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ORIGINAL RESEARCH

# Tumor Necrotic Factor-Alpha (−308A/G, rs1800629) and Interleukin-13 (R130Q, rs20541) Gene Polymorphisms as Predictors of Severe Asthma in Pilot Cohort of Kuwaiti Population: Influence of Age, Nasal Polyps, Fractional Exhaled Nitric Oxide, and Blood Eosinophil Count

Mona Al-Ahmad[1,2,*], Asmaa Ali[2–4,*], Ahmed Maher[2], Mohammad Z Haider[5]

[1]Department of Microbiology, College of Medicine, Kuwait University, Kuwait City, Kuwait; [2]Department of Allergy, Al-Rashed Allergy Center, Ministry of Health, Kuwait City, Kuwait; [3]Department of Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang, People's Republic of China; [4]Department of Pulmonary Medicine, Abbassia Chest Hospital, Ministry of Health, Cairo, Egypt; [5]Department of Pediatrics, College of Medicine, Kuwait University, Kuwait City, Kuwait

*These authors contributed equally to this work

Correspondence: Mona Al-Ahmad, Department of Microbiology, College of Medicine, Kuwait University, P.O. Box 24923, Safat, 13110, Kuwait, Tel +965-24636515, Fax +965-25332719, Email mona.alahmad@ku.edu.kw



**Background:** Genetic factors, along with sociodemographic characteristics, are believed to play a significant role in asthma pathogenesis.

**Objective:** This study investigated the role of Interleukin-13 (IL-13) and Tumor Necrosis Factor-alpha (TNF-α) gene polymorphisms, in conjunction with clinical characteristics, in predicting asthma severity.

**Methods:** A total of 214 asthma patients (98 mild, 116 severe) and 121 healthy individuals were genotyped using PCR-RFLP for IL-13 (R130Q, rs20541) and TNF-α (−308A/G, rs1800629) polymorphisms. Sociodemographic and clinical data were collected for statistical analysis.

**Results:** Compared to controls, the "Q" allele of the IL-13 gene increased the risk of mild asthma twofold but had no significant impact on severe asthma. Conversely, the "G" allele of the TNF-α gene increased the risk of mild asthma twofold and severe asthma threefold. Additionally, the TNF-α "GG" genotype was associated with a sixfold increased risk of asthma, while the "AG" genotype had a protective effect. In the comparison of mild versus severe asthma, the IL-13 "QQ" pattern was protective, while the TNF-α "GG" genotype increased the risk of severe asthma threefold, with "AG" being protective. Severe asthma patients were older, significantly associated with comorbid nasal polyposis (NP), had higher levels of FeNO and blood eosinophils. Logistic regression analysis identified the TNF-α "GG" genotype as independent significant predictor of asthma severity, whereas IL-13 polymorphism showed no association.

**Conclusion:** The TNF-α "GG" genotype emerges as a significant independent predictor of asthma severity, substantially increasing the risk of both mild and severe asthma. In contrast, IL-13 polymorphism, while associated with mild asthma, plays no significant role in severe asthma. Furthermore, severe asthma was strongly linked to older age, nasal polyposis, elevated FeNO levels, and blood eosinophils.

**Keywords:** severe asthma, type 2 inflammatory markers, IL-13 gene polymorphism, TNF-alpha gene polymorphism

## Introduction

Asthma, often perceived as a childhood ailment, manifests in adulthood for around half of middle-aged patients, as evidenced by longitudinal studies.[1] This proportion of adult-onset asthma rises with age.[1,2] The annual incidence of

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asthma among adults is estimated at 0.5%, comparable to the childhood rates.[3] It remains uncertain whether adult-onset asthma constitutes a distinct condition from childhood-onset asthma. The trajectory of adult-onset asthma is complicated due to its heterogeneity.[4] Unlike childhood-onset asthma, which is mostly mild with frequent remissions, adult-onset asthma tends to be less prone to remission and often progresses to more severe forms.[3,4]

The ERS/ATS and GINA define severe asthma based on medication levels, typically requiring high daily doses of inhaled corticosteroid (ICS) with additional controllers or oral corticosteroids for control.[5,6] Previous studies estimate severe asthma prevalence at 4–8% in clinic and registry samples,[7–10] and around 0.5% at the population level.[7–9] Severe asthma is characterized by more symptoms, exacerbations, and comorbidities compared to mild-to-moderate asthma.[8,11] It also leads to lower asthma control, poor quality of life, and impaired lung function.[11–13]

Type 2 inflammation (driven by CD4+ T cells producing IL-4, IL-5, and IL-13) is central to severe asthma pathogenesis.[14] This response, associated with elevated IgE and eosinophilia, activates epithelial cells and recruit effector cells, promoting airway remodeling.[14,15] Structural changes like smooth muscle thickening, mucus hyperplasia, and vascular shifts heighten sensitivity to inhaled triggers.[14–16] Genetic variants in IL-4 receptor (IL4Rα) have been linked to severe asthma, especially in African Americans.[17–19] Moreover, therapies targeting IgE, IL-5, and IL-13 effectively suppress Type 2 inflammation and reduce exacerbations.[16,20,21]

The *IL-13* gene is crucial for type 2 immune responses. It resides on chromosome 5 and encodes a cluster of other Th2 cytokines as well.[22] IL-13 plays a key role in type 2 diseases, promoting inflammation, airway remodeling, and mucus production.[23] While sharing some functions with IL-4, IL-13 appears to have a more pronounced effect on fibrosis and mucus secretion.[24,25] Their distinct effects may be due to variations in receptor usage and timing of release.[23] Overall, both cytokines contribute significantly to the hallmarks of type-2 asthma.[24,25]

The role of IL-13 gene polymorphism in asthma has been reported in several studies.[19,26–29] Specifically, the IL-13 R130Q A/G genotype may influence total IgE and serum IL-13 levels, implicating IL-13 in asthma-related signaling pathways.[19] A promoter polymorphism at position −1055 (C to T) has also been associated with allergic asthma and altered IL-13 regulation, likely due to increased nuclear protein binding at this site.[28] However, its correlation with asthma severity remains unaddressed in prior research.

The inflammatory process in asthma involves multiple mediators, with TNF-α playing a prominent role, particularly in allergic responses and disease pathogenesis. Elevated TNF-α levels in asthmatic patients contribute to airway hyperresponsiveness and inflammation by recruiting neutrophils and eosinophils.[30]

Genetic polymorphisms in the TNF-α gene promoter, particularly at position −308 bp, have been linked to increased TNF-α production,[31] though their impact on asthma remains unclear. Findings are inconsistent; a study in a Pakistani population found no significant association between TNF-α variants and asthma,[31] while an Indian study reported significant differences, especially when stratified by asthma type (seasonal vs perennial) and age of onset (childhood vs late-onset), suggesting subtype-specific associations.[32] Although the role of TNF-α polymorphisms in asthma is still debated, targeting TNF-α activity shows therapeutic potential and may help reduce glucocorticosteroid dependence.[30,31]

Based on the aforementioned studies, we hypothesized that the IL-13 (R130Q, rs20541) and TNF-alpha (−308A/G, rs1800629) gene polymorphisms are associated with asthma susceptibility and disease severity in Kuwaiti adults. Furthermore, we proposed that these genetic polymorphisms, in combination with other sociodemographic and clinical features, could influence disease severity and treatment response. To test this hypothesis, the present study investigated the role of IL-13 (R130Q, rs20541) and TNF-alpha (−308A/G, rs1800629) gene polymorphisms in Kuwaiti adult patients with asthma and evaluated their association with disease severity alongside sociodemographic and clinical characteristics.

# Methods

## Patients and Study Design

This case-control study was conducted at Al-Rashed Allergy Center, Kuwait, spanning from April 2023 to December 2023. The study included 98 patients with mild asthma, 116 with severe asthma, and 121 healthy controls for comparison. Diagnosis of asthma (in both mild and severe) was based on GINA guidelines,[5] involved a history of

fluctuating respiratory symptoms such as wheezing, shortness of breath, chest tightness, and cough, alongside variable expiratory airflow limitation confirmed by spirometry (FEV1/FVC < 0.75–0.80 in adults) and significant improvement in FEV1 post-bronchodilator (>12% and >200 mL). Severe asthma was defined according to ERS/ATS guidelines; patients required high-dose inhaled corticosteroids (ICS) plus a second controller (and/or systemic corticosteroids) to prevent uncontrolled asthma or remained uncontrolled despite this therapy.[6] Patients meeting one of the following criteria: requiring high-dose ICS and a second controller for the previous year or systemic corticosteroids for ≥50% of the previous year to prevent uncontrolled asthma, or remaining uncontrolled despite therapy, were classified as having severe asthma.[6,32,33] However, patients were classified as having mild asthma if they required either step 1 or 2 treatment: as-needed low-dose ICS-formoterol combination therapy; or ICS with each use of short-acting beta-agonists (SABA).[5,34]

## Ethics Approval and Consent to Participate

Ethical clearance was secured from Kuwait University and the Ministry of Health, aligning with the principles outlined in the Helsinki Declaration protocol (Research study number MI02/22), ensuring adherence to globally accepted standards. All participants, provided informed consent, signifying their full understanding of the study's objectives and willingly agreeing to participate.

## Sample Size

The sample size was determined using Minitab 17.1.0.0 for Windows software (Minitab Inc., 2013, Pennsylvania, USA). With the aim of achieving 80% power, we factored in an odds ratio of 2, a disease prevalence of approximately 1%, a minor allele frequency of 5%, complete linkage disequilibrium (LD), and a 5% error rate in an allelic test. Thus, the minimum total sample size required for this study was calculated to be 248, maintaining a 1:1 case-to-control ratio. Furthermore, to ensure adequate representation of varying degrees of asthma severity (mild and severe), an equal number of mild asthma cases would be necessary, assuming a case-to-case ratio similar to the control-to-case ratio, in order to achieve 80% study power.

## Sample Collection and Preparation

Under sterile conditions, 10 mL of venous blood was drawn from each participant using a plastic syringe. The blood was divided into two tubes: one without anticoagulant and the other containing EDTA to aid DNA extraction. Following centrifugation at 4,000 g for 10 minutes, serum, plasma, and the buffy coat containing leukocytes were separated. The QIAamp Blood Kits (QIAGEN, Germany) were utilized to isolate genomic DNA following the manufacturer's recommended procedure. The quantity and purity of the isolated DNA were assessed by measuring the absorbance at wavelengths 260 nm and 280 nm using a Nanodrop 8000 spectrophotometer (Thermo-Scientific, Delaware, USA). DNA purity was determined by the A260/A280 ratio, with the target range set between 1.8 and 2.0. To estimate DNA concentration, the optical density (O.D). at 260 nm was measured, and the concentration (mg/mL) was calculated using the formula: Concentration = O.D. 260×50 x dilution factor (x 100). The final DNA concentration was determined directly using the Nanodrop 8000 spectrophotometer software, typically ranging between 107 and 552 ng/μL.

## Genotyping

### IL-13 Gene Polymorphism (R130Q; rs20541)

*IL-13* gene polymorphism (R130Q; rs20541) genotypes were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, as previously described.[34] The PCR primers used were as follows: Sense primer: 5′-CTTCCGTGAGGACTGAATGAGACGGTC-3′ and Antisense primer: 5′-GCAAATAATGATGCTTTCGAAGTTTCAGTGGA-3′. Amplification reactions were performed at 94°C for 5 minutes followed by 35 cycles of 94°C for 45 seconds, 67°C for 45 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. The PCR products were then digested with the restriction enzyme NlaIV (0.5 U) at 37°C for 3 hours and analyzed by agarose gel electrophoresis, following established protocols. For the normal R130- genotype (QQ), the expected product sizes were 210 bp and 26 bp, while for the mutant −130Q (RR) genotype, the expected product sizes were 178 bp, 32 bp, and 26 bp, respectively.

### TNF-Alpha Gene Polymorphism (−308A/G; rs1800629)

The genotypes for the *TNF-alpha* gene (−308A/G; rs1800629) polymorphism were determined using a PCR-RFLP method, following previously established protocols.[35] The PCR reactions were conducted in a total volume of 25 µL, comprising 100 ng of genomic DNA, 10 pmoles of each primer, 2 mm MgCl2, 0.2 mm deoxynucleotides (dNTPs), 1x buffer, and 2U of Taq polymerase. Amplification involved incubation at 94°C for 5 minutes, followed by 35 cycles consisting of 94°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute, with a final extension step at 72°C for 10 minutes. Polymorphism detection was achieved by NcoI restriction endonuclease digestion of the PCR-amplified fragment at 37°C for 1 hour. Expected product sizes were 107 bp for the A-allele and 87 bp and 20 bp for the G-allele. The resulting fragments were analyzed by electrophoresis on a 3% agarose gel and visualized under UV light after staining with Ethidium bromide, following standard procedures.

## Statistical Analysis

Demographic information of both patients and control groups, along with the genotype of each participant, was recorded in an Excel spreadsheet. Statistical analysis was conducted using Minitab for Windows (version 17.1.0.0, Minitab Inc., Pennsylvania, USA). Numerical data are presented as mean and standard deviation or median and inter quartile range, while categorical data are expressed as number and percentage. The comparison between two means was performed using an independent *t*-test, and between two median using Mann Whitney test, while comparing two frequencies was done using a Chi-square test. Logistic regression analysis, employing both adjusted and non-adjusted methods, was utilized to assess the predictive capability of *IL-13* and *TNF-alpha* gene polymorphisms for severe asthma. All tests were two-sided, and a p-value of less than 0.05 was considered statistically significant.

# Results

## Demographic Profile of Participants

In Table 1, the study evaluated 98 patients with mild asthma and 116 with severe asthma, comparing them to 121 normal healthy volunteers (control group). The patients and control groups were matched by sex and BMI. However, patients with severe asthma were found to be significantly older than those with mild asthma, p<0.001, as well as control participants, p=0.001. Moreover, 90.52% of patients with severe asthma were non-smokers, while only 78.51% of the control group were non-smokers, p=0.03.

## The Frequency of IL-13 and TNF-Alpha Gene Polymorphisms in Mild and Severe Asthma

Table 2 expressed the pattern of IL-13 and TNF-α gene polymorphisms and their association with mild asthma and severe asthma versus control participant. Interestingly, no significant correlation was observed between IL-13 gene polymorphism and mild asthma patients in comparing with control group. However, a compelling finding emerged regarding the Q alleles, demonstrating a twofold increase in the likelihood of mild asthma. The frequency of Q alleles was notably

**Table 1** Demographic Characteristics of the Studied Groups

| Factors | Control (n=121) | | Mild asthma (n=98) | | Severe asthma (n=116) | | $P_1$ | $P_2$ | $P_3$ |
|---|---|---|---|---|---|---|---|---|---|
| Age (mean, SD) | 38.8 | 14.7 | 40.3 | 14 | 52.8 | 11.3 | 0.44 | **0.001**† | **<0.001**† |
| F-sex (n, %) | 83 | 68.6 | 66 | 67.35 | 80 | 68.97 | 0.84 | 0.95 | 0.81 |
| BMI (mean, SD) | 30.92 | 5.81 | 30.23 | 6.96 | 31.46 | 5.75 | 0.42 | 0.47 | 0.16 |
| Smoking status (n, %) | | | | | | | | | |
| Smoker | 12 | 9.91 | 10 | 10.2 | 5 | 4.31 | 0.06 | **0.03*** | 0.19 |
| Non-smoker | 95 | 78.51 | 85 | 86.73 | 105 | 90.52 | | | |
| Ex-smoker | 14 | 11.58 | 3 | 3.06 | 6 | 5.17 | | | |

**Notes**: † independent *t*-test, * chi square test, p<0.05 considered significant, the bold numbers represent significant correlation.
**Abbreviations**: N, number; SD, standard deviation; BMI, body mass index (kg/m$^2$); $p_1$, control vs mild asthma; $p_2$, control vs severe asthma; $p_3$, Mild vs severe asthma.

**Table 2** The Genotype and Allele Frequencies of IL-13 and TNF-Alpha Gene Polymorphism in Patients with Mild and Severe Asthma and Comparison with the Controls

| Factors | Control (n=121) | | Mild Asthma (n=98) | | Severe asthma (n=116) | | OR1 | 95% CI | OR2 | 95% CI | OR3 | 95% CI | p1 | p2 | p3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IL-13 genotype (n, %) | | | | | | | | | | | | | | | |
| Co-dominant | | | | | | | | | | | | | | | |
| IL-13-QQ | 62 | 51.24 | 63 | 64.29 | 46 | 39.66 | 0.58 | 0.338; 1.008 | 1.6 | 0.955; 2.677 | 2.74 | 1.571; 4.776 | 0.07 | 0.09 | **<0.001*** |
| IL-13-RQ | 54 | 44.63 | 33 | 33.67 | 62 | 53.45 | 0.63 | 0.363; 1.093 | 1.43 | 0.854; 2.376 | 2.26 | 1.298; 3.941 | 0.13 | 0.21 | **0.01*** |
| IL-13-RR | 5 | 4.13 | 2 | 2.04 | 8 | 6.9 | 0.48 | 0.0917; 2.547 | 1.72 | 0.545; 5.415 | 3.56 | 0.737; 17.153 | 0.62 | 0.35 | 0.17 |
| Dominant | | | | | | | | | | | | | | | |
| QQ+RQ | 116 | 95.87 | 96 | 97.96 | 108 | 93.1 | 0.483 | 0.0917; 2.547 | 1.72 | 0.545; 5.415 | 3.56 | 0.737; 17.153 | 0.62 | 0.35 | 0.09 |
| RR | 5 | 4.13 | 2 | 2.04 | 8 | 6.9 | Reference† | | Reference† | | Reference† | | | | |
| Alleles | | | | | | | | | | | | | | | |
| R | 69 | 26.5 | 37 | 18.9 | 78 | 33.6 | Reference† | | Reference† | | Reference† | | | | |
| Q | 178 | 73.5 | 159 | 81.1 | 154 | 66.4 | 1.67 | 1.059; 2.620 | 1.41 | 1.0961; 1.8176 | 2.18 | 1.4895; 3.1772 | **0.03*** | 0.19 | **0.007*** |
| TNF-α genotype | | | | | | | | | | | | | | | |
| Co-dominant | | | | | | | | | | | | | | | |
| TNF-AA | 19 | 15.7 | 12 | 12.24 | 6 | 5.17 | 1.34 | 0.613; 2.905 | 3.42 | 1.312; 8.888 | 0.39 | 0.141; 1.084 | 0.46 | **0.01*** | 0.11 |
| TNF-AG | 80 | 66.12 | 53 | 54.08 | 43 | 37.07 | 0.6 | 0.349; 1.043 | 0.3 | 0.177; 0.514 | 0.5 | 0.289; 0.865 | 0.09 | **<0.001*** | **0.01*** |
| TNF-GG | 22 | 18.18 | 33 | 33.67 | 67 | 57.76 | 2.29 | 1.225; 4.262 | 6.15 | 3.408; 11.110 | 2.69 | 0.213; 0.649 | **0.01*** | **<0.001*** | **<0.001*** |
| Dominant | | | | | | | | | | | | | | | |
| AA+AG | 99 | 81.82 | 65 | 66.33 | 49 | 42.24 | 2.29 | 1.225; 4.262 | 6.15 | 3.408; 11.110 | 2.69 | 1.542; 4.705 | **0.01*** | **<0.001*** | **<0.001*** |
| GG | 22 | 18.18 | 33 | 33.67 | 67 | 57.76 | Reference† | | Reference† | | Reference† | | | | |
| Alleles | | | | | | | | | | | | | | | |
| A | 118 | 48.8 | 77 | 39.2 | 55 | 23.7 | 1.47 | 1.004; 2.154 | 3.06 | 2.066; 4.540 | 2.07 | 1.4491; 2.9723 | **0.05*** | **<0.001*** | **0.001*** |
| G | 124 | 51.2 | 119 | 60.8 | 177 | 76.3 | Reference† | | Reference† | | Reference† | | | | |

**Notes**: † (Reference): Genotype in homogenous subject with RR genotype and R allele of IL13 gene, and GG genotype and G allele of TNF-α gene were considered as reference for calculation of statistical significance, *: chi square test, p<0.05 considered significant, the bold numbers represent significant correlation.

**Abbreviations**: N, number; OR, Odd ratio; CI, confidence interval; $p_1$, control vs mild asthma; $p_2$, control vs severe asthma; $p_3$, Mild vs severe asthma.

higher in the mild asthma group than in the control group, at 81.1% versus 73.5%, yielding an odds ratio (OR) of 1.67 (p=0.03). On the other hand, the GG TNF-α gene polymorphism exhibited a noteworthy association with mild asthma in both codominant and dominant models, revealing a twofold increase in the likelihood of mild asthma (OR=2.29, p=0.01). Furthermore, the frequency of G alleles in mild asthma displayed a significant twofold likelihood of association (OR=1.47, p=0.05).

A parallel strategy to that observed in mild asthma conducted with severe asthma, with no discernible correlation between IL-13 gene polymorphism and severe asthma patients compared to the control group. Strikingly, no significant association was identified with Q alleles (p=0.19). In the same line, mirroring the findings in mild asthma, the GG TNF-α gene polymorphism demonstrated a substantial and notable association with severe asthma in both codominant and dominant models. The likelihood of experiencing severity surged six-fold (OR=6.15, p<0.001). Furthermore, an intriguing observation emerged concerning the AA and AG polymorphism patterns, which exhibited a protective effect against severe asthma with odds ratios of 3.42 and 0.3, respectively, demonstrating statistical significance (p<0.001 for both). Moreover, the frequency of G alleles was significantly elevated in severe asthma, escalating the likelihood of severity threefold (OR=3.01, p<0.001).

However, comparing mild and severe asthma regarding IL-13 and TNF-α gene polymorphisms showed that, within the codominant models of IL-13 gene polymorphism, the homogenous "QQ" pattern emerged as a protective factor against severe asthma. In contrast, the heterogeneous "QR" pattern exhibited a significant association with severity, resulting in a twofold increase in the likelihood of severe asthma (p<0.001 and 0.01, respectively). Furthermore, the presence of R alleles was notably higher in severe asthma, contributing to a twofold increase in the likelihood of severity (OR=2.18, p=0.007). Conversely, the GG polymorphism pattern of the TNF-α gene was linked to a threefold increase in the likelihood of severe asthma in both codominant and dominant patterns (OR=2.69, p<0.001). Intriguingly, the heterogeneous "AG" pattern exerted a protective effect against severe asthma, with an odds ratio of 0.5 and statistical significance at p=0.01. Additionally, the frequency of G alleles was significantly elevated in severe asthma, resulting in a twofold increase in the likelihood of severity compared to mild asthma (OR=2.01, p=0.001).

## Factors Influencing the Severity of Asthma

In Table 3, a univariate analysis showed that patients with severe asthma were significantly older than the mild asthma patients, p<0.001, and were associated with higher frequency rate of AR and NP, p<0.001. Additionally, they had a significant higher level of markers of type 2 inflammation: total IgE, BEC and FeNO, p<0.001, 0.002 and 0.001,

**Table 3** Factors Associated with Severe Asthma

| Factors | Mild asthma (n=98) | | Severe asthma (n=116) | | p |
|---|---|---|---|---|---|
| Age (mean, SD) | 40.3 | 14 | 52.8 | 11.3 | **<0.001**† |
| F-sex (n, %) | 66 | 67.35 | 80 | 68.97 | 0.81 |
| BMI (mean, SD) | 30.23 | 6.96 | 31.46 | 5.75 | 0.16 |
| Smoking status (n,%) | | | | | |
| Smoker | 10 | 10.2 | 5 | 4.31 | 0.19 |
| Non-smoker | 85 | 86.73 | 105 | 90.52 | |
| Ex-smoker | 3 | 3.06 | 6 | 5.17 | |
| Disease onset (n, %) | | | | | |
| Adulthood | 62 | 63.27 | 84 | 72.41 | 0.15 |
| Childhood | 36 | 36.73 | 32 | 27.59 | |
| Comorbidity (n,%) | | | | | |
| DM | 6 | 6.12 | 9 | 7.76 | 0.64 |
| AR | 46 | 46.94 | 85 | 73.28 | **<0.001*** |
| NP | 8 | 8.16 | 57 | 49.14 | **<0.001*** |
| Eczema | 7 | 7.14 | 8 | 6.9 | 0.94 |

(*Continued*)

**Table 3** (Continued).

| Factors | Mild asthma (n=98) | | Severe asthma (n=116) | | p |
|---|---|---|---|---|---|
| Type 2 inflammation marker | | | | | |
| IgE (median, IQR) | 114 | (35–298) | 275 | (130–566) | **<0.001**†† |
| BEC (median, IQR) | 0.22 | (0.11–0.46) | 0.41 | (0.19–0.73) | **0.002**†† |
| FeNO (median, IQR) | 14.5 | (8–25) | 23 | (11–46) | **0.001**†† |
| Asthma medication (n, %) | | | | | |
| High dose ICS | 0 | 0 | 31 | 26.72 | **<0.001*** |
| Medium dose ICS | 9 | 9.18 | 10 | 8.62 | 0.88 |
| Low dose ICS | 89 | 90.81 | 0 | 0 | **<0.001*** |
| High dose ICS + LABA | 0 | 0 | 85 | 73.27 | **<0.001*** |
| Low dose ICS+LABA | 77 | 78.57 | 0 | 0 | **<0.001*** |
| LABA | 90 | 91.83 | 108 | 93.1 | 0.79 |
| SABA | 88 | 89.79 | 99 | 85.34 | 0.41 |
| Leukotriene receptor antagonists | 90 | 91.83 | 45 | 38.79 | **<0.001*** |
| LAMA | 10 | 10.2 | 80 | 68.96 | **<0.001*** |
| OCS | 0 | 0 | 5 | 4.31 | 0.06 |

**Notes**: †: independent *t*-test, ††: Mann Whitney test, *: chi square test or Fisher Exact test, p<0.05 considered significant, the bold numbers represent significant correlation.

**Abbreviations**: N, number; SD, standard deviation; BMI, body mass index ($kg/m^2$); DM, diabetes millets; AR, allergic rhinitis; NP, Nasal polyp; IgE, immune globulin E (KU/l); BEC, blood eosinophils count ($10^9$/L); FeNo, Fractional exhaled nitric oxide; ICS, Inhaled corticosteroids; OCS, oral corticosteroids; LABA, long acting B2 agonist; SABA, short acting B2 agonist; LAMA, Long acting muscarinic antagonist.

respectively. Additionally, the table highlighted significant differences in asthma medication usage between patients with mild and severe asthma. Patients with severe asthma were more likely to be prescribed higher-dose ICS and combination therapies (high dose ICS-LABA), $p < 0.001$ for both. However, low dose ICS and combination therapies (low dose ICS-LABA) were more likely to be prescribed in mild asthma group, $p<0.001$. Furthermore, leukotriene receptor antagonists were more commonly utilized in mild asthma cases, whereas LAMA use was more prevalent in the severe asthma group ($p < 0.001$). While LABAs were frequently prescribed to both mild and severe asthma patients for bronchodilation and symptom relief, SABAs were commonly used across both groups for acute symptom relief, with no significant difference between the groups.

Table 4 illustrated the independent predictors of severe asthma. In both unadjusted and adjusted logistic regression models, the GG patterns of TNF-α gene dominant models emerged as a significant independent predictor of asthma

**Table 4** Independent Predictors of Asthma Severity

| Factors | OR | 95% CI | P* |
|---|---|---|---|
| A- Unadjusted model | | | |
| *IL-13* genotype | | | |
| QQ+RQ | Reference† | | |
| RR | 3.4 | (0.6622,16.4061) | 0.11 |
| *TNF-α* genotype | | | |
| AA+AG | Reference† | | |
| GG | 2.7 | (1.5148,4.6518) | **0.001** |
| B-Adjusted model | | | |
| Age | 1.08 | (1.0441,1.1183) | **<0.001** |
| F-sex | 0.52 | (0.1850,1.4434) | 0.19 |
| AR | 2.04 | (0.7296,5.7187) | 0.16 |

(*Continued*)


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**Table 4** (Continued).

| Factors | OR | 95% CI | P* |
|---|---|---|---|
| NP | 7.97 | (2.4783,25.6029) | **<0.001** |
| FeNO | 1.02 | (1.0024,1.0384) | **0.02** |
| BEC | 0.96 | (0.8920,1.0263) | **0.02** |
| IgE | 1.00 | (0.9995,1.0022) | 0.19 |
| *IL-13* genotype | | | |
| QQ+RQ | Reference† | | |
| RR | 3.81 | (0.5349,27.1522) | 0.18 |
| *TNF-α* genotype | | | |
| AA+AG | Reference† | | |
| GG | 3.33 | (1.3486,8.2018) | **0.007** |

**Notes**: *: the test of significant: logistic regression analysis with unadjusted and adjusted models, p<0.05 considered significant, † (Reference): The dominant genotype form of IL13 and TNF-α gene polymorphisms were considered as a reference group for calculating the OR of homogenous recessive genotype, the bold numbers represent significant correlation.

**Abbreviations**: OR, Odd ratio; CI, confidence interval; AR, allergic rhinitis; NP, Nasal polyp; IgE, immune globulin E (KU/l); BEC, blood eosinophils count ($10^9$/L); FeNo, Fractional exhaled nitric oxide.

severity, with the likelihood of severity increasing threefold, OR=2.7 and 3.3, p=0.001 and 0.007, respectively. In contrast, IL-13 gene polymorphism showed an insignificant association in both models. Furthermore, being an older asthmatic patient and having associated NP increased the likelihood of severe asthma (OR=1.08 and 7.9, respectively, p<0.001). Additionally, for every one-unit increase in FeNO, the likelihood of severe asthma increased by one-fold. In contrast, the likelihood also increased for every one-unit decrease in BEC (OR=1.02 and 0.96, respectively, p=0.02).

## Discussion

The *IL-13* gene located on chromosome 5q31, encodes a 13-kDa glycoprotein.[27] Two common single nucleotide polymorphisms (SNPs) within this gene, rs20541 (R130Q or Arg130Gln) in exon 4 and rs1800925 (1112C/T) in the promoter region, have been extensively studied in relation to asthma susceptibility.[36] It has been shown that the presence of rs20541 polymorphism results in reduced IL-13 receptor affinity and an increased IL-13 expression in asthma patients.[37] On the other hand, the rs1800925 polymorphism, influences IL-13 expression by altering STAT transcription factor binding to the IL-13 promoter.[37,38]

Despite extensive research, the association between IL-13 polymorphisms and asthma remains inconsistent. In our study, IL-13 variants showed no significant correlation with asthma severity overall. However, the Q allele was linked to a twofold increased risk of mild asthma (p=0.03), with no significant association in severe asthma (p=0.19). Notably, within codominant models, the QQ genotype appeared protective against severe asthma, while the QR genotype and R allele were each associated with a twofold increased risk of severity.[39] This finding suggested that *IL-13* polymorphism modulates asthma progression and severity, rather than clearly distinguishing it from healthy states. While R130Q influences IL-13 receptor binding, affecting airway remodeling and inflammation, its impact varies with asthma phenotype, ethnicity, and gene-environment interactions.[40] In this case, other inflammatory pathways may mask IL-13's effect in severe, treatment-resistant asthma. Thus, IL-13 polymorphisms appear to contribute to asthma susceptibility and phenotypic modulation, aligning with the disease's multifactorial, polygenic nature, but are not direct biomarkers for severe asthma.[39–42]

A recent meta-analysis aimed to resolve inconsistencies in previous research regarding the association between interleukin *IL-13* gene polymorphisms and asthma risk and analyze the most recent data on two specific *IL-13* gene polymorphisms, rs20541 and rs1800925.[38] The researchers gathered data from 45 case-control studies for rs20541 and 31 case-control studies for rs1800925, encompassing a total of over 20,000 individuals. The pooled analysis revealed

a statistically significant association between rs20541 polymorphism and asthma risk overall. Subgroup analyses showed significant associations between rs20541 polymorphism and asthma risk in different populations, including Europeans, Asians, and Caucasians, across various genetic models. The difference in findings between the meta-analysis and our results could be due to several factors, including variations in sample size, study design, population demographics, and effect sizes of *IL-13* gene polymorphism on asthma risk. Additionally, population-specific differences in genetic backgrounds and environmental factors could contribute to the varying results. Furthermore, publication bias in the meta-analysis, where studies with significant results are more likely to be included, may impact the overall findings.

Our study found that the Q allele of the IL-13 gene was associated with a twofold increased risk of mild asthma, but not with severe asthma. In contrast, the R allele was more frequent in severe cases and linked to a twofold increase in severity, suggesting distinct roles for IL-13 variants in asthma phenotypes. The QQ genotype appeared protective against severe asthma, highlighting the complex influence of IL-13 polymorphisms on disease severity. Similarly, in pediatric populations, a case-control study reported that the rs20541 SNP in the IL-13 gene was associated with allergic traits like positive skin prick tests.[43] This variant may affect IL-13 receptor binding efficiency,[44] and animal studies suggest that the Gln110 variant enhances IL-13 activity and persistence.[45,46] It has also been linked to asthma and atopic dermatitis,[47,48] with additional studies supporting its role in eosinophilia, elevated IgE, and allergic rhinitis,[27,49–52] even when no direct association with asthma was observed.[50–52]

In addition to IL-13, our study examined the TNF-α −308G>A polymorphism and its association with asthma severity. The GG genotype was linked to a twofold increased risk of mild asthma and a sixfold increased risk of severe asthma in both co-dominant and dominant models. The G allele alone also conferred increased risk: twofold for mild and threefold for severe asthma. In contrast, AG and AA genotypes were protective against severe asthma. Among asthma patients, those with mild asthma and the GG genotype were three times more likely to progress to severe disease, while the AG genotype remained protective.

Findings across populations vary: a Pakistani study found no association with TNF-α polymorphisms,[31] whereas a European-American study did.[53] These discrepancies likely reflect population differences. TNF-α, though traditionally linked to Th1 immunity, also contributes to the multicellular inflammatory response in asthma, alongside Th2 cytokines such as IL-4, IL-5, and IL-13.[54–57]

TNF-alpha plays a crucial role in the innate immune response, acting as an immediate defense mechanism against invading pathogens before the adaptive immune system is fully engaged.[57] Macrophages primarily produce TNF-alpha in response to the recognition of common bacterial cell surface components. Initially synthesized as a membrane-bound precursor, TNF-alpha is then cleaved to its active form by TNF-alpha-converting enzyme.[58] The released protein forms active homotrimers that bind to TNF-α receptors 1 and 2 (TNFR1 and TNFR2), initiating intracellular signaling pathways without internalization of the complex.[59] This signaling cascade leads to the activation of pro-inflammatory genes, including *TNF-alpha* gene, through phosphorylation of nuclear factor-κB.[59,60] The response to TNF-alpha activation is regulated by shedding the extracellular domain of TNF-alpha receptors, maintaining a balance in the immune response.[60] A subset of asthma patients manifests severe, refractory symptoms despite maximal therapy, presenting a significant clinical challenge.[61] TNF-alpha has been implicated in the airway pathology of asthma, particularly in severe cases.[62]

Patients with severe asthma in our cohort were significantly older than those with mild asthma and had higher rates of comorbidities such as AR and NP. They also showed elevated type 2 inflammation markers (total IgE, BEC, and FeNO). The GG genotype of the TNF-α gene emerged as an independent predictor of severe asthma, tripling the risk in both unadjusted and adjusted models. In contrast, IL-13 polymorphisms showed no significant link to severity. A large international study reported a mean age of 55 among severe asthma patients, with notable regional differences in biomarkers and treatment patterns.[63] In our setting, high-dose ICS, combination therapy, and LAMA were more commonly prescribed for severe cases. Although asthma management in the Gulf aligns with GINA guidelines,[64] regional challenges (such as comorbidity detection and phenotyping) require more adapted strategies.

Previous studies from Kuwait reported that patients with severe asthma were significantly older, had a higher frequency of allergic rhinitis (AR) and nasal polyps (NP),[65,66] and exhibited elevated levels of type 2 inflammation markers.[66]

## Strength, Limitation and Future Prospective

This study offers valuable genetic insights into asthma, though its single-center design may limit generalizability. While IL-13 polymorphisms showed no significant association with severe asthma, the TNF-α GG genotype emerged as a strong predictor of severity. The G allele was also linked to an increased risk of both mild and severe asthma, highlighting TNF-α's more prominent role compared to IL-13 in our cohort.

Severe asthma was further associated with older age, nasal polyposis, elevated FeNO, and increased blood eosinophils. These findings underscore the need for broader, multicenter studies and point toward the potential for personalized treatment approaches based on genetic and clinical profiles.

## Data Sharing Statement

The datasets used during the current study are available from the corresponding author on reasonable request.

## Ethics Approval and Consent to Participate

The study was approved by the Ethics committee of Kuwait University and the Ministry of Health (Research project number MI02/22). Informed consent has been obtained from all participants involved in the study, to ensure that they are fully aware of the nature and purpose of the research and have given their voluntary and informed consent to participate.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, took part in drafting, revising the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

All authors declare no conflicts of interest in this work.

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