Advances in World Medical Research, 2025, 1(1), 61-70
https://doi.org/10.71204/s8ksf005

Two-Sample Mendelian Randomization Analysis of the Correlation Between Allergic Rhino-Conjunctivitis and Concomitant Diseases and Peripheral Blood Eosinophil Count

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Received: 12 April 2025/ Accepted: 12 May 2025/ Published online: 20 May 2025

Abstract

To explore the causal relationship between allergic rhino-conjunctivitis, asthma, and eczema and peripheral blood eosinophil count from the perspective of single nucleotide polymorphisms through two-sample Mendelian randomization analysis. Five methods, including inverse variance weighting, MR-Egger regression, median weighting, simple model, and weighted model, were used to evaluate the relationship between allergic rhino-conjunctivitis, asthma, and eczema and peripheral blood eosinophil count. Sensitivity analysis (pleiotropy, heterogeneity, and leave-one-out test) was used to evaluate the robustness of the results. There was a significant causal association between SNPs of allergic rhinitis , allergic conjunctivitis, asthma, and eczema and SNPs of peripheral blood eosinophil count. The sensitivity analysis of SNP instrumental variables showed no significant heterogeneity or pleiotropy, indicating that the results are robust. Evidence shows that peripheral blood eosinophil count is associated with an increased risk of allergic rhino-conjunctivitis, asthma, and eczema.

Keywords: Mendelian Randomization Analysis; Allergic Rhino-Conjunctivitis; Eosinophil Count

1. Introduction

Allergic rhinitis (AR) is a local allergic reaction disease, predominantly presenting with symptoms such as nasal itching, sneezing, clear nasal discharge, and nasal obstruction. It falls under type IV hypersensitivity reaction mediated by immunoglobulin E (IgE). Studies have demonstrated that in patients with AR, there is an infiltration of mast cells and eosinophils in the local area of the nasal cavity within the peripheral blood. The main pathological changes in the nasal mucosa mainly involve enhanced vascular permeability, tissue edema, and infiltration of

mast cells and eosinophils. Clinically, the infiltration of eosinophils in the peripheral blood can be utilized to predict the hormone resistance status and treatment outcomes of patients.

Allergic diseases are a common category of chronic immune disorders, encompassing AR, allergic conjunctivitis (AC), asthma, eczema (atopic dermatitis), food allergy, and so on. AR is frequently accompanied by diseases such as AC, asthma, and eczema, and this is even more prevalent in seasonal AR. It severely impacts the social life of patients and is often accompanied by emotional disorders. AR and AC are often collectively referred to as allergic rhino-conjunctivitis (ACR). The incidence of AR complicated with asthma ranges from 74% to 100% (Wang et al., 2023), the incidence of AR complicated with AC is between 30% and 71% (Leonardi et al., 2015), and the incidence of AR complicated with eczema is 9.2% (Camilla et al., 2020).

Eosinophilic asthma (allergic asthma) accounts for 70% to 80%. The allergic reaction in the human body mainly refers to the process in which when the same allergen re-enters the body, it specifically binds to the antibodies on the surface of the sensitized basophil granulocytes (BASO), leading to the release of bioactive mediators mainly composed of histamine from these cells. During this process, eosinophils (EOS) among white blood cells exhibit an antagonistic effect with basophil granulocytes (BASO). EOS release histaminase and arylsulfatase to inactivate the histamine and leukotrienes released by BASO, phagocytize the particles expelled by BASO, and simultaneously produce prostaglandin E1 to inhibit the degranulation process of BASO (Ogawa et al., 2024). Research has revealed that EOS is associated with the onset of allergic diseases such as ACR and asthma. The release of extracellular vesicles and DNA traps by EOS affects both local and systemic immune responses, thereby further influencing the pathophysiological processes of allergic diseases such as airway inflammation, chronic sinusitis, and atopic dermatitis (Weihrauch et al., 2024). Animal experiments have found that ribonuclease 2 related to EOS in mice can exacerbate the allergic reaction (Nguyen et al., 2024). The aforementioned studies mainly focus on observational studies and basic experimental research, and lack evidence of causal associations. This study aims to start from Mendel's laws of inheritance, use genetic variations strongly correlated with exposure factors as instrumental variables, and infer the causal effects between exposure and outcomes at the genetic level. In this study, we employ the method of Mendelian randomization (MR) to explore the potential causal relationships between EOS and genetic variations associated with ACR, asthma, and eczema.

2. Methods

2.1. Research Design

This study employed a two-sample Mendelian randomization (MR) method to evaluate the causal effect relationships between eosinophils (EOS) and allergic rhino-conjunctivitis (ACR), asthma, and eczema. The MR analysis adheres to the following three core assumptions (Davey et al., 2020): (1) Association assumption: The instrumental variables of single nucleotide polymorphisms (SNPs) must be closely related to the exposure factors; (2) Independence assumption: Genetic variations are independent of the confounding factors that affect both the

exposure and the outcome; (3) Exclusivity assumption: SNPs can only influence the outcome through the exposure.

2.2. Data Sources

The data for allergic rhinitis (AR), allergic conjunctivitis (AC), asthma, and eczema were derived from the Genome-Wide Association Study (GWAS) summary data. AR included 217,914 European individuals with 16,380,461 SNPs, and the summary data was publicly available from https://gwas.mrcieu.ac.uk/datasets/finn-b-ALLERG_RHINITIS/; AR included 218,792 European individuals with 16,380,466 SNPs, and the summary data was publicly available from https://gwas.mrcieu.ac.uk/datasets/finn-b-H7_ALLERGICCONJUNCTIVITIS/; Asthma included 361,194 European individuals with 10,443,939 SNPs, and the summary data was publicly available from https://gwas.mrcieu.ac.uk/datasets/ukb-d-J10_ASTHMA/; Eczema included 218,792 European individuals with 16,380,466 SNPs, and the summary data was publicly available from https://gwas.mrcieu.ac.uk/datasets/finn-b-L12_DERMATITISECZEMA/. The peripheral blood EOS count was obtained from the GWAS summary data, which included 563,946 European individuals, and the summary data was publicly available from https://gwas.mrcieu.ac.uk/datasets/ieu-b-33/. The research data was sourced from publicly available GWAS databases, and there were no ethical issues involved.

2.3. Selection of Instrumental Variables

The instrumental variables were required to satisfy the condition that the selected SNPs were closely related to AR, AC, asthma, and eczema. The threshold for instrumental variables was set at $P < 5\times10\text{-}8$. Linkage disequilibrium (LD) was removed to ensure that the selected SNPs were independent of each other (Slatkin et al., 2008). The screening criteria were $R2 < 0.001$ and KB = 10,000, and the screening was carried out using the two-sample MR package in R software. The F statistic ($F = \beta2/sx2$) was calculated to further verify the association assumption and to exclude the bias of weak instrumental variables ($F < 10$), so as to determine the final instrumental variables.

2.4. Statistical Methods

2.4.1. Main Causal Relationship Assessment

In this study, five methods, namely inverse variance weighting (IVW), MR-Egger, weighted median, simple model, and weighted mode, were used to evaluate the causal relationships between AR, AC, asthma, eczema, and the peripheral blood EOS count. IVW is the main evaluation method. When all SNPs are valid instrumental variables, the test efficiency of the IVW causal relationship is the highest. The MR-Egger regression slope coefficient is used to evaluate the true causal effect. When there are pleiotropic instrumental variables, the reliability of the causal evaluation will be affected (Bowden et al., 2015). The weighted median method and the IVW method complement each other. Even if half of the instrumental variables are invalid, they can still provide consistent causal relationships. The weighted median method can also be used as a sensitivity analysis to assess the robustness of the causal association (Bowden et al., 2016). The simple model and the weighted mode method can be used as auxiliary methods for the causal

effect test. The results of the causal effect evaluation are usually expressed by the odds ratio (OR) and the 95% confidence interval (CI).

2.4.2. Sensitivity Analysis

In this study, the heterogeneity of the causal effect was evaluated by the Cochran Q test. When the result heterogeneity is large, a random effects model is selected; conversely, a fixed effects model is selected. When $P < 0.1$, it indicates the presence of heterogeneity (Burgess & Bowden, 2017). MR-Egger and MR-PRESSO were used to test the horizontal pleiotropy in the MR analysis. When there is no statistical difference between the MR-Egger intercept and zero, it indicates the absence of pleiotropy (Burgess & Thompson, 2017). The leave-one-out method tests the effect size of the remaining SNPs by removing SNPs one by one to evaluate the robustness of the results (Hemani et al., 2018). All data analyses were performed in R software version 4.2.2, using the Two Sample MR software package (version 4.41).

3. Results

3.1. Determination of Instrumental Variables

The R software was used to screen for single nucleotide polymorphisms (SNPs) that were closely related to the exposure. The fact that all F statistics were > 10 indicated the absence of weak instrumental variables. A total of 448 SNPs were extracted as instrumental variables from the Genome-Wide Association Study (GWAS) data of the peripheral blood eosinophil (EOS) count. Among them, 440 SNPs were significantly associated with allergic rhinitis (AR), 440 SNPs were significantly associated with allergic conjunctivitis (AC), 154 SNPs were significantly associated with asthma, and 145 SNPs were significantly associated with eczema.

3.2. Analysis of Main Results

The results of the inverse variance weighting (IVW) method for the peripheral blood EOS count data suggested a clear causal relationship between allergic rhino-conjunctivitis (ACR), asthma, eczema, and the risk of the peripheral blood EOS count (Odds Ratio [OR] = 1.507, 95% Confidence Interval [CI]: 1.383–1.643; OR = 1.335, 95% CI: 1.249–1.428; OR = 1.228, 95% CI: 1.118–1.350; OR = 1.228, 95% CI: 1.161–1.300). The results of the MR-Egger, weighted median, simple model, and weighted mode methods supported the above conclusions, as shown in Table 1.

Table 1. Results of the Mendelian Randomization (MR) Analysis of the Correlation between Allergic Rhinitis, Allergic Conjunctivitis, Asthma, Eczema, and the Peripheral Blood Eosinophil Count

Indicators	Methods	β	SE	OR（95%CI）	P value
AR	IVW	0.410	0.044	1.507(1.383, 1.643)	<0.01
	MR Egger	0.273	0.110	1.314(1.059, 1.631)	0.014

	Simple Mode	0.319	0.210	1.376(0.912,2.074)	0.129
	Weighted Median	0.365	0.078	1.440(1.235,1.679)	<0.01
	Weighted Mode	0.406	0.132	1.501(1.158,1.946)	0.002
AC	IVW	0.289	0.034	1.335(1.249, 1.428)	<0.01
	MR Egger	0.229	0.084	1.258(1.067,1.483)	0.007
	Simple Mode	0.224	0.176	1.251(0.886,1.768)	0.204
	Weighted Median	0.297	0.062	1.345(1.191,1.519)	<0.01
	Weighted Mode	0.296	0.0987	1.345(1.108,1.632)	0.003
asthma	IVW	0.003	0.000	1.228(1.118,1.350)	<0.01
	MR Egger	0.001	0.001	1.001(0.999, 1.004)	0.301
	Simple Mode	0.003	0.002	1.003(0.999,1.008)	0.108
	Weighted Median	0.003	0.001	1.003(1.002,1.005)	<0.01
	Weighted Mode	0.002	0.002	1.002(0.998,1.006)	0.374
eczema	IVW	0.206	0.029	1.228(1.161,1.300)	<0.01
	MR Egger	0.0979	0.114	1.103(0.882, 1.378)	0.391
	Simple Mode	0.001	0.135	1.001(0.768,1.305)	0.993
	Weighted Median	0.086	0.052	1.090(0.984,1.208)	0.098
	Weighted Mode	0.057	0.085	1.059(0.897,1.251)	0.500

3.3. Results of Sensitivity Analysis

In the Cochran Q heterogeneity test, the Q values of both the inverse variance weighting (IVW) method and the MR-Egger method were greater than 0.1, indicating the absence of heterogeneity, as shown in Table 2. In the leave-one-out method analysis, no single single nucleotide

polymorphism (SNP) that significantly affected the results and undermined the robustness of the results was found, as shown in Figure 1.

Table 2. Results of the Sensitivity, Heterogeneity, and Horizontal Pleiotropy Tests of the Instrumental Variables

Eosinophil	Cochran Q value	P value
AR	702.63	1.462231e-15
AC	672.76	4.501209e-13
asthma	264.44	4.311666e-08
Eczema	388.45	5.272859e-25

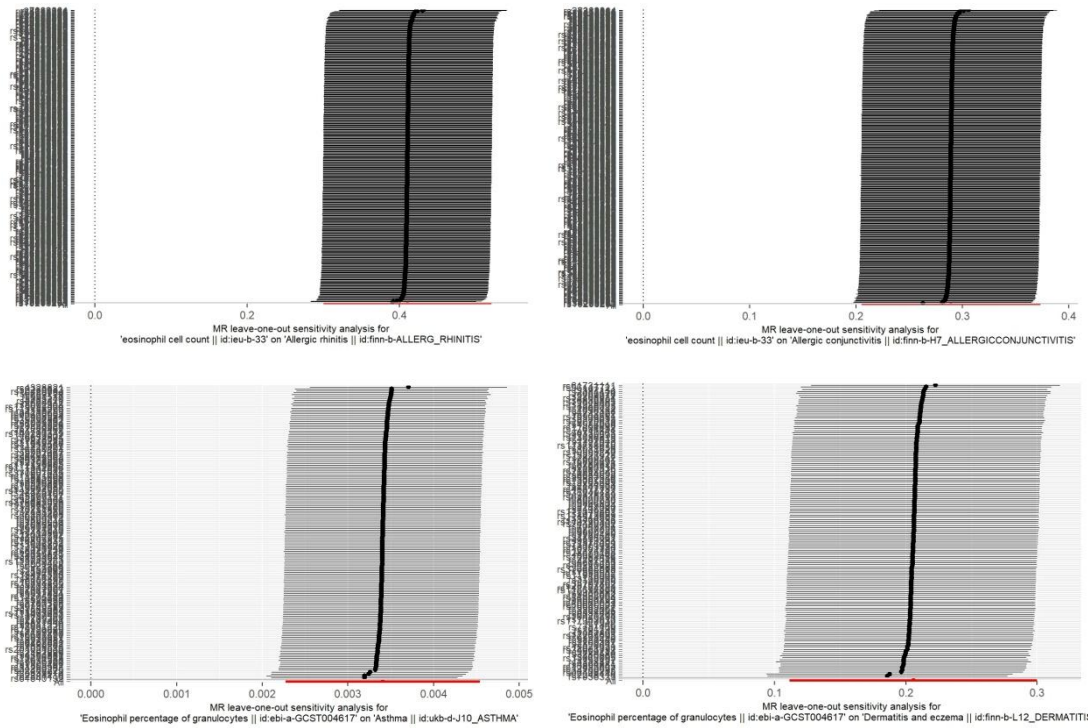

Figure 1. Sensitivity Test by the Leave-One-Out Method

4. Conclusion

In this study, the publicly available data on peripheral blood eosinophil (EOS) count, allergic rhino-conjunctivitis (ACR), asthma, and eczema from the Genome-Wide Association Study (GWAS) database were utilized to conduct a two-sample Mendelian randomization (MR) analysis. The results showed a significant correlation between the peripheral blood EOS count and the occurrence of ACR, asthma, and eczema. A potential causal association was found between the peripheral blood EOS count and the risk of allergic rhinitis (AR), which is consistent with the

results of previous studies on the peripheral blood EOS count and AR (Pranavi et al., 2024), once again verifying the causal effect between the peripheral blood EOS count and AR at the gene level. In this study, the GWAS database was used, and the MR analysis method was adopted to further explore the associations between EOS and allergic conjunctivitis (AC), asthma, and eczema. The results suggested a significant correlation between the peripheral blood EOS count and the occurrence of these three diseases. For the first time, it was proposed that the peripheral blood EOS count is a risk factor for ACR, asthma, and eczema, and it was found that there are also causal effects between the peripheral blood EOS count and ACR, asthma, and eczema at the gene level. The above findings suggest that controlling the peripheral blood EOS count may be beneficial for the prevention and management of the occurrence and development of ACR, asthma, and eczema, providing important evidence for future research on drug interventions for such diseases.

Allergic diseases (such as allergic rhinitis) are caused by the body's abnormal type 2 immune response to harmless antigens in the environment. They include atopic dermatitis, allergic asthma, AR, AC, and allergic urticaria, etc. (Cao et al., 2024). These are inflammatory comorbidities with a genetic predisposition and a chronic and recurrent manifestation. Currently, in the fields of genomics, transcriptomics, proteomics, epigenetics, metagenomics, and metabolomics, some biomarkers and potential targets of reference value have been discovered (Hao et al., 2025). EOS and mast cells are key effector cells in allergies (Gangwar et al., 2021). If the late phase of the allergic reaction does not subside, it can develop into the chronic stage, that is, the stage of allergic inflammatory reaction. This involves a large number of mast cells and EOS coexisting in the inflamed tissues during the late phase and chronic stage. The bidirectional interaction between them is mediated through cell surface receptors (such as CD48, 2B4 and their respective ligands), intercellular contact, and the release of mediators (such as various specific granular mediators, metabolites of arachidonic acid, cytokines, and chemokines), which keeps the allergic inflammation in a continuous cycle. The latest research has found that the degranulation of EOS is regulated by the miR-223-3p/FBXW7 targeting, thereby enhancing the allergic inflammation of AR. The miR-223-3p/FBXW7 axis related to EOS provides a new method for the treatment of AR (Wu et al., 2023). Observational studies (Fernandez et al., 2022) have shown that EOS plays a central role in the pathophysiology of chronic ocular allergies such as AC in spring and in maintaining the immune response. Therefore, research on EOS-related cell subtypes, receptors, and mediator release is helpful for the precise treatment of allergic diseases. The activation state of EOS in the blood and airways and its state after encountering inflammatory mediators determine the multiple roles of EOS in type 2 inflammation. Airway epithelial cells play a sentinel role, which can guide the aggregation and migration of EOS and play an important role in the pathogenesis of asthma (Steffan et al., 2024). Different from clinical observational studies and laboratory studies, the GWAS data used in this study has the advantages of a large sample size and being unaffected by external confounding factors. Therefore, the results are reliable. This study explains the causal relationship between EOS and ACR, asthma, and eczema at the gene level, and further confirms the relationship between EOS and allergic diseases as well as the relevance of the pathological mechanisms. It is a powerful complement to clinical observations and laboratory research.

Author Contributions:

Conceptualization, W. Z and J. Y.; methodology, G. L.; software, G. L.; validation, W. Z.; formal analysis, G. L.; investigation, W. Z.; resources, W. Z and G. L.; data curation, G. L.; writing—original draft preparation, W. Z.; writing—review and editing, J. Y.; visualization, W. Z.; supervision, G. L.; project administration, W. Z., G. L.; funding acquisition, W. Z., J. Y. All authors have read and agreed to the published version of the manuscript.

Funding:

This research was funded by National Population Health Science Data Center of Chinese Academy of Medical Sciences, grant number NCMI202409A08; National Natural Science Foundation of China, grant number 82205206; Talent Training Project of Dongzhimen Hospital of Beijing University of Chinese Medicine, grant number DZMG-ONGG0006.

Institutional Review Board Statement:

Not applicable.

Informed Consent Statement:

Not applicable.

Data Availability Statement:

Not applicable.

Acknowledgments:

The authors acknowledge for efforts of the authors.

Conflict of Interest:

The authors declare no conflict of interest.

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