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Association Between IL-4 (rs2243250) and IL-13 (rs20541) Gene Polymorphisms and Total IgE Levels in Bronchial Asthma: A Study from Aswan Governorate, Egypt

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ABSTRACT

Background & Objective: Bronchial asthma is a persistent inflammatory disorder of the respiratory system, characterised by the involvement of numerous immune cells and mediators. Interleukin-4 (IL-4) and interleukin-13 (IL-13) are Th2 cytokines involved in asthma pathophysiology. This study aimed to assess the impact of IL-4 rs2243250 and IL-13 rs20541 genetic variants, in combination with total blood IgE levels, on asthma patients from Aswan governorate, Egypt.

Methods: This case-control study included 50 patients with asthma and 30 healthy controls matched for age and environment. Serum levels of IL-4, IL-13, and IgE were quantified using ELISA. IL-4 and IL-13 polymorphisms were identified using PCR-RFLP genotyping.

Results: Serum IgE levels showed strong correlations with IL-4 and IL-13 levels (Spearman r = 0.742 and 0.937, respectively; p = 0.001). Genotype distributions of IL-4 rs2243250 and IL-13 rs20541 differed significantly between asthma cases and controls ($p < 0.05$), with enrichment of the IL-13 rs20541 A allele among cases. IL-13 rs20541 genotype distribution did not differ significantly across asthma severity categories; however, severity-stratified analyses were underpowered. After Benjamini–Hochberg FDR adjustment for the prespecified genetic family (m = 4), IL-4 genotype distribution (p = 0.0027, q = 0.0107) and T-allele enrichment (p = 0.0185, q = 0.037) remained significant. For IL-13 rs20541, genotype distribution (p = 0.009, q = 0.0105) and A-allele enrichment (p = 0.0005, q = 0.0035) were also significant.

Conclusion: This study supports Th2-skewed immune activation in bronchial asthma, with higher serum IgE, IL-4, and IL-13 levels in cases. Genetic analyses suggest associations between IL-4 rs2243250 and IL-13 rs20541 polymorphisms and asthma susceptibility. Given the modest control sample and absence of A/A cells among controls, the rs20541 finding should be interpreted as suggestive and hypothesis-generating. Larger, ancestry-matched cohorts with objective phenotyping and standardized treatment sampling are warranted.

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Introduction

Asthma is a chronic respiratory condition characterized by recurrent airway inflammation, bronchial hyperplasia, and reversible airflow limitation. This ongoing airway inflammation leads to airway structural remodeling, a contributing factor to disease progression and persistent illness. Asthma is a substantial global health issue, affecting health-related quality of life and placing a considerable burden on health care systems (1). Over the last several decades, the burden of asthma-related sickness and death has been persistently increasing and affects an estimated 1–18% of the populations of countries around the world (2). The most prominent cytokines involved in asthma pathogenesis are Interleukin-4 (IL-4) and Interleukin-13 (IL-13), both secreted primarily by T helper 2 (TH2) cells. These cytokines significantly promote immunoglobulin E (IgE) isotype switching in B lymphocytes by enhancing MHC class II and CD23

expression, thereby affecting immune cell development, differentiation, and inflammatory signaling (3).

IL-13 alters macrophage activation and reduces the synthesis of proinflammatory cytokines such as IL-1α, IL-1β, IL-8, TNF-α, and TNF-6. The effects of IL-4 and IL-13 are mediated by receptor dimerization. Receptor dimerization occurs when the cytokine binds to the α-subunit of the IL-4 receptor (IL-4Rα), initiating a downstream signaling cascade (4). Polymorphisms in IL-13, IL-4Rα, and MS4A2 genes are associated with asthma sensitivity. However, the strength and significance of these links vary across ethnic groups and age groups, indicating a complex relationship between genetic factors and environmental risks in the development of asthma.

Materials and Methods

Study Design and Participants

This case–control study included 50 patients diagnosed with bronchial asthma and 30 apparently healthy, non-asthmatic controls. Participants were recruited from the Asthma and Allergy Clinic at Aswan Governorate Hospital between October 2019 and June 2021. Controls were recruited from the same catchment area and were frequency-matched to cases by age, sex, and living environment (residency: urban/rural). To minimize population stratification, cases and controls were recruited from the same region, and participants reported similar Egyptian ancestry.

The diagnosis of asthma was validated by a specialist physician utilizing the criteria established by the European Community Respiratory Health Survey (ECRHS), which stipulate that asthma is characterized by experiencing an episode within the preceding 12 months or by the consistent use of asthma medication (6). Controls were screened using the same ECRHS-based assessment and clinical evaluation to exclude physician-diagnosed asthma, asthma-like symptoms within the preceding 12 months, and current use of asthma medication. Controls with a personal history of atopy/allergic disease were excluded; family history was recorded as a background characteristic. Participants were unrelated to each other.

History and Clinical Assessment

A structured questionnaire was used to obtain a detailed medical history and to evaluate asthma severity. Severity was operationalized in accordance with the Canadian Consensus Guidelines (7), using a standardized checklist that captured daytime symptom frequency, nocturnal symptoms, activity limitation, reliever (short-acting β2-agonist) use, and current controller therapy step. Where available, recent exacerbation history (systemic corticosteroid bursts, emergency department visits, and/or hospitalizations in the preceding 12 months) was reviewed to corroborate severity categorization and to ensure consistent classification across participants.

Pulmonary function and treatment status at sampling: Spirometry (FEV1, FVC, FEV1/FVC, and bronchodilator reversibility) was not routinely available for all participants in this setting and therefore could not be systematically reported; asthma case definition relied on specialist-verified ECRHS criteria and medication history. In addition, medication use at the time of venipuncture was not uniformly captured; given that serum cytokines are treatment-sensitive, cytokine-related genotype associations should be interpreted cautiously and are presented as hypothesis-generating.

Exclusion Criteria

Asthmatic group: Patients with other respiratory disorders such as chronic bronchitis, emphysema, tuberculosis, or pneumonia were excluded.

Control group: Individuals were excluded if they presented with asthma-like symptoms, had known allergies, a history of smoking, or a family history of asthma or allergic conditions.

Laboratory Procedures

Blood Sample Collection

From each participant, 5 mL of venous blood was collected under sterile conditions and fractionated as follows: 2.5 mL was placed in a plain tube, allowed to clot, and then centrifuged at 3000 rpm for 5 minutes. The serum was then stored at –80 °C in sterile Eppendorf tubes. The other 2.5 mL was transferred into an EDTA tube and used for genomic DNA extraction and subsequent genotyping.

Measurement of IL-4, IL-13, and IgE Levels

Serum levels of IL-4, IL-13, and total IgE quantification were done using enzyme-linked immunosorbent assay (ELISA) kits. IL-4 was assessed using the Human IL-4 ELISA Kit (Cat. No. E0092Hu; Biosafety Technology Laboratory). Samples were tested in duplicate. IL-13 was measured using the Human IL-13 ELISA kit from the Biosafety Technology Laboratory, according to the manufacturer's protocol. Total IgE determination was done using the Human IgE ELISA Kit from Koma Biotech, Korea.

Genetic Analysis

DNA Extraction

Genomic DNA was isolated from EDTA-preserved blood specimens using the Quick-DNA™ MiniPrep Kit (Cat. No. D3025) according to the manufacturer's guidelines.

Polymerase Chain Reaction (PCR)

For the IL-4 and IL-13 genes, specific primers were designed to their corresponding gene boundaries for amplification. For precision and verification, all primer sequences were screened using NCBI tools. For IL-4, the forward primer was 5′-ACTAGGCCTCACCTGATACG-3′, and the reverse primer was 5′-GTTGTAATGCAGTCCTCCTG-3′,

yielding a product size of 252 bp. For IL-13, the forward primer was 5′-TAAACTTGGGAGAACATGGT-3′, and the reverse primer was 5′-TGGGGAAAGATAGAGTAATA-3′, which had a product size of 236 bp.

PCR Conditions

For each gene of interest, specific thermal cycling protocols were followed for PCR. In the case of IL-4, the PCR protocol comprised an initial denaturation step of 95 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 57 °C for 1 minute, extension at 72 °C for 1 minute, and a final extension of 72 °C for 5 minutes. For IL-13, the conditions were: initial denaturation at 93 °C for 3 minutes, followed by 35 cycles of denaturation at 93 °C for 30 seconds, annealing at 62 °C for 30 seconds, and extension at 72 °C for 2 minutes, followed by a final step at 72 °C for 10 minutes.

RFLP-PCR Analysis

The polymorphisms in IL-4 and IL-13 were detected using specific restriction enzymes BsmFI and NLAIV, respectively. Complete digestion reactions were incubated for 4 hours at the optimal temperature to ensure complete digestion. The DNA fragments were separated by gel electrophoresis on a 3% agarose gel and visualized under UV light. All the gels were photographed using a digital imaging system for further analysis. Fig. 1 illustrates the PCR-RFLP genotyping patterns for the IL-4 (C/T) polymorphism after digestion with BsmFI.



Fig. 1. Agarose gel electrophoresis image showing PCR-RFLP analysis of IL-4 (−589 C/T; rs2243250) polymorphism. DNA was digested using the BsmFI restriction enzyme.

- Lane 1: 50 bp DNA ladder.
- Lanes 2–4: Heterozygous genotype (C/T) showing three bands at 252 bp, 192 bp, and 60 bp.
- Lanes 5, 6, 9, 10: Homozygous mutant genotype (T/T) with a single uncut band at 252 bp.
- Lanes 7, 8: Homozygous wild-type genotype (C/C) with two bands at 192 bp and 60 bp.

Figure 2 illustrates the genotyping patterns of IL-13 (G/A) polymorphisms using PCR-RFLP. The products were digested with NlaIV, revealing apparent differences among G/G, A/A, and G/A genotypes.

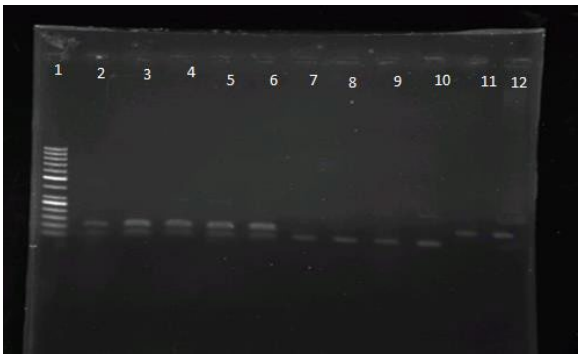

Fig. 2. Agarose gel electrophoresis showing PCR-RFLP genotyping of IL-13 (rs20541; G/A) polymorphism following dIgEstion with the *NlaIV* enzyme.

Lane 1: 100 bp DNA ladder (Ultra Low Range).

- Lanes 2–6: Heterozygous G/A genotype, showing two visible bands at 210 bp and 178 bp (the expected 32 bp fragment is too small to resolve).
- Lanes 7–10: Homozygous G/G genotype, showing a single visible band at 178 bp.
- Lanes 11–12: Homozygous A/A genotype, with a single uncut band at 210 bp.

Statistical Analysis and Power Considerations

Data were analyzed using standard statistical methods. Continuous variables were summarized as mean ± SD when approximately normally distributed and as median (IQR) when skewed. Categorical variables were presented as counts and percentages.

Normality of continuous variables was assessed using the Shapiro–Wilk test together with visual inspection of histograms and Q–Q plots. Homogeneity of variances was evaluated using Levene's test. If data were approximately normal (or became approximately normal after log10 transformation for right-skewed variables), between-group comparisons were conducted using the independent-samples t test (Welch's correction was applied when variances were unequal). If the normality assumptions were not met, nonparametric alternatives were used (Mann–Whitney U test for two-group comparisons and Kruskal–Wallis test for comparisons across severity categories). Correlations were assessed using Pearson's correlation for approximately normally distributed variables and Spearman's rank correlation for skewed or ordinal variables. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

Genotype and allele frequencies were compared using Pearson's chi-square test; Fisher's exact test was used when expected cell counts were small. Odds ratios (OR) and 95% confidence intervals (CI) for asthma susceptibility were estimated using univariable logistic regression with asthma status as the dependent variable.

Hardy-Weinberg equilibrium (HWE) was assessed in the control group for each SNP using a chi-square goodness-of-fit test.

Given the modest sample size and the marked imbalance in family history between groups, fully adjusted multivariable logistic regression models were not deemed sufficiently stable for reliable inference; therefore, genetic associations are reported from univariable analyses and interpreted as exploratory.

Genotypes were analyzed (i) as a categorical predictor with the common homozygous genotype as the reference category, and (ii) under standard genetic models: additive (coded 0/1/2 for the number of minor alleles), dominant (minor-allele carriers vs. common homozygotes), and recessive (minor-allele homozygotes vs. others), as appropriate. Allele-based ORs were additionally derived from 2×2 allele count tables.

To control for multiple testing, p values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure (q = 0.05) within pre-specified families of related analyses (genetic association tests and correlation analyses).

All analyses were performed using IBM SPSS Statistics version 26.

Because the sample size was constrained by recruitment feasibility, we also report the achieved (post hoc) power for the key IL-13 rs20541 analyses. Achieved power for chi-square tests was estimated using the noncentral chi-square approach based on the observed effect size $w = \sqrt{(\chi^2/N)}$, where N is the total sample size for the contingency table, and df is the test degrees of freedom. Power was computed as $P(\chi^2 df,\lambda > \chi^2 crit)$, where $\lambda = N \cdot w^2$ and $\chi^2 crit$ is the critical value at $\alpha = 0.05$.

Results

Participant Characteristics

The research comprised 50 asthmatic patients (case group) and 30 healthy participants (control group). No substantial differences were seen in baseline age, gender, residence (urban versus rural), or concomitant conditions between patients and controls ($p > 0.05$ for all comparisons). However, a family history of asthma was far more common in the asthmatic group (54% vs. 6.7% in controls), a highly significant difference ($\chi^2 = 17.923$, $p < 0.001$) (Table 1).

Asthma Severity Distribution

Among the 50 asthma patients, 18 were classified as having mild asthma. (36%), moderate in 21 patients (42%), and severe in 11 patients (22%). This distribution reflects the range of disease intensity in the case group (categorised according to standard consensus criteria) (Table 2).

Table 1. Demographic data of the studied groups

Parameter	Case Group (n=50)	Control Group (n=30)	Test Statistic	p-value
Age (years)	42.71 ± 18.33 (6–63)	39.41 ± 15.29 (6–57)	t = -0.828	0.410
Gender				
Male	31 (62%)	21 (70%)	$X^2 = 0.521$	0.471
Female	19 (38%)	9 (30%)		
Residency				
Rural	28 (56%)	14 (46.7%)	$X^2 = 0.725$	0.216
Urban	22 (44%)	16 (53.3%)		
Family History				
Yes	22 (54%)	2 (6.7%)	$X^2 = 17.923$	< 0.001*
No	28 (46%)	28 (93.3%)		

Table 2. Distribution of asthma severity in the case group

Severity Level	Number (n=50)	Percentage (%)
Mild	18	36%
Moderate	21	42%
Severe	11	22%

Serum IgE and Cytokine Levels

Asthmatic patients had markedly elevated immunological markers compared to controls. The average total IgE level in patients with asthma was 426.31 ± 117.24 kIU/L (range, 18-640), which was more than 12 times higher than the control level of 34.11 ± 12.83 kIU/L (range, 14-52; $p < 0.001$). Similarly, Serum IL-4 concentrations were markedly elevated in individuals (109.24 ± 96.36 pg/mL) than in controls (41.62 ± 15.74 pg/mL; $p < 0.001$). The same trend was observed for IL-13 levels, with asthmatics

averaging 83.71 ± 67.22 pg/mL, compared with 38.33 ± 11.95 pg/mL in controls ($p < 0.001$). These results indicate that individuals with asthma exhibit substantially elevated Th2-related cytokines and IgE levels compared with healthy individuals. (Table 3)

Table 3. Serum levels of IgE, IL-4, and IL-13 in the study groups

Parameter	Case Group (n=50)	Control Group (n=30)	t-test	p-value
IgE (kIU/L)	426.31 ± 117.24 (18–640)	34.11 ± 12.83 (14–52)	-18.212	< 0.001*
IL-4 (pg/mL)	109.24 ± 96.36 (37.73–471.24)	41.62 ± 15.74 (21.44–57.19)	-3.804	< 0.001*
IL-13 (pg/mL)	83.71 ± 67.22 (33.12–430.87)	38.33 ± 11.95 (17.58–51.37)	-3.654	< 0.001*

IL-4 Gene Polymorphism (rs2243250)

Significant changes were observed in the IL-4 gene (−589 C/T, rs2243250) polymorphism between asthmatic individuals and control subjects. In the asthma group, the distribution of IL-4 genotypes was 42% C/C, 24% C/T, and 34% T/T, whereas in the control group, it was 76.7% C/C, 16.6% C/T, and 6.7% T/T. The T allele was much more prevalent in patients (46% of alleles) than in controls (15%). The T/T genotype was significantly more prevalent among individuals with asthma (one-third) than in the control group (6.7%; odds ratio [OR] ≈ 7.6, 95% confidence interval 1.7–39.1; $p = 0.006$). In contrast, the wild-type C/C genotype was less prevalent among cases than among controls (42% versus 76.7%). The heterozygous C/T genotype exhibited no significant difference across groups (24% vs. 16.6% in controls, $p = 0.436$). The presence of the IL-4 T allele was associated with an increased risk of asthma (OR = 3.2 for allele frequency comparison; $p < 0.01$). Table 4. Genotype distribution in controls was consistent with Hardy-Weinberg equilibrium (chi-square $p = 0.058$).

Table 4. Genotype and allele frequencies of IL-4 in case and control groups

Genotype/Allele	Case Group (n=50)	Control Group (n=30)	OR (95% CI)	p-value
C/C	21 (42%)	23 (76.7%)	Reference	0.003*
C/T	12 (24%)	5 (16.6%)	1.28 (0.38–4.47)	0.436
T/T	17 (34%)	2 (6.7%)	7.63 (1.67–39.11)	0.006*
C allele	54 (54%)	51 (85%)	Reference	0.005*
T allele	46 (46%)	9 (15%)	3.15 (1.46–6.31)	0.037*

Control group Hardy-Weinberg equilibrium: chi-square p = 0.058.

The IL-4 polymorphism was also related to IgE levels in the study subjects. Among asthmatic patients, those with the T/T genotype had the highest IgE levels (approximately 935.5 kIU/L), followed by those with the C/T genotype (approximately 614.2 kIU/L). Patients with the C/C genotype had much lower IgE levels (about 126.2 kIU/L). Patients with the C/C genotype had much lower IgE levels (≈ 126.2 kIU/L). This suggests that the IL-4 T allele (particularly in the homozygous form) is associated with increased IgE production in asthma. In the control group, IgE levels were mainly low for all genotypes (averages of 41.8, 25.8, and 261.2 kIU/L for C/C, C/T, and T/T, respectively). They were much lower than those in asthmatics ($p < 0.001$ for each genotype when comparing cases to controls). Notably, even in controls, the two individuals with the T/T genotype exhibited higher IgE levels than individuals with other control genotypes, mirroring the trend observed in patients (Table 5).

Table 5. Serum IgE levels in different IL-4 genotypes

Genotype	Case Group (Mean ± SD)	Control Group (Mean ± SD)	t-test	p-value
C/C	126.18 ± 42.73	41.75 ± 16.51	-10.347	< 0.001*
C/T	614.22 ± 131.88	25.83 ± 11.47	-24.320	< 0.001*
T/T	935.53 ± 172.51	261.23 ± 97.15	-19.595	< 0.001*

IL-13 Gene Polymorphism (rs20541)

For the IL-13 gene (Arg130Gln, rs20541) polymorphism, genotype distributions differed significantly between bronchial asthma cases and controls ($\chi^2 = 9.37$, df = 2, $p = 0.009$; Table 6). A-carrier genotypes (G/A or A/A) were more frequent among cases (24/50, 48%) than controls (5/30, 16.7%), conferring increased odds of asthma under a dominant model (OR = 4.62, 95% CI 1.52–13.99; Fisher's exact

p = 0.0076). Allele-based analysis similarly indicated enrichment of the A allele in cases (32/100, 32%) compared with controls (5/60, 8.3%) (allelic OR = 5.18, 95% CI 1.89–14.17; Fisher's exact p = 0.0005).Genotype distribution in controls was consistent with Hardy-Weinberg equilibrium (chi-square p = 0.619).

Within the asthmatic group, the distribution of rs20541 genotypes did not differ across mild, moderate, and severe asthma (χ^2 = 3.34, df = 4, p = 0.50; Table 7). However, this severity-stratified analysis was underpowered (achieved power ≈ 27%), so the absence of significance should be interpreted cautiously. Given the modest control sample and sparse genotype cells, the IL-13 rs20541 case–control association should be regarded as exploratory and hypothesis-generating rather than conclusive.

Table 6. Genotype and allele frequencies of IL-13 (rs20541) in case and control groups

Genotype/Allele	Cases (n=50)	Controls (n=30)	Effect / Test	p-value
G/G	26 (52.0%)	25 (83.3%)	Overall χ^2 = 9.37; df = 2	0.009
G/A	16 (32.0%)	5 (16.7%)		
A/A	8 (16.0%)	0 (0.0%)		
A-carriers (G/A + A/A) vs G/G	24 vs 26	5 vs 25	OR = 4.62 (95% CI 1.52–13.99)	0.0076*
G allele	68 (68.0%)	55 (91.7%)		
A allele	32 (32.0%)	5 (8.3%)	OR = 5.18 (95% CI 1.89–14.17)	0.0005*

Key genetic associations remained significant after Benjamini–Hochberg FDR correction (q = 0.05); however, given the modest control cohort and sparse genotype cells for rs20541 (including absence of A/A in controls), the IL-13 susceptibility finding should be interpreted cautiously as suggestive and hypothesis-generating pending replication. Control group Hardy-Weinberg equilibrium: chi-square p = 0.619.

Table 7. Genotype and allele distribution of IL-13 based on asthma severity

Genotype/Allele	Mild (n=18)	Moderate (n=21)	Severe (n=11)	p-value
G/G	8 (44.4%)	11 (52.4%)	7 (63.6%)	0.317
G/A	5 (27.8%)	8 (38.1%)	3 (27.3%)	0.151
A/A	5 (27.8%)	2 (9.5%)	1 (9%)	0.083
G allele	21 (58.3%)	33 (78.6%)	14 (63.6%)	0.239
A allele	15 (41.7%)	9 (21.4%)	8 (36.4%)	0.511

Correlation Analysis

Spearman's correlation analysis revealed strong positive relationships between total IgE levels and the Th2 cytokines. In asthmatic patients, serum IgE showed a high correlation with IL-13 levels (r = 0.937, p = 0.001) and a substantial correlation with IL-4 levels (r = 0.742, p = 0.001). This finding indicates that individuals with higher circulating IL-4 or IL-13 levels tend to have markedly higher IgE levels, consistent with the role of these interleukins in promoting IgE production. By contrast, IgE levels did not significantly correlate with patient age (r = 0.146, p = 0.460) or gender (r = 0.316, p = 0.228). These findings highlight a direct link between the Th2 immune response (IL-4/IL-13) and IgE in asthma, irrespective of age or sex (Table 8).

Table 8. Correlation between serum IgE and studied parameters

Parameter	Correlation Coefficient (r)	p-value
Age	0.146	0.460
Male Gender	0.316	0.228
IL-4	0.742	0.001*
IL-13	0.937	0.001*

Note: FDR-adjusted q-values (Benjamini–Hochberg, q = 0.05) for the correlation p-values were: Age q = 0.46, Male gender q = 0.304, IL-4 q = 0.002, IL-13 q = 0.002.

Discussion

In this observational case–control study, we examined serum IgE, IL-4, and IL-13 levels and evaluated two cytokine gene polymorphisms (IL-4 rs2243250 and IL-13 rs20541) in relation to asthma susceptibility and severity. Demographic variables (age, sex, residency, and comorbidities) did not differ significantly between cases and controls, whereas family history was significantly more frequent among cases ($p < 0.001$). Asthma patients demonstrated markedly higher serum IgE, IL-4, and IL-13 concentrations than controls (all $p < 0.001$), supporting a heightened type-2 inflammatory profile in this cohort and aligning with previous reports describing elevated IgE and Th2-related cytokines in asthma (12,14,15,17,19).

Because IL-4 and IL-13 were measured in serum, these concentrations should be interpreted as systemic proxies and may not directly reflect airway mucosal cytokine activity.

Consistent with the literature, IgE levels were positively associated with cytokine levels, and higher IgE has been linked to greater disease burden in some cohorts (8,12,14,15). Previous pediatric studies have reported higher IgE levels in patients with moderate versus mild asthma, findings broadly consistent with our severity-related patterns (16,17). Together, these findings support an association between immunological markers and asthma phenotype, while recognizing that such associations do not establish causality in a case–control design.

Regarding IL-4 rs2243250, we observed an association with asthma susceptibility in this cohort, with differences in genotype/allele distribution between cases and controls and higher IgE levels among T-allele carriers. Similar relationships between IL-4 pathway variants and IgE or asthma susceptibility have been reported, although results vary across populations and study designs (8, 13-15,18,19). This heterogeneity is expected in a multifactorial disease influenced by environmental exposures and gene–environment or gene–gene interactions, underscoring the importance of replication in larger, well-characterized cohorts (8, 13-15).

For IL-13 rs20541 (Arg130Gln), we observed enrichment of the A allele among cases, consistent with a possible susceptibility association. However, this finding should be interpreted cautiously as hypothesis-generating because the control cohort was small ($n = 30$) and genotype cells were sparse, with no A/A controls (Table 6). This structure can inflate odds ratios and widen confidence intervals in small samples. Moreover, the severity-stratified analysis did not show a significant genotype difference across severity categories and was underpowered; therefore, the absence of association with severity should not be interpreted as evidence of no effect. Prior studies evaluating rs20541 have reported heterogeneous results across ancestries and phenotypic definitions, and independent replication in larger, well-matched cohorts with population-structure control is required to assess robustness and to clarify whether any severity-related effects exist (21–26). Although IL-13 has been implicated in IgE-related pathways, this biological plausibility does not provide evidence of a mechanism in the present observational data (20).

Several limitations should be emphasized. The modest sample size—particularly the control cohort—may increase the likelihood of both false-positive and false-negative findings and can yield unstable effect estimates. In particular, sparse-genotype cells (e.g., the absence of the A/A genotype in controls for rs20541) can inflate odds ratio estimates and widen confidence intervals. Although multiple testing procedures were applied, the current findings remain hypothesis-generating and require validation. Although predictive modeling approaches using circulating biomarkers have been explored in prior literature, we did not perform predictive modeling in the present study; therefore, we do not make claims regarding the prediction of severity or clinical utility from serum cytokine measurements.

At this stage, our findings do not support mechanistic or clinical claims, particularly regarding IL-13, and independent replication in larger, adequately powered cohorts is necessary before inferring biological or clinical relevance (21–24-27).

Future Directions

Future studies should recruit larger, well-matched case–control cohorts, pre-specify power calculations, and apply appropriate multiple-testing correction. Independent replication across populations and, where feasible, functional validation experiments are needed to clarify the relevance of IL-13 rs20541 without implying clinical utility at this stage.

Strengths and Limitations

This investigation gains considerable strength from its targeted analysis of biologically critical genetic variants—specifically IL-4 rs2243250 and IL-13 rs20541. By pairing patients with appropriately matched control subjects and by correlating these findings with immunological measures of IL-4, IL-13, and immunoglobulin E levels, the study can paint a more detailed picture of the underlying biology. The observed statistically significant relationships, particularly those linked to the IL-4 polymorphisms, lend credible support to the authors' main conclusions.

That said, the research is not without its constraints. The cohort is relatively modest, which can diminish the power of some analyses; further, data on environmental exposure factors known to influence immune responses and phenotype outcomes were not collected. Because all samples were drawn from a single center, caution is warranted when generalizing the results to broader, more diverse populations. Moreover, critical clinical metrics such as eosinophil counts and objective measures of lung function were omitted, leaving

significant questions about the functional significance of the genetic findings unanswered.

In addition, because family history differed substantially between cases and controls, residual confounding cannot be excluded, and the genetic association findings should be interpreted cautiously as hypothesis-generating.

In addition, medication use and disease control at the time of blood sampling were not standardized, and serum cytokine concentrations can be substantially modified by controller therapy. Accordingly, residual treatment-related confounding and phenotype misclassification cannot be excluded.

Additionally, genetic association findings may be sensitive to population structure. Although cases and controls were recruited from the same catchment area and shared a similar self-reported ancestry background, we did not genotype ancestry-informative markers or apply principal component adjustment. Therefore, residual population stratification and sampling-structure effects cannot be excluded entirely and should be considered when interpreting the association of IL-13 rs20541.

A notable limitation of the present investigation is the modest sample size, particularly in the control cohort. The limited control size may increase the probability of both false-positive and false-negative associations. It may yield unstable estimates with wider confidence intervals, especially for IL-13 rs20541, where some genotype cells are sparse. Notably, the A/A genotype was absent in controls for rs20541, which can inflate odds ratio estimates and widen confidence intervals when cell counts are sparse.

Conclusion

Accordingly, the observed association with IL-13 should be viewed as suggestive rather than definitive, and independent replication with larger, well-matched control cohorts is required. Such circumscribed numbers may have attenuated the statistical power of some analyses and hampered the identification of weaker, albeit potentially relevant, associations, notably those involving interleukin-13 polymorphisms. Moreover, the absence of a prespecified power analysis at the study's conception further complicates the generalizability of the outcomes. In post hoc analyses, the achieved power for the rs20541 case–control genotype comparison (2×3 table; df = 2) was ~79% at $\alpha = 0.05$, whereas the achieved power for the rs20541 severity-stratified genotype comparison (3×3 table; df = 4) was ~27%. Approximately 179 asthma cases would be required to reach ~80% power for an effect size of similar magnitude in severity-stratified analyses. Hence, subsequent investigations should include larger cohorts, a priori power calculations, and appropriately sized control groups to corroborate and extend the findings reported herein.

Abbreviations

IL-4: Interleukin-4
IL-13: Interleukin-13
IgE: Immunoglobulin E
PCR: Polymerase Chain Reaction
RFLP: Restriction Fragment Length Polymorphism
ELISA: Enzyme-Linked Immunosorbent Assay
Th2: T-helper Type 2
IRB: Institutional Review Board
OR: Odds Ratio
CI: Confidence Interval
SD: Standard Deviation
GWAS: Genome-Wide Association Studies
NGS: Next-Generation Sequencing
FOXO3: Forkhead Box O3 gene
MHC: Major Histocompatibility Complex
TCR: T Cell Receptor (if needed)
BALF: Bronchoalveolar Lavage Fluid

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Authors' Contributors

AAE and ASA designed the study. SBA and MMA collected clinical data. MMA performed the laboratory work. AAE and MMA analysed the data and drafted the manuscript. All authors read and approved the final version of the manuscript.

Data Availability

The data supporting the results of this study are available upon request from the corresponding author.

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Ethics Approval

This study was approved by the Institutional Review Board of the Faculty of Medicine at Aswan University (Approval No. MIC007/1/200/10/2019). All participants provided informed consent before enrolment.

Consent to participate:

Not applicable.

Conflict of Interest

The authors declared no conflict of interest.

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