in cases and 81.7% in controls. This means that GG genotype has a higher rate than other genotypes in both cases and controls, but it is elevated in controls more than cases.

In the present study, a statistically significant difference was detected between the studied groups regarding AA genotype (p= 0.042), also there was statistically significant difference when compared AA genotype to GG genotype as AA genotype was three times at risk for asthma (p1=0.031) (OR=3.95) and A allele increased the risk of asthma by about three times (OR=3.2). No difference was found between the study groups concerning GA genotype when compared to GG genotype (p2=0.70). Similar results were mentioned by Farhan et al., 2021.[29] They showed that GG genotype has a higher rate than other genotypes in both cases and controls, but it is elevated in controls (77%) more than cases (54%). They also showed that AA genotype was 18% in cases while was 7% in controls. A statistically significant difference was found between cases and controls regarding AA genotype (p=0.021) (OR=3.21). A statistically significant difference existed between cases and controls regarding A allele (p=0.0002) (OR=2.745). However, they found statistically significant difference between cases and controls regarding GA genotype (p=0.0167) (OR=2.515). Also, the study by Noureldin et al., 2014 showed similar results.[31] They reported that GG genotype was significantly more common in the control group than in asthmatic patients (93.3% vs. 58%, respectively, p =0.001), and the frequency of A allele was significantly greater in asthmatic patients (28%) as opposed to control individuals (5%) (p<0.001). However, it different from our study as it showed that GA genotype was significantly more common in asthmatic patients than in the control group (28% vs. 3.3%, respectively, p =0.007), and there was no statistically significant difference between cases and controls regarding AA genotype (p=0.247). On the other hand, the study by Hafez et al., 2022 and Sharifi et al., 2021 showed that there was no difference between patients and controls regarding IL-13 rs20541 polymorphism.[18,12] The difference in the result between the various studies may be due to the different sample size between the studies and the nature of the studies.

In the present study, a statistically significant relation was observed between IL-13 rs20541 gene polymorphism and FEV1% and FEV1/FVC in the cases group (p<0.001 and p= 0.002, respectively), as AA genotype had the worst function. This is consistent with findings of the study by Noureldin et al., 2014 who showed that patients with more severe asthma were significantly more likely to have the A allele of the IL-13+2044 G/A polymorphism (p< 0.001), while G allele was significantly more common in less severe asthmatics (mild patients) (p< 0.001).[31] However, the study by Imraish et al., 2021 mentioned that no significant relation was found between IL-13 rs20541 gene polymorphism and FEV1/FVC (p=0.8187).[17]

The current work found a statistically significant relation between IL13 rs20541 gene polymorphism and median serum level of IL-13 (p= 0.04), as AA genotype showed higher median serum level of IL-13. The study by Arima et al., 2002 reported similar results. They found that the presence of A allele was associated with elevated serum level of IL-13.[32] On the

other hand, the study by Hafez et al., 2022 showed that there was no significant association between serum IL-13 levels and IL-13 rs20541 gene polymorphism among allergic asthma patients [18]. The variation in this result could be attributed to the intricate interplay between the genetic and epigenetic components of various societies and their influence on gene expression. Also, in the current study, there was a statistically significant relation between IL-13 rs20541 gene polymorphism and the median serum level of total IgE in the cases group (p= 0.024) as AA gene showed higher median serum level of total IgE ($p2$=0.005). This was consistent with the findings of the study by Abdulla & Shaheed, 2022 who reported similar results.[33]

It has been reported that FOXP3 is a particular marker with an essential role in the development and function of the Tregs to negatively regulate the immune responses, so it has a significant role in allergic disorders.[34] FOXP3 gene polymorphisms were linked to different allergic diseases but it is unclear how they contribute to the occurrence of allergic asthma.[35] In the current study, regarding FOXP3 rs3761548 polymorphism, 31.7% of allergic asthma patients have genotype AA, 29.3% genotype AC and 39% genotype CC, while 7.3% of the normal controls have genotype AA, 29.3% genotype AC and 63.4% genotype CC. Allele A was detected in 46.3% of the cases group and in 21.9% of the controls. The C allele was present in 53.7% of the allergic asthma cases group and in 78.1% of the controls. This may indicate that CC genotype has a higher rate than other genotypes in both cases and controls, but it is elevated in controls more than the allergic asthma cases. Also, a statistically significant difference was found in AA genotype between the study groups (p= 0.013), and there was statistically significant difference when AA genotype was compared to CC genotype as AA genotype had 7 times risk for asthma ($p1$=0.003) (OR=7.04) and A allele increased the risk of asthma by about 3 times (p<0.001) (OR=3.07). However, no statistically significant difference was found in AC genotype between the studied groups ($p2$=0.347). This comes in consistent with findings of a study by Beigh et al., 2020 who found significant difference between cases and controls in the AA genotype of FOXP3 rs3761548 gene polymorphism [p = 0.009; OR, 3.52) and significant difference in the A allele between cases and controls (p = 0.001; OR, 1.75) which may indicate that individuals with the A allele are more susceptible to allergic asthma.[15] Also, the study by Hassannia et al., 2011 showed similar results.[36] On the other hand, the study by Sarhan et al., 2022 mentioned that the most prevalent genotype was the heterozygous mutant form AC followed by the wild form CC then homozygous mutant form AA.[20] They reported no significant difference between asthmatic patients and the control group. These contradictory findings could be attributed to disparities in study methodology.

In the current study, a statistically significant relation was detected between FOXP3 rs3761548 gene polymorphism and FEV1% and FEV1/FVC in the cases group (p<0.001 and p=0.001, respectively), as the AA genotype had the worst function. In the current work, a statistically significant relation was detected between FOXP3 rs3761548 gene polymorphism and the median serum level of IL-13 in the cases group (p= 0.021), as the AA genotype showed higher median serum level of IL-13 when compared to CC genotype ($p2$=0.007). But no significant relation was found when comparing serum level of IL13 in AA genotype with its level in AC genotype ($p1$=0.277) and when comparing serum level of IL13 in AC genotype with its level in CC genotype ($P3$=0.109). On the other hand, the study of Beigh et al., 2020 showed that serum level of IL-13 was found high in each genotype of FOXP3 rs3761548 gene polymorphism. However, no significant association was found between the serum level of IL-13 and various genotypes of FOXP3 rs3761548.[15]

In the current work, there was a statistically significant relation between FOXP3 rs3761548 gene polymorphism and median serum level of total IgE in the cases group as AA genotype showed higher median serum level of total IgE when compared to CC genotype (p= 0.030) ($p2$=0.006). But no significant relation was found when comparing total IgE level in AA

genotype with its level in AC genotype ($p1$=0.265) and when comparing total IgE level in AC genotype with its level in CC genotype ($p3$=0.246). Conversely, the study by Beigh et al., 2020, showed that serum level of total IgE was high in each genotype of FOXP3 rs3761548 gene polymorphism. However, no significant association was detected between the serum level and various genotypes of FOXP3 rs3761548.[15]

There are certain limitations to this work, including the limited sample size and the absence of evaluation of additional coding SNPs in the IL-13 and FOXP3 genes. In conclusion, patients with AA genotype of IL-13 rs20541 and AA genotype of FOXP3 rs3761548 have a higher risk for developing allergic asthma. IL-13 could be a valuable tool for predicting allergic asthma. Total IgE could be considered an appropriate diagnostic and prognostic biomarker for allergic bronchial asthma.

Author Contributions

ARMH; Study design and Manuscript review. AGMA; Sample processing, laboratory work, and Manuscript preparation. NAI; Sample collection and Manuscript review. YAF; Study design, Manuscript preparation and Manuscript review.

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Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University (approval no. IRB#:9155–14-12-2021).

Informed consent

A signed informed consent was obtained from each patient and control individual before included in the study.

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