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Original Article

Serum IL-18R1 and its effect on immune cell infiltration in asthma

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Abstract: Objective: Asthma is a common chronic respiratory disease, affecting 5-10% of the global population. Despite its widespread impact, underdiagnosis remains a significant concern. This study aimed to explore the diagnostic potential of interleukin 18 receptor 1 (IL-18R1) and its relationship to immune cell infiltration in asthma. Methods: We retrospectively collected clinical and laboratory data of all participants. The expression of serum IL-18R1 in patients with asthma and healthy controls was quantified using enzyme linked immunosorbent assay (ELISA). The associations of IL-18R1 with clinical laboratory indicators and the diagnostic efficacy of IL-18R1 for asthma were assessed. Bioinformatic analysis was used to explore the biological function of IL-18R1 in asthma. Results: Peripheral blood white blood cells (WBC), neutrophils (NE), eosinophils (EO) and serum IL-18R1 were significantly elevated in patients with asthma compared to heathy controls. Patients with high IL-18R1 also had increased blood lymphocytes. The expression of IL-18R1 was associated with diverse immune cell infiltration and inflammatory pathways. Mendelian randomization (MR) analysis revealed a positive relationship between IL-18R1 and asthma. The area under the receiver operating characteristic (ROC) curve of the combination of IL-18R1 with WBC was 0.884, with a sensitivity and specificity of 84.8% and 81.8%. Conclusion: Elevated expression of IL-18R1 is linked to the infiltration of various immune cells and the activation of inflammatory pathways. The combination of IL-18R1 and WBC may be a biomarker with high diagnostic accuracy for asthma.

Keywords: IL-18R1, white blood cell, immune cells infiltration, ROC curve, asthma

Introduction

Asthma is a prevalent chronic inflammatory airway disorder, affecting approximately 5-10% of the global population [1]. It is characterized by airway hyperresponsiveness, intermittent airflow obstruction, and chronic airway inflammation, causing a substantial social burden and deaths worldwide [1]. Asthma diagnosis remains challenging due to its complex interplay of genetic, environmental, and immunological factors. Conventional diagnostic methods, such as clinical symptoms and pulmonary function tests, have limitations, leaving 20-70% of cases undiagnosed or misdiagnosed [2]. Given these challenges, a deeper understanding of asthma's pathophysiology and the search for better biomarkers is crucial.

Common biomarkers for asthma diagnosis, such as serum IgE, fractional exhaled nitric oxide, and eosinophil or neutrophil counts, are often influenced by other factors or diseases. Recent studies have highlighted cytokines and chemokines as potential asthma biomarkers. Generally, based on Th2 inflammation, asthma is classified into two subgroups: T helper (Th) 2-high and Th2-low types [3]. Elevated Th2 cytokines, including interleukin 4 (IL-4), IL-5, and IL-13, are key features of "Th2-high asthma", which accounts for over half of asthma cases [4]. IL-25 and IL-33 derived from epithelium,

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also induce Th2 inflammation by activating type 2 innate lymphoid cells (ILC2) [5]. IL-9 has pleiotropic effects and is associated with asthma pathogenesis. It activates Th2 cells and recruits mast cells to facilitate Th2 inflammation in asthma. It also induces macrophages secreting chemokine CCL5 to recruit eosinophils, T cells and monocytes, which contribute to lung inflammation [6]. IL-18 has demonstrated overexpression in active asthma. It causes airway inflammation by promoting Th2 responses and inducing the release of immunoglobulin E (IgE) and histamine [7]. On the other hand, IL-17 is likely to play important roles in "Th2-low asthma". Elevated IL-17 was observed in serum, sputum and bronchoalveolar lavage fluid of asthmatics, and related to disease severity [8]. IL-17 recruited neutrophils to the lung and induced inflammation. Tumor necrosis factor-α (TNF-α) was also elevated in neutrophilic and severe asthma, and was proposed to have a synergistic effect with IL-17 [9]. Notably, IL-18R1, as a typical inflammatory cytokine encoded by IL-18R1 and IL-18RAP genes, has been identified as a key genetic locus associated with asthma [10, 11]. Our previous study using an Olink panel showed that serum IL-18R1 levels differed between asthma patients and healthy controls and correlated with disease severity and lung function [12]. However, the clinical significance and biological functions of serum IL-18R1 in asthma remain unclear.

This study aimed to clarify the clinical significance of serum IL-18R1 in asthma. Clinical and laboratory data were collected from asthma patients. Serum IL-18R1 levels were quantified using enzyme linked immunosorbent assay (ELISA) to assess its relationship with clinical characteristics, diagnostic efficacy, and potential risk factors. Bioinformatic analysis was also performed to explore the association of IL-18R1 with immune cells infiltration in asthma, providing new insight for diagnosis and management.

Materials and methods

Study population

A total of 343 patients with asthma from July 2021 to June 2024 in The First Affiliated Hospital of Soochow University were enrolled in this study, 350 healthy individuals in the same period as the control group. The clinical laboratory data of all participants were retrospectively collected, including peripheral blood cell analysis, lung function test and serum IgE. The main observation indicators were peripheral blood cell analysis and lung function test, white blood cell (WBC) count, neutrophil (NE) count, lymphocyte (LY) count, monocyte (MO) count, eosinophil (EO) count, basophil (BA) count, percentage of neutrophil (NE%), percentage of lymphocyte (LY%), percentage of monocyte (MO%), percentage of eosinophil (EO%), percentage of basophil (BA%), red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet (PLT) count, platytroctid (PCT), mean platelet volume (MPV), platelet distribution width (PDW), forced expiratory volume in one second (FEV1), FEV1/forced vital capacity (FEV1/FVC) and fractional exhaled nitric oxide (FeNO). This study was approved by the institutional review board of the First Affiliated Hospital of Soochow University (No. 2024-616).

Sample collection and measurement of IL-18R1

To further confirm the result of the Olink panel [12], we collected blood samples and centrifuged them at 3500 r/min for 10 minutes, then serum was separated, stored at -80°C for use. The concentration of serum IL-18R1 in asthma patients and healthy individuals was measured by human interleukin 18 receptor 1 (IL18R1) ELISA kit (SAB Signalway Antibody). Operation steps of the ELISA were done according to the manufacturer's manual. The results were detected and read at a wavelength of 450 nm using the Microplate ELx808 (BioTek, USA).

Differential expression analysis of IL-18R1 in asthma

Gene expression profiles of two datasets (GSE76262, GSE137268) were downloaded from the Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo/). The data were then preprocessed and normalized using R software (version 4.3.1, https://www.r-project.org/). Differential analysis of data for 105 severe asthma patients and 36 controls were performed using the "limma" package. To

quantify the immune cells infiltration in each sample, we utilized the single-sample gene set enrichment analysis (ssGSEA). As described in our previously published studies [12-14], ssGSEA was employed to calculate the enrichment levels of 23 immune cell types in samples from both severe asthma patients and control subjects. In addition, the correlation between IL-18R1 and the infiltration of the 23 immune cells was assessed using Spearman's correlation coefficient. Based on the median IL-18R1 expression, patients with severe asthma were classified into IL-18R1 low and high expression groups. "limma" and "ggplot2" packages were used to analyze and plot the volcano maps of DEGs between IL-18R1 high and low expression groups. $P<0.05$ and |log2 fold change (FC)|>0.5 were adopted as screening criteria. In addition, Gene Set Enrichment Analysis (GSEA) was conducted to explore functional differences and associated pathways between the IL-18R1 high and low expression groups. GSEA of all genes in the IL18R1 high and low expression group samples was performed using the "clusterProfiler" package and the Hallmark signature gene set from the Molecular Signature Database (MSigDB) (HYPERLINK, https://www.gsea-msigdb.org/gsea/msigdb/index.jsp).

Mendelian randomization analysis

In addition, we performed Mendelian randomization (MR) analysis to investigate the causal relationship between IL-18R1 and asthma. SNPs with a $P<5\times10^{-8}$ were extracted as phenotypically associated genetic instrumental variables (IVs) for subsequent analyses. R2< 0.001 and genetic distance criterion of 10,000 kb were used as exclusion criteria for linkage disequilibrium (LD). In addition, we calculated the F-statistic for each SNP and excluded SNPs with F-statistics less than 10 to mitigate the potential effect of weak instrumental bias. Inverse variance weighting (IVW) method was primarily employed for MR analysis. MR-Egger regression was used to detect the effect of potential horizontal pleiotropy, with $P>0.05$ indicating the absence of horizontal pleiotropy. To assess heterogeneity, Cochran's Q statistic was performed, with $P>0.05$ indicating no significant heterogeneity. In addition, we performed leave-one-out sensitivity analysis to assess the effect of each SNP on the outcome. All MR analyses were performed using the "TwoSampleMR" packages in the R software (version 4.3.1).

Statistical analysis

Data were presented as median and interquartile intervals. Two group comparisons were made using a Mann-Whitney test. Univariate and multivariate logistic regression analyses were employed to assess the association between experimental index and the likelihood of having asthma as previously described [15, 16]. The receiver operating characteristic (ROC) curves were used to evaluate the diagnostic accuracy for asthma. All statistical analyses were performed with SPSS 27.0 and $P<0.05$ was considered significant.

Results

Clinical significance and bioinformatic analysis of IL-18R1 in asthma

Clinical and laboratory data of all participants were collected and the expression of IL-18R1 in serum was quantified using ELISA. Bioinformatics analysis was used to explore the relationship of IL-18R1 expression with immune cells infiltration. As shown in **Figure 1**, the expression of IL-18R1 differed in the two groups and was associated with clinical features. ROC curve of the combination of IL-18R1 with WBC exhibited a good diagnostic accuracy. Bioinformatic analysis revealed the expression of IL-18R1 was associated with certain types of immune cell infiltration and inflammatory pathways. MR analysis showed a causal relationship between IL-18R1 and asthma.

Characteristics of the study participants

The clinical characteristics of study participants are summarized in **Table 1**. Compared to healthy controls, patients with asthma had higher levels of WBC, NE, MO, EO, BA, NE%, EO%, RDW and PLT in peripheral blood ($P<0.05$). In contrast, LY%, MO%, RBC, HGB, HCT, MCV, MCH and MPV were significantly lower in asthma patients ($P<0.05$). There was no significant difference in LY, BA%, MCHC, PCT and PDW between the two groups ($P>0.05$).

Elevated expression of serum IL-18R1 in asthma patients

Our previous study found that patients with asthma had a higher level of IL-18R1 than healthy individuals [12]. To further verify this point, ELISA was used to measure the concen-

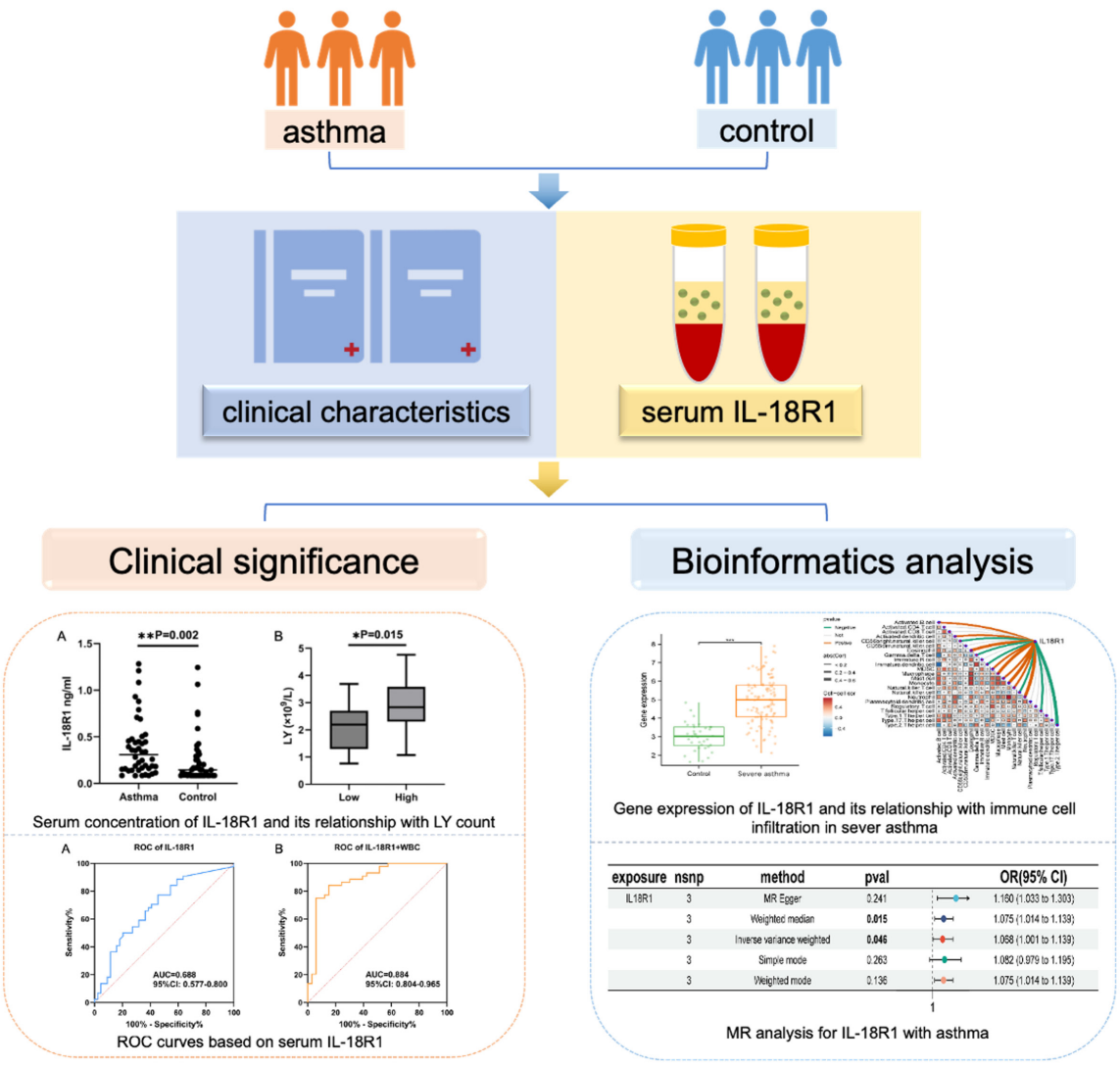

Figure 1. Clinical significance and bioinformatic analysis of IL-18R1 in asthma. The concentration of IL-18R1 was quantified by ELISA. ROC curve was drawn to assess the diagnostic value. Bioinformatic analysis showed a correlation between IL-18R1 expression and immune cell infiltration. MR analysis showed a causal relationship between IL-18R1 and asthma.

tration of IL-18R1 in serum both of asthma patients and healthy controls. The results showed that serum IL-18R1 in patients with asthma (Median: 0.309 ng/ml) was significantly elevated compared to healthy controls (Median: 0.146 ng/ml) (P<0.05, **Figure 2A**). Subsequently, in order to explore the relationship between IL-18R1 and laboratory indicators, patients with asthma were stratified into a low-expression group and high-expression group based on serum IL-18R1 level. The results suggested that blood LY in high-expression group were also increased compared to the low-expression group (P<0.05, **Figure 2B**).

Association of laboratory data with the occurrence of asthma

Both univariate and multivariate logistic regression analyses were conducted to assess the association of laboratory data with the onset of asthma, investigating risk factors for asthma. The results of univariate logistic regression showed that WBC, NE%, LY%, EO%, RBC, HGB and PLT were associated with asthma (P<0.05). In order to reduce the influence of confounding factors, MO% and BA% which had no statistical significance in univariate regression were excluded from multivariate logistic regression.

Table 1. Comparison of clinical characteristics and laboratory data between asthma patients and healthy controls

Variable	Median in asthma (P25, P75)	Median of controls (P25, P75)	*P* value
Gender	140/179	142/208	0.386
Age	40 (32-56)	41 (33-53)	0.888
WBC	7.22 (6.07-9.06)	5.73 (5.03-6.67)	<0.001
NE	4.08 (3.19-5.52)	3.14 (2.60-3.80)	<0.001
LY	2.13 (1.63-2.69)	2.02 (1.75-2.36)	0.079
MO	0.50 (0.37-0.65)	0.41 (0.34-0.48)	<0.001
EO	0.21 (0.08-0.47)	0.11 (0.07-0.17)	<0.001
BA	0.04 (0.03-0.06)	0.03 (0.02-0.04)	<0.001
NE%	57.60 (50.75-65.13)	54.45 (50.00-59.80)	<0.001
LY%	29.85 (24.13-34.88)	35.75 (30.18-40.10)	<0.001
MO%	6.45 (5.20-8.08)	7.00 (6.10-8.00)	0.001
EO%	2.85 (1.30-6.58)	1.90 (1.20-2.90)	<0.001
BA%	0.60 (0.40-0.80)	0.60 (0.40-0.70)	0.831
RBC	4.73 (4.41-5.05)	4.87 (4.62-5.07)	<0.001
HGB	140.00 (130.00-153.00)	148.00 (142.00-154.00)	<0.001
HCT	0.42 (0.40-0.45)	0.44 (0.43-0.46)	<0.001
MCV	89.80 (87.40-92.60)	91.60 (89.1-93.7)	<0.001
MCH	30.00 (29.08-30.90)	30.40 (29.70-31.20)	<0.001
MCHC	333.00 (325.00-339.25)	332.00 (326.00-339.00)	0.558
RDW	12.60 (12.10-13.10)	12.30 (12.00-12.70)	<0.001
PLT	245.00 (203.00-290.00)	219.00 (193.75-246.00)	<0.001
PCT	0.24 (0.20-0.27)	0.23 (0.21-0.26)	0.08
MPV	9.80 (9.20-10.30)	10.60 (9.90-11.20)	<0.001
PDW	11.90 (10.50-15.70)	12.25 (10.90-13.60)	0.795
FEV1/FVC (%)	72.59 (63.61-78.80)		
FEV1 (%)	84.95 (71.00-95.00)		
FeNO (ppb)	48.00 (25.00-87.00)		
IgE	96.30 (31.30-289.00)		

WBC, white blood cells; NE, neutrophis; LY, lymphocytes; MO, monocytes; EO, eosinophils; BA, basophils; NE%, percentage of neutrophils; LY%, percentage of lymphocytes; MO%, percentage of monocytes; EO%, percentage of eosinophils; BA%, percentage of basophils; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; PLT, platelets; PCT, plateletcrit; MPV, mean platelet volume; PDW, platelet distribution width; FEV1, forced expiratory volume in one second; FEV1/FVC, FEV1/forced vital capacity; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E.

After adjustment for confounding factors, multivariate regression analyses revealed that WBC had an independent positive association with asthma, while HGB had an independent negative association with asthma. The odds ratio (OR) and 95% confidence interval (CI) were 1.705 (1.434-2.028) and 0.905 (0.870-0.942) for WBC and HGB, respectively (**Table 2**).

Diagnostic value of serum IL-18R1 for asthma

In order to assess the ability of IL-18R1 to distinguish asthma from healthy individuals, ROC curves were constructed based on IL-18R1 alone or IL-18R1 combined WBC. The area under the ROC curve (AUC) of IL-18R1 alone was 0.688 (95% CI: 0.577-0.800, *P*=0.002). Sensitivity and specificity were 54.5% and 77.3%, respectively (**Figure 3A**). When IL-18R1 combined WBC, the AUC was 0.884 (95% CI: 0.804-0.965, *P*<0.001), with sensitivity and specificity 84.8% and 81.8% (**Figure 3B**).

Gene expression and functional enrichment analysis of IL-18R1 in asthma

Differential analysis of gene expression profiles in patients with severe asthma and healthy

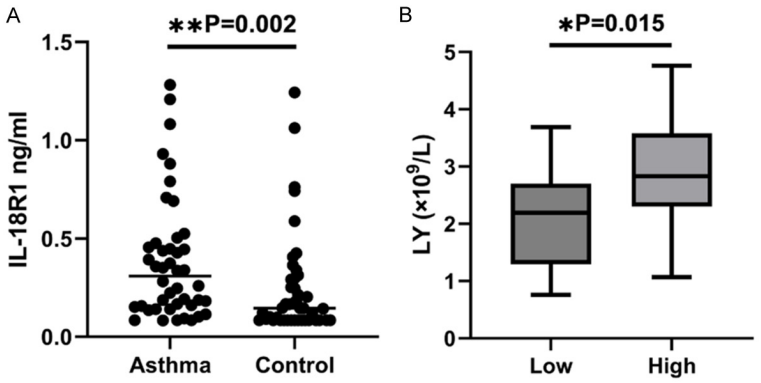

Figure 2. Serum IL-18R1 and its relationship with blood LY. A. The concentration of IL-18R1 was quantified in asthma patients and healthy controls by ELISA. B. Blood LY was significantly higher in the high IL-18R1 group than in the low IL-18R1 group. *P<0.05, **P<0.01; IL-18R1, interleukin 18 receptor 1; LY, lymphocytes.

Table 2. Risk factor analysis for the occurrence of asthma

Variable	Univariate logistic regression analyses			Multivariate logistic regression analyses		
	OR	OR (95% CI)	*P* value	OR	OR (95% CI)	*P* value
WBC	1.845	1.618-2.103	<0.001	1.705	1.434-2.028	<0.001
NE%	1.050	1.029-1.071	<0.001	1.101	0.424-2.859	0.843
LY%	0.900	0.877-0.924	<0.001	1.029	0.395-2.684	0.953
MO%	0.906	0.820-1.001	0.053			
EO%	1.335	1.232-1.448	<0.001	1.590	0.590-4.286	0.360
BA%	1.619	0.856-3.060	0.138			
RBC	0.437	0.279-0.684	<0.001	2.289	0.764-6.862	0.139
HGB	0.949	0.933-0.964	<0.001	0.905	0.870-0.942	<0.001
PLT	1.013	1.009-1.017	<0.001	1.004	0.999-1.010	0.116

WBC, white blood cells; NE%, percentage of neutrophils; LY%, percentage of lymphocytes; MO%, percentage of monocytes; EO%, percentage of eosinophils; BA%, percentage of basophils; RBC, red blood cells; HGB, hemoglobin; PLT, platelets; OR, odds ratio; CI, confidence interval.

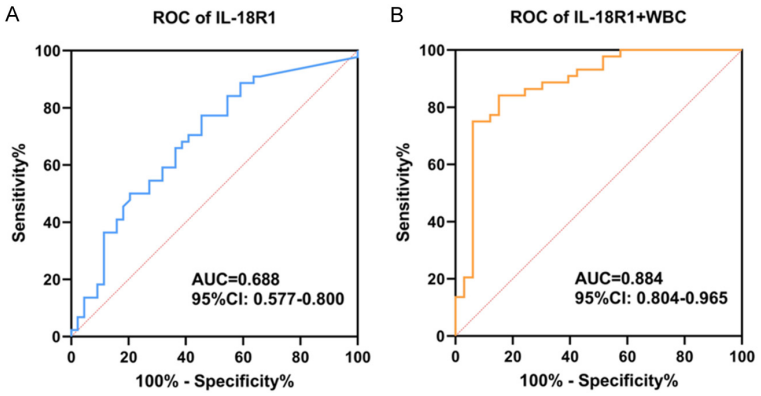

Figure 3. ROC curves for distinguishing asthma patients from healthy individuals based on serum IL-18R1. A. ROC curves of IL-18R1 alone. B. ROC curves of IL-18R1 combined with WBC. IL-18R1, interleukin 18 receptor 1; ROC, receiver operating characteristic; WBC, white blood cells; AUC, area under the ROC curve; CI, confidence interval.

controls from the GSE76262 and GSE137268 datasets was performed. Consistent with previous results, IL-18R1 expression was significantly increased in severe asthma compared to healthy controls (**Figure 4A**). To explore the correlation between IL-18R1 expression and immune infiltration, the ssGSEA enrichment scores of different immune cell subsets were quantified. We found that Activated B cells, Activated CD4 T cells, Activated dendritic cells (DC), CD56 bright natural killer (NK) cells, EO, Gamma delta (γδ) T cells, Myeloid-derived suppressor cells (MDSC), Macrophage, Mast cells, MO, NK T cell, NE, Plasmacytoid DCs, Regulatory T cells, Type 1 T helper (Th1) cells, and Type 17 T helper (Th17) cell enrichment scores differed significantly between healthy controls and patients with severe asthma (**Figure 4C**). Moreover, in patients with severe asthma, IL18R1 was positively associated with infiltration of Activated B cells, Activated DC, EO, MDSC, Macrophage, Mast cell, MO, NK T cells and NE (**Figure 4B**).

To further explore the molecular mechanism of IL-18R1 in severe asthma, the DEGs between high and low IL-18R1 expression groups in severe asthma were analyzed. As shown in the volcano diagram, a total of 577 DEGs were identified, including 331 down-regulated genes and 246 up-regulated genes in the high IL-18R1 expression group (**Figure 4D**). In addition, GSEA enrichment analysis revealed that several pathways such as TNF-α signaling via NfκB, Inflammatory Response, IL6 Jak STAT3 Signaling, Apoptosis, and Hypoxia

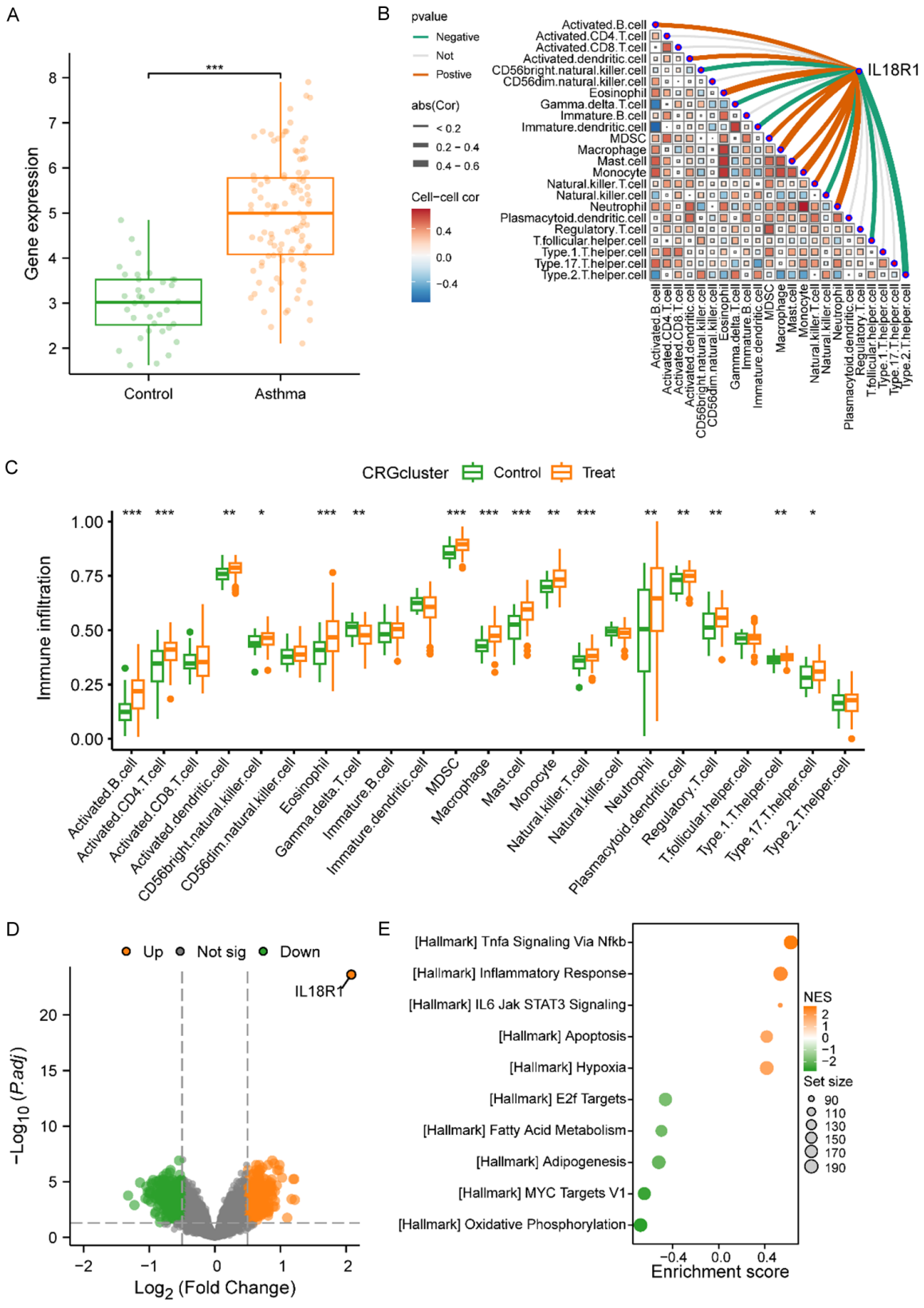

Figure 4. Gene expression and functional enrichment analysis of IL18R1 in asthma. A. Differential expression of IL-18R1 in healthy controls and patients with severe asthma. B. Correlation between IL-18R1 expression and immune cell infiltration in severe asthma. C. Differences in immune cell infiltration in healthy controls and patients with

severe asthma. D. Volcano plot for DEGs between high and low IL-18R1 expression groups. E. Differences in HALLMARK pathway enrichment between high and low IL-18R1 expression groups. *P<0.05, **P<0.01, ***P<0.001; MDSC, myeloid-derived suppressor cells; NES, normalized enrichment score.

were activated in the IL-18R1 high expression group. E2f Targets, Fatty Acid Metabolism, Adipogenesis, MYC Targets V1 and Oxidative Phosphorylation pathway were activated in IL-18R1 low expression group (**Figure 4E**).

MR analysis for IL-18R1 with asthma

MR analysis showed a causal relationship between IL-18R1 and asthma. IL-18R1 expression was positively correlated with asthma, by the IVW method $P<0.05$ (OR: 1.068, 95% CI: 1.001, 1.139) (**Figure 5A-C**). Notably, Cochran's Q statistic showed no heterogeneity ($P>0.05$, **Table 3**). In addition, there was no indication of pleiotropy in the MR Egger regression ($P>0.05$, **Table 3**). Furthermore, funnel plots and leave-one-out sensitivity analysis demonstrated the reliability and robustness of the results (**Figure 5D**, **5E**).

Discussion

Exploration of serum markers for asthma is vital to help clinicians more often achieve an accurate and timely diagnosis. In this study, we found that IL-18R1 expression was elevated in serum of asthmatics and was associated with infiltration of diverse immune cells. MR analysis showed that IL18R1 expression was positively correlated with asthma. Moreover, when IL-18R1 was combined with WBC, the ROC curve exhibited good diagnostic accuracy for asthma.

Environmental triggers like allergens, infections, smoking, and cold air can cause episodic bronchoconstriction and reversible airway narrowing [3]. These factors activate innate and adaptive immune responses, driving chronic airway inflammation - a key aspect of asthma pathophysiology [17]. Peripheral blood WBC count and classification are routine indicators of inflammation. Our results showed that patients with asthma had elevated levels of blood WBC, NE, EO, NE% and EO%, indicating an active inflammatory state. Bioinformatic analyses also demonstrated this point because Activated B cells, Activated CD4 T cells, Activated DC, CD56 bright NK cells, EO, Gamma delta T cells, MDSC, Macrophage, Mast cells, MO, NK T cells, NE, Plasmacytoid DC, Regulatory T cells, Th1 cells and Th17 cells enrichment scores differed significantly between healthy controls and patients with severe asthma. Of note, there are two inflammatory phenotypes in the airway of asthma, including eosinophilic-dominated and neutrophil-dominated inflammation [18]. Eosinophils, as key effector cells, mediate airway inflammation and exacerbation in eosinophilic asthma, exhibiting increased numbers and activation states in the asthmatic airway [19]. Several studies have shown that blood EO and EO% were significantly increased and strongly associated with disease activity of asthma [20, 21]. Airway eosinophils secrete cytokines and chemokines such as IL-4, IL-5, IL-9, and IL-13, which promote type 2 immune responses [22]. The novel biologics targeting IL-5 to induce blood eosinophil depletion can significantly improve asthma symptoms and reduce exacerbations [23]. Neutrophilic asthma, another sub-phenotype distinct from eosinophilic asthma, is characterized by markedly increased neutrophils in blood and sputum, and is related to IL-17 [24]. Neutrophilic inflammation is observed in persistent asthma and is associated with a poor response to inhaled corticosteroids [25]. Elevated NE was also reported to have a relationship with poor clinical outcomes of severe asthma [26]. Elevated EO and NE were observed, meanwhile, in asthmatics in this study, which indicated that the patients enrolled in our study contained diverse asthma phenotypes.

Asthma's immune pathology is complex, involving Th1, Th2, and Th17 responses, with Th2-driven inflammation by IL-5, IL-4 and IL-13 being central [5, 27]. Allergens stimulate Th2 cells to secrete these cytokines, activating B cells to produce IgE and mast cells to release mediators, and causing bronchoconstriction [28]. Airway epithelial cytokines further amplify this process in response to environmental triggers [28]. We found that inflammatory IL-18R1 was significantly elevated in asthmatics. IL-18R1 specially binds to IL-18 and determines the bioactivity of IL-18. IL-18 is a pleiotropic cytokine and its function depends on the host microenvironment [29]. It acts as an effective inducer

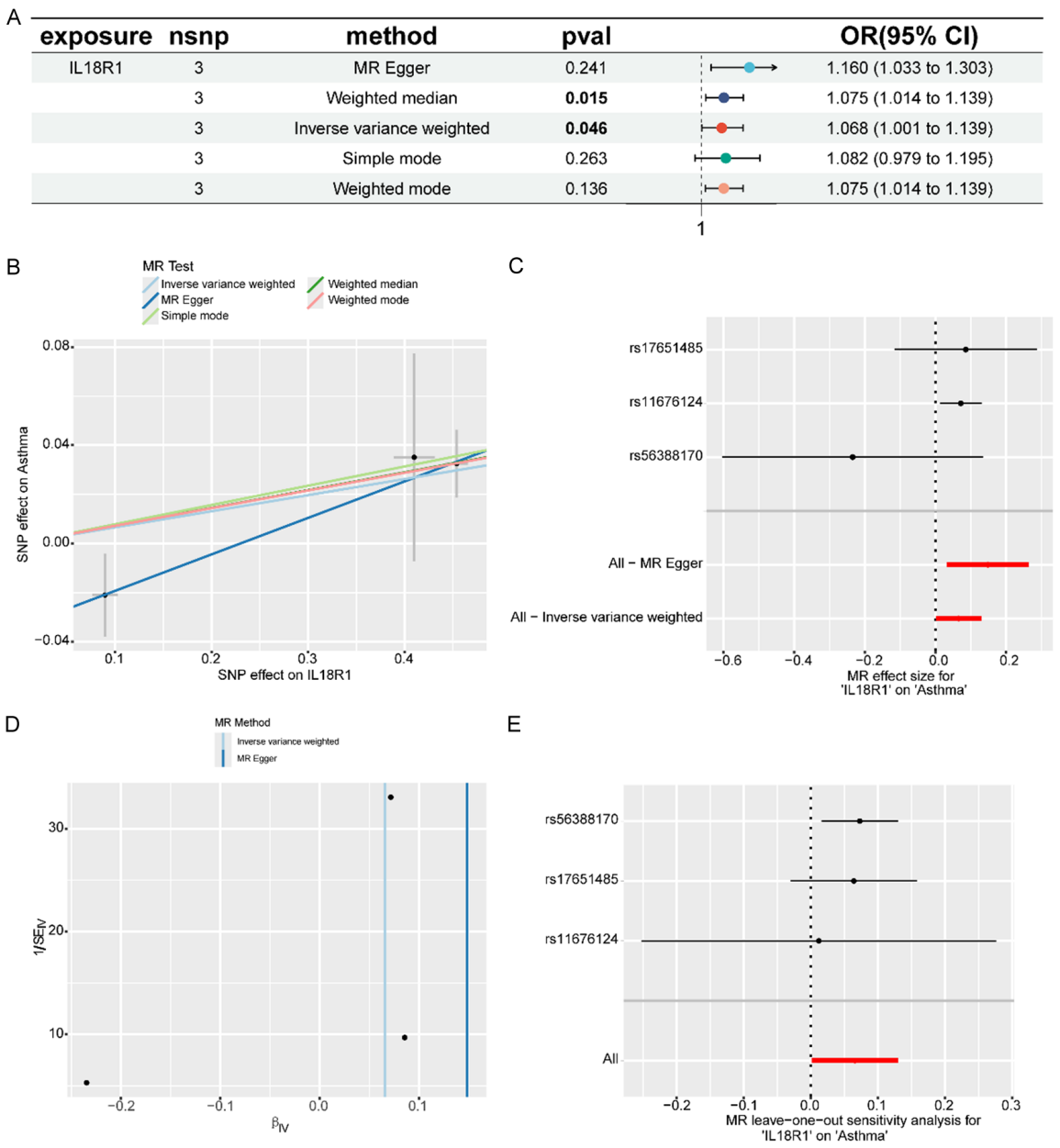

Figure 5. MR analysis for IL-18R1 with asthma. A. Forest plot of the results of five methods of MR analysis. B. Correlation between IL-18R1 and asthma depicted as a scatter plot. C. Forest plot of MR for IL-18R1 with asthma. D. Funnel plot of MR for IL-18R1 with asthma. E. Leave-one-out analysis of MR for IL-18R1 with asthma. IL-18R1, interleukin 18 receptor 1; MR, mendelian randomization; OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism.

Table 3. Heterogeneity and pleiotropy in MR analysis

Exposure	Outcome	Heterogeneity test				Pleiotropy test		
		IVW		MR Egger		MR Egger regression		
		Cochran Q	*P*	Cochran Q	*P*	Intercept	se	*P*
IL18R1	Asthma	2.618	0.270	0.042	0.837	-0.034	0.021	0.355

IL-18R1, interleukin 18 receptor 1; MR, mendelian randomization; IVW, inverse variance weighting.

of interferon-gamma in NK and Th1 cells; on the other hand, it also plays a role in modulating Th2 and Th17 responses, as well as the activity of CD8 cytotoxic cells and neutrophils [29]. The activated IL-18/IL-18R1 signaling axis was demonstrated to have an association with chronic inflammatory disorders [30]. In inflammatory bowel disease, IL-18R1 expression was elevated in both effector and regulatory $CD4^+$ T cells and IL-18 differentially regulated Th17 or Treg subsets during homeostatic and inflammatory conditions [30]. IL-18R1 signaling was required for Treg cells to control intestinal inflammation [30]. Similarly, IL-18R1 was also essential for Treg cells selectively suppressing the Th17 response to recover lung homeostasis after infection [31]. Our study demonstrated that asthmatics with high IL-18R1 expression had elevated blood LY, suggesting that expression of IL-18R1 was associated with lymphocytes. This is consistent with the finding that IL-18R1 was mainly expressed in immune cells, such as T cells and DCs [32].

To further explore the function of IL-18R1, we assessed the relationship between IL-18R1 expression and immune cell infiltration. The results showed that IL18R1 was associated with various immune cells, including Activated B cells, Activated DC, EO, Macrophages, Mast cells, MO, NK T cells, and NE. These immune cells play critical roles in asthma pathology. As is well known, a high level of IgE derived from B cells is a feature of allergic diseases, including asthma. IgE mediates allergic reactions and is essential for antigens entering into immune cells. In the asthmatic airway, IgE binds to high-affinity receptor FcεRI on the surfaces of mast cells and basophils, triggering them to release pro-inflammatory mediators, which induce airway vasodilation and smooth muscle contraction [28]. It was reported that IgE was associated with asthma severity, and anti-IgE antibodies helped to improve symptoms in asthma patients [33, 34]. Mast cells, as a multifunctional kind of cell, accumulate in the asthmatic airway and express diverse receptors. They initiate rapid responses to stimulants and release various mediators. These mediators recruit T cells and DCs and enhance their functions, which establishes a link between innate and adaptive immunity [34]. This process is essential for asthma immunopathology. Macrophages abundantly exist in the lung and are also involved in the inflammation of asthma. According to their microenvironment, macrophages are prone to polarize into two distinct phenotypes, M1 and M2 macrophages. These two phenotypes are respectively associated with Th1 and Th2 polarization of T cells [35]. A recent study revealed that M1 macrophages were the major effector cells of non-allergic asthma, whereas M2 macrophages predominated in allergic asthma [35]. These results suggested that IL-18R1 may play a role in asthma pathology by regulating types of immune cell infiltration.

Pathway enrichment analysis showed that in severe asthma, high expression of IL-18R1 was associated with several inflammatory pathways, such as TNF-α signaling via NfκB, Inflammatory Response, IL6 Jak STAT3 Signaling. The nuclear factor-kappa B (NfκB) signaling pathway is a typical transcriptional activator of inflammatory cytokines. It is initiated by active mast cells and results in the production of inflammatory mediators such as TNF-α and IL-6. The latter induce muscular contraction and microvascular dilation during an asthma episode [36]. Elevated IL-6 was associated with mixed eosinophilic/neutrophilic inflammation and degenerated lung function [37]. IL-18/IL-18R1 complex contains Toll-IL-1 receptor (TIR) domains and sequentially recruits myeloid differentiation primary response protein (MyD) 88, four IL-1 receptor associate kinases (IRAKs), and tumor necrosis factor receptor associated factor (TRAF)-6, leading to NfκB activation [32]. Based on this, we presumed that in asthma, IL-18R1 interacted with IL-18, initiating activation of the NF-κB pathway, which eventually led to airway inflammation. The IL-18R1-expressing immune cells and their regulatory networks constitute the major effector ingredients contributing to the pathology of asthma. This may provide a novel perspectives for us to understand and manage asthma. However, the molecular mechanism of IL-18R1 involved in asthma still needs to be precisely determined.

This study has several limitations. First, there was no sputum and airway tissue to verify the expression of IL-18R1. Second, a lack of cell and animal experiments to verify the inflammatory pathways. Therefore, further investigations are essential to confirm the clinical and functional roles of IL-18R1 in asthma. In the future,

we will collect sputum and bronchoalveolar lavage fluid specimens to further verify the expression of IL-18R1 and incorporate bronchial biopsies or *in vivo* models to confirm the role of IL-18R1 in asthma pathogenesis.

In conclusion, increased EO and NE represent distinct asthma phenotypes. Elevated IL-18R1 was quantified in asthmatics and was related to infiltration of certain immune cells and inflammation pathways. IL-18R1 may induce inflammation by activating the NF-κB pathway and play a critical role in asthma.

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Disclosure of conflict of interest

The authors state that they conducted the research without any commercial or financial relationships that could be considered a potential conflict of interest.

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