Exploring the Causal Effects of 731 Immune Cell Phenotypes on Asthma: A Bidirectional Two-Sample Mendelian Randomization Study

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Article

Keywords: Asthma, mendelian randomization, immune cells, causal inference, Dual Samples

Posted Date: June 27th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4560690/v1

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Additional Declarations: No competing interests reported.
Abstract

**Background:** Asthma is a common chronic respiratory condition characterized by reversible airflow obstruction, bronchial hyperresponsiveness, and inflammation, influenced by genetic predispositions, environmental exposures, and immune responses. Current treatments focus primarily on symptom management, underscoring the need for a deeper understanding of the disease mechanisms.

**Methods:** This study employed Mendelian randomization (MR) to investigate the causal relationships between 731 immune cell phenotypes and asthma susceptibility. Using genetic variants as instrumental variables, we aimed to address confounding and reverse causation biases typical of observational studies. Data were sourced from the FinnGen database's GWAS summary statistics and immune trait data from the GWAS catalog. Various MR methods, including MR Egger, Weighted Median, Inverse Variance Weighted, Simple Mode, and Weighted Mode, were utilized.

**Results:**

Our study has identified six immune cell phenotypes that exhibit potential causal relationships with asthma. After adjustments for a False Discovery Rate (FDR) less than 0.05, the expression of HLA-DR on plasmacytoid dendritic cells (DCs) was significantly associated with asthma, with an odds ratio (OR) of 1.054 and a 95% confidence interval (CI) ranging from 1.029 to 1.080 (P = 2.02E-05, PFDR = 0.015). The following immune phenotypes also demonstrated notable associations: CD62L- CD86+ myeloid DC percentage ($p = 3.354E-04$; PFDR = 0.078; 95% CI = 1.031 to 1.049), CD3 expression on CD4+ regulatory T cells (Tregs) ($p = 1.661E-04$; PFDR = 0.061; 95% CI = 0.959 to 0.980), CD33 expression on dimly expressed CD33 HLA-DR+ CD11b− cells ($p = 5.471E-04$; PFDR = 0.078; 95% CI = 1.019 to 1.030), CD33 on monocytic myeloid-derived suppressor cells (Mo MDSCs) ($p = 6.0433E-04$; PFDR = 0.078; 95% CI = 1.018 to 1.028), HLA-DR expression on CD33− HLA-DR+ cells ($p = 4.472E-04$; PFDR = 0.078; 95% CI = 1.064 to 1.102). Similarly, we conducted reverse MR analysis, which revealed no significant association between immune traits and asthma at a significance level of 0.05.

**Conclusions:** Our findings emphasize the significant role of specific immune cell phenotypes in asthma pathogenesis and suggest potential targets for precision medicine strategies.

**Introduction**

Asthma is a widespread chronic respiratory condition characterized by reversible airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation[1, 2]. The pathophysiology of asthma involves complex interactions between genetic predispositions, environmental exposures, and immune system responses[3, 4]. Key triggers such as allergens, air pollution, respiratory infections, and stress can initiate and intensify the inflammatory processes within the airways[5, 6]. Current therapeutic strategies focus on symptom control and managing exacerbations with inhaled corticosteroids, beta-agonists, and biologic agents targeting specific immune pathways[7, 8]. However, these treatments variably affect the diverse patient population and do not address the root causes of asthma, highlighting
the need for more targeted interventions based on a deeper understanding of the disease mechanisms[9].

The role of immune cells in asthma is critical, as they orchestrate the inflammatory response underlying the disease's symptomatic manifestations. Various immune cell phenotypes, including T cells, B cells, eosinophils, and neutrophils, play distinct roles in asthma pathology[10]. For example, T helper cells (Th2) are known to secrete cytokines such as IL-4, IL-5, and IL-13, which promote eosinophilic inflammation—a hallmark of many asthma phenotype[11]. In contrast, neutrophils are predominant in the inflammatory response of severe asthma, releasing proteases and reactive oxygen species that worsen airway damage[12, 13]. These interactions are dynamic and complex, influencing the onset and progression of the disease. Recent studies indicate that variations in immune cell activity can predict asthma severity and influence responses to treatment, suggesting potential for immune-targeted therapeutic strategies[14].

Mendelian randomization (MR) analysis is a robust approach to identifying causal relationships between exposures and outcomes in observational studies[15, 16]. By using genetic variants as instrumental variables, MR can address confounding and reverse causation biases typical of traditional observational studies[17]. In asthma and immune cell biology, MR analysis is promising for uncovering the causal pathways linking immune cell phenotypes and asthma susceptibility. Employing genetic variants as proxies for immune cell traits, MR analysis can elucidate the causal roles of specific immune cell subsets in asthma pathogenesis, potentially guiding the development of precision medicine strategies for asthma management. This study utilizes Mendelian randomization to explore the causal relationships between 731 immune cell phenotypes and asthma susceptibility, aiming to illuminate the immunological mechanisms driving asthma and identify new therapeutic targets.

**Experimental Strategy**

**Methodology**

To mitigate issues with duplicate analysis, we employed two-sample MR to explore the causal relationship between 731 immune cell phenotypes and asthma. MR is a statistical approach that uses genetic variations as instrumental variables (IVs) to discern causality. For the causal inference to be robust, the IV must satisfy three core criteria: (1) The genetic variation must be strongly associated with the exposure of interest, meaning that the genetic variation can influence immune cell characteristics, which may, in turn, affect asthma[18]. (2) There should be no confounding factors that could influence both the exposure and the outcome associated with the genetic variation; if the genetic variation is linked to potential confounders, it cannot serve as a valid IV[19]. (3) The only pathway through which the genetic variation should affect asthma is by altering immune cell characteristics; outside of the exposure, the genetic variation should not affect the outcome through any other mechanism[20].

**Sources Of Asthma Data**
To ensure originality, the asthma data was derived from the GWAS summary statistics available in the FinnGen database (https://www.finngen.fi/en). The GWAS study included 266,418 European individuals (N_case = 46,684, N_control = 219,734).

Source of Immune Cell Genome-Wide Association Study(GWAS) Data

The GWAS catalog encompasses an extensive array of immune trait data, spanning from project GCST0001391 to GCST0002121[21]. The study involved 3,757 Europeans and investigated 731 distinct immune cell phenotypes. These include 32 morphological parameters, 192 types of relative cell counts, 118 types of absolute cell counts, and median fluorescence intensities across various immune cell types, aiming to provide a comprehensive overview of immunological characteristics.

Choosing Appropriate IVs

To identify significant IVs for each immune trait, we set a p-value threshold of $1 \times 10^{-5}$[22]. For selecting independent variables, we referred to the linkage disequilibrium (LD) data panel from the 1000 Genomes Project, ensuring that the LD index ($R^2$) did not exceed 0.001 and that the separation distance between independent loci was no more than 10,000 kb[23]. Additionally, variables with an F-statistic less than 10 were excluded to avoid the use of weak instruments. For asthma research specifically, we implemented a more stringent significance threshold of $5 \times 10^{-8}$.

Statistical Analysis

We utilized five distinct MR methodologies for our analysis. The MR Egger method was employed to assess the causal relationship between genetic variation and exposure factors, while also scrutinizing the assumption of balance. The Weighted Median approach estimated the causal effect by computing the weighted median of genetic variation, thus alleviating the requirement for balance. The Inverse Variance Weighted(IVW) method[24], a classical MR analysis technique, estimated the causal effect by weighting the effect size of genetic variation. The Simple Mode approach straightforwardly aggregated the effect estimates of each genetic variation, whereas the Weighted Mode method considered the discrepancies in weight among different genetic variations. MR-Egger intercept testing was used to evaluate potential pleiotropy[25], and Cochran's Q test was utilized to assess residual heterogeneity[26], both at a significance level of $p < 0.05$. For the final results, we additionally employed the False Discovery Rate (FDR) method for correction[27]. Our aim was to minimize bias to the fullest extent possible and obtain reliable estimates of the causal relationship between the exposure of interest and the outcome. Lastly, to explore whether there exists a causal relationship between asthma and immune cells, reverse MR analysis was conducted. All statistical analyses in this study were performed using the R software package (v4.2.2). The primary R package employed was TwoSampleMR.

Results
Causal Relationship Between Immune Cell Phenotypes And Asthma

Our findings indicate that six immune cell types exhibit a potential causal association with asthma. Following adjustment for the PFDR < 0.05, one immune phenotype was found to have a causal link with asthma. Specifically, HLA-DR expression on plasmacytoid dendritic cells (DCs) has been identified as a risk factor for asthma, with an odds ratio (OR) of 1.054 and a 95% confidence interval (CI) ranging from 1.029 to 1.080 (P = 2.02E-05, PFDR = 0.015). After further correction for FDR (PFDR < 0.08), several markers were recognized as risk factors for asthma: the percentage of CD62L - CD86 + myeloid DCs, CD33 expression on dimly expressed CD33 HLA-DR + CD11b - cells, CD33 on monocytic myeloid-derived suppressor cells (Mo MDSCs), and HLA-DR on CD33 - HLA-DR + cells. Conversely, CD3 expression on CD4 + regulatory T cells (Tregs) was identified as a protective factor. The IVW analysis yielded the following results: CD62L - CD86 + myeloid %DC (p = 3.354E-04; 95%CI = 1.031 (1.014,1.049)), CD3 on CD4 Treg (p = 1.661E-04; 95%CI = 0.959 (0.938,0.980)), CD33 on dimly expressed CD33 HLA-DR + CD11b - (p = 5.471E-04; 95%CI = 1.019 (1.008,1.030)), CD33 on Mo MDSC (p = 6.043E-04; 95%CI = 1.018 (1.008,1.028)), HLA-DR on CD33 - HLA-DR + (p = 4.472E-04; 95%CI = 1.064 (1.028,1.102)) (Fig. 1).

Additionally, our investigation did not reveal any evidence of pleiotropy (see Supplementary Table S1). Scatter plots and forest plots were utilized to validate the reliability of our study results (refer to Supplementary Figures S1 and S2).

Causal Relationship Between Asthma And Immune Cell Phenotype

To investigate the causal relationship between asthma and immune phenotypes, we conducted a two-sample MR analysis, utilizing the IVW method as the primary analytical tool. After multiple testing adjustments using the FDR method, no significant associations were detected between asthma and immune traits at a significance level of 0.05.

DISCUSSION

Asthma, as a complex inflammatory disease, is influenced by an intricate interplay among genetic predispositions, environmental exposures, and immune responses[28, 29]. Our study underscores the critical role of immune cells in orchestrating the inflammatory milieu that characterizes asthma. The findings from our MR analysis highlight how specific immune cell phenotypes contribute distinctively to asthma pathogenesis.

HLA-DR on plasmacytoid dendritic cells (pDCs) plays a pivotal role in the pathogenesis of asthma through its involvement in immune regulation and response to inflammation[30]. Plasmacytoid DCs are primarily known for their ability to produce large amounts of type I interferons in response to viral infections, which are critical in the innate immune defense[31, 32]. The expression of HLA-DR, a major histocompatibility complex class II molecule, on these cells facilitates their role in antigen
In asthma, HLA-DR on pDCs allows them to present allergen-derived peptides to T cells, particularly initiating the differentiation of naive T cells into Th2 cells during the sensitization phase of allergic asthma. This Th2 cell differentiation leads to the production of cytokines like IL-4, IL-5, and IL-13, which further drive the inflammatory processes characteristic of asthma, such as eosinophilic inflammation and hypersecretion of mucus in the airways. By modulating the activity of pDCs or their interaction with T cells, it may be possible to influence the course of asthma, highlighting the importance of these cells in both the initiation and exacerbation of the disease.

CD62L−CD86+ myeloid dendritic cells (DCs) play a crucial role in asthma by modulating immune responses, particularly in inflammation and T cell activation. The absence of CD62L suggests these DCs are activated or mature, primarily located in peripheral tissues or inflammation sites, while the presence of CD86 indicates readiness to activate T cells by presenting antigens and providing co-stimulatory signals. In asthma, these cells facilitate the activation of naive T cells into Th2 cells, which produce cytokines like IL-4, IL-5, and IL-13, driving key asthma features such as eosinophil recruitment, mucus production, and airway hyperresponsiveness.

CD33, a sialic acid-binding immunoglobulin-like lectin, marks a subset of myeloid cells known as CD33dim HLA-DR+CD11b−, which play a complex role in asthma's immune landscape. These cells, with moderate CD33 expression and high levels of HLA-DR but no CD11b, potentially modulate immune responses to reduce inflammation, influencing asthma severity and symptom progression. Additionally, CD33 expression on monocytic myeloid-derived suppressor cells (Mo MDSCs) significantly impacts asthma by suppressing immune responses, particularly through inhibiting T cell proliferation and cytokine production, which can perpetuate inflammation and affect disease resolution.

CD3 on CD4+ regulatory T cells (Tregs) plays a crucial role in modulating the immune response associated with asthma, primarily through their immunoregulatory functions. On CD4+ Tregs, CD3 is involved in the activation of these cells, which are essential for maintaining immune tolerance and preventing autoimmunity. In the context of asthma, CD4+ Tregs help to control and suppress excessive immune responses to allergens, which are a common trigger for asthma attacks. They achieve this by inhibiting the proliferation of effector T cells and reducing the production of pro-inflammatory cytokines. This suppressive activity helps to prevent the overactive inflammatory responses that lead to airway hyperresponsiveness and remodeling, characteristic features of asthma.

Enhancing the function or number of Tregs might provide a therapeutic strategy to reduce inflammation and improve asthma outcomes, highlighting the importance of these cells in maintaining immune homeostasis and controlling asthma exacerbations. Our study also explores the causal relationship between asthma and immune phenotypes. Following adjustment for multiple testing using the FDR method, we did not observe significant associations between asthma and immune traits at a significance level of 0.05. This finding suggests that the pathogenesis of asthma may be a multifactorial process influenced not only by the immune system but also by other regulatory factors.

The use of MR analysis in our study provides a robust framework for understanding the causal impact of immune cell phenotypes on asthma susceptibility. By leveraging genetic variants as instrumental variables, MR helps in mitigating confounding factors typical of observational studies. This approach is particularly valuable in dissecting the complex genetic architecture underlying immune responses in asthma.
asthma. The identification of causal relationships between specific immune cell phenotypes, such as the expression of HLA-DR on plasmacytoid dendritic cells and asthma risk, potentially opens new avenues for targeted therapeutic interventions. These immune cells could serve as biomarkers for predicting asthma severity or as direct targets for biologic therapies aimed at modulating their function. While our findings provide significant insights into the immunological underpinnings of asthma, several challenges remain. The complexity of asthma phenotypes and the heterogeneity in patient responses to treatments indicate that multiple genetic and environmental factors are at play. Future research should focus on integrating comprehensive genomic data with detailed environmental exposure data to better characterize the gene-environment interactions that drive asthma pathogenesis. Furthermore, the clinical translation of these findings requires validation in diverse populations. Given the genetic diversity and varying environmental exposures across populations, the generalizability of our results needs to be rigorously tested in multi-centric, international cohorts. In conclusion, our MR analysis has elucidated several key immune cell phenotypes that hold causal associations with asthma. These findings enhance our understanding of the immunological mechanisms of asthma and underscore the potential of immune-focused therapies. By continuing to leverage advanced genetic analytical techniques like MR, the path toward precision medicine in asthma looks promising, with the potential to tailor interventions based on individual immune profiles to improve patient outcomes.

**Declarations**

**Author contributions**

JS drafted the manuscript, HL critically reviewed and edited the manuscript, and all authors reviewed and provided approval for the final version.

**Conflicts of interest**

It is claimed that the authors do not have any conflicts of interest.

**Ethical approval and participant consent**

The study was a public database study, so it did not require informed consent and ethical approval from the committee. All the authors agreed to the publication of the paper.

**Acknowledgments**

We extend our gratitude to all the authors for their invaluable contributions.

**Availability of data and materials**

The data obtained can be accessed for investigation through the IEU Digital Repository at https://gwas.mrcieu.ac.uk and can be freely downloaded from http://ftp.ebi.ac.uk/pub/databases/gwas/summary statistics/. The asthma data originates from the
publicly available Finnish database, which can be accessed at https://www.finngen.fi/en and is also available for unrestricted download. For further information, please contact the corresponding author.

References


**Figures**
### Figure 1

Forest plot illustrating the causal relationships between immune cell phenotypes and asthma. IVW; Inverse Variance Weighted. CI; Confidence Interval. GD; Graves' Disease; OR: Odds Ratio.

### Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigureS1.pdf](https://example.com/SupplementaryFigureS1.pdf)
- [SupplementaryFigureS2.pdf](https://example.com/SupplementaryFigureS2.pdf)
- [SupplementaryTableS1.xls](https://example.com/SupplementaryTableS1.xls)