



Original Article

## Proteome-wide Mendelian randomization identifies therapeutic targets for asthma

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### ABSTRACT

**Objectives:** Asthma is a prevalent chronic inflammatory disease that involves a complex interaction between various cells of the innate and adaptive immune systems and epithelial cells. Anti-inflammatory therapeutic approaches have proven superior in controlling asthma and reducing exacerbation rates and are receiving increasing attention. The treatment of asthma is a potentially actionable target pending further validation. This study presented integrative evidence that supports both previously reported and emerging causal proteins, as well as potential drug targets, for asthma.

**Methods:** Mendelian randomization (MR) analysis, colocalization (COLOC) analysis, phenome-wide association (PWAS) analysis, transcriptome analysis, single-cell data analysis, protein-protein interaction medication targets analysis.

**Results:** Nephronectin (NPNT) and neuronal growth regulator 1 (NEGR1) were found to be statistically significantly associated with a noticeable protective effect against asthma, tumor necrosis factor, alpha-induced protein 3 (TNFAIP3) showed a suggestive statistical association with a protective effect against asthma in MR analyses. COLOC indicated that NPNT and NEGR1 have greater potential as drug targets. Compared with normal tissues, NPNT exhibited a higher expression level in Type II pneumocytes; NEGR1 was upregulated in smooth muscle cells, regulatory T cells, plasmacytoid dendritic cells, helper T cells; TNFAIP3 expression was decreased in Cluster of differentiation 4 (CD4)-positive alpha-beta T cells, lung ciliated cell 1, smooth muscle cells, whereas it was elevated in CD8-positive alpha-beta T cell 1, helper T cells, and myelocytes in asthma lung tissues. The interaction between Integrin alpha-8 (ITGA8) and NPNT, as well as the interaction between melanocortin-4 receptor and NEGR1, might serve as potential therapeutic targets.

**Conclusion:** NPNT and NEGR1 could be promising targets for current asthma medications. NEGR1 was first identified in MR of asthma, and we further performed cross-omics validation to verify the roles of NPNT, NEGR1, and TNFAIP3.

**Keywords:** Alpha-induced protein 3, Asthma, Mendelian randomization, Nephronectin, Neuronal growth regulator 1, Mendelian randomization analysis

### INTRODUCTION

Asthma is a prevalent condition, affecting nearly 1 in 10 children and 1 in 12 adults, with approximately 300 million individuals impacted globally. This chronic inflammatory disease

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of the conducting airways involves complex interactions between innate and adaptive immune cells and epithelial cells, leading to bronchial hyperresponsiveness (BHR). Asthma symptoms are predominantly driven by airway inflammation, which triggers a series of pathological processes, including increased mucus production, airway wall remodeling, and BHR.<sup>[1,2]</sup> Moreover, damage to the airway epithelial barrier plays a crucial role in asthma pathophysiology, while repair of this barrier is integral to airway wall remodeling.<sup>[3,4]</sup>

Current asthma treatments mainly include  $\beta$ 2-agonists, corticosteroids, and certain biological agents. Short-acting  $\beta$ 2-agonists provide rapid relief of acute symptoms, while daily inhaled corticosteroids remain the standard of care for persistent asthma. Additional treatment options include long-acting muscarinic antagonists and biological agents that target key proteins involved in asthma pathogenesis, such as omalizumab, mepolizumab, and reslizumab.<sup>[5]</sup> Recent clinical research highlights the critical role of inhaled corticosteroids in achieving optimal asthma control. The onset and exacerbation of symptoms indicate increased inflammation, and the associated risks can be mitigated through combination therapy with maintenance inhaled corticosteroids and as-needed long-acting  $\beta$ 2-agonists.<sup>[6]</sup> Anti-inflammatory treatment is considered vital in asthma management, with anti-inflammatory relievers proving superior in controlling asthma and reducing exacerbation rates.<sup>[7,8]</sup>

Proteins represent the most effective biomarkers and therapeutic targets,<sup>[9,10]</sup> as they are key functional components of cellular and biological processes and the end products of gene expression.<sup>[11]</sup> Mendelian randomization (MR) analysis is a widely used tool for discovering new therapeutic targets, employing genetic variation as instrumental variables (IVs) to infer causal relationships between exposures and diseases.<sup>[12]</sup> Genome-wide association studies (GWAS) have identified specific single-nucleotide polymorphisms (SNPs) that regulate protein expression, known as protein quantitative trait loci (pQTLs).<sup>[13]</sup> These pQTLs are utilized as IVs to explore causal associations between exposures and outcomes, facilitating the identification of potential drug targets and biomarkers. Furthermore, PWAS integrates gene and protein expression data with GWAS results, offering distinct advantages over traditional GWAS approaches.<sup>[14]</sup>

To discover novel drug targets for asthma, we analyzed a cohort of 54,306 participants from the UK biobank-Precision Medicine Project (UKB-PPP) and conducted a comprehensive evaluation of the associations between 2,923 serum proteins and asthma risk.<sup>[15]</sup> This large-scale integration of the plasma proteome with genetic and disease data enabled us to determine the genomic architecture underlying protein levels, aiming to identify plasma proteins as potential therapeutic targets for asthma. First, we obtained the pQTL datasets from the UKB-PPP and performed MR analysis to explore potential

therapeutic targets for asthma. Next, we employed COLOC to integrate the positively identified MR proteins and pQTL data to validate the reliability of our results. Subsequently, we conducted PWAS to examine associations between the positively identified MR proteins and a wide range of phenotypes across the entire phenome. The positive MR proteins were further verified at the transcriptomic level. In addition, single-cell data analysis enabled the identification of the positive MR proteins in both normal and asthmatic tissues. Finally, we explored the interactions between the identified proteins and the targets of current asthma medications.

Nephronectin (NPNT) has been reported in MR analyses of chronic obstructive pulmonary disease (COPD) and asthma, with an indirect effect of NPNT on COPD risk mediated by the FEV1/FVC ratio. Moreover, 24 proteins were reported as druggable targets in the drug gene interaction database, among which 8 were reported to interact with drugs. Zhao *et al.*,<sup>[16]</sup> whereas neuronal growth regulator 1 (NEGR1) and tumor necrosis factor, alpha-induced protein 3 (TNFAIP3) were first identified using this approach. We further conducted cross-omics validation to verify the functional roles of NPNT, NEGR1, and TNFAIP3, which constitute the added value of the present study.

## MATERIALS & METHODS

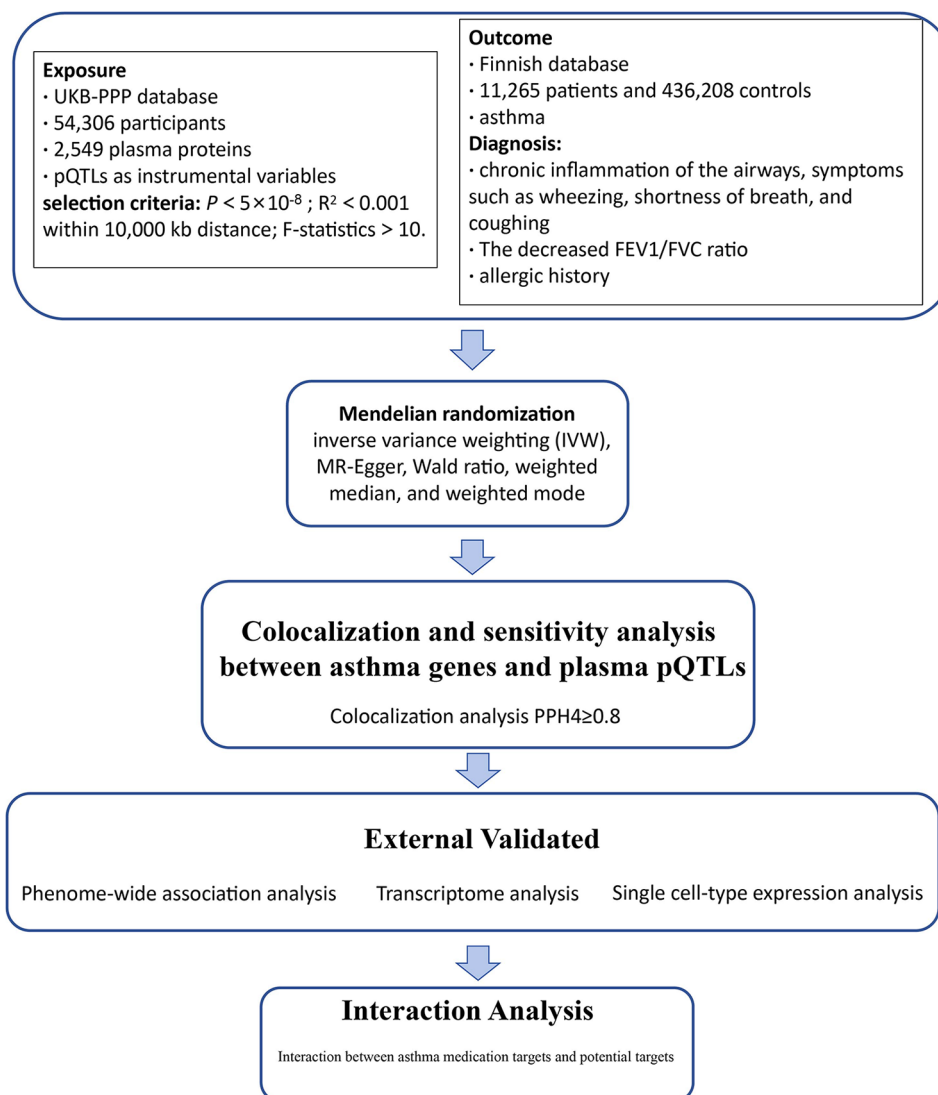
### Study design

In this study, we utilized pQTL data from a large-scale proteomic investigation to explore its associations with asthma through MR analysis. Subsequently, we validated the proteins identified as significant by MR using COLOC. We then performed PWAS analyses to assess potential side effects associated with these proteins and their suitability as therapeutic targets. Next, we employed transcriptomic analysis to evaluate genes linked to the positive MR findings. In addition, single-cell analysis further delineated the cell specificity of the genes related to these positive MR proteins. Finally, we investigated the interactions between the identified proteins and the targets of existing asthma medications. The workflow of this study is illustrated in Figure 1.

### Plasma pQTLs

Plasma pQTLs were extracted from the UKB-PPP database, which includes data on 2,923 plasma proteins. The genomic position of pQTLs in relation to specific proteins is typically located within proximity to their corresponding genes, with a set threshold of 1,000 kb. In this study, we used pQTLs as IVs based on the following criteria:

1. SNPs located within  $\pm 1$  Mb around the gene region.
2. A stringent threshold for highly correlated SNPs is defined as  $P < 5 \times 10^{-8}$ .



**Figure 1:** Study workflow- We conducted a comprehensive omics integration analysis to identify the plasma proteins and explored the potential causal proteins and drug targets for asthma diseases. We first identified proteins associations by Mendelian randomization, and further verified proteins with a potential causal role in asthma using colocalization. Then, we performed external validated using phenome-wide association, transcriptome analysis and single cell-type analysis. Finally, we conducted interaction analyses using protein-protein interaction network.

3. A linkage disequilibrium (LD) threshold of 0.001 for the LD parameter ( $r^2$ ) and a genetic distance of 10,000 kb to ensure the selection of independent SNPs and minimize the impact of LD.
4. An  $F > 10$ , which is a critical criterion for IVs to ensure that they are sufficiently strong to provide reliable estimates.

#### GWAS statistics of asthma

In this research, genetic data related to asthma were sourced from the Finnish database (finngen\_R12\_ALLERG\_

ASTHMA), which includes information from 11,265 patients and 436,208 controls. Asthma is a prevalent respiratory condition characterized by chronic inflammation of the airways, leading to symptoms such as wheezing, shortness of breath, and coughing.

#### MR analysis

We employed several methods – including inverse variance weighted (IVW), MR-Egger, Wald ratio, weighted median, and weighted mode – to evaluate the causal relationship between plasma proteins and asthma. These methods

were utilized to assess potential violations of horizontal pleiotropy. Our MR analysis used plasma proteins from the UK Biobank data as the exposure and asthma as the outcome. The IVW method estimates the linear causal effect of the exposure on the outcome by calculating the Wald ratio of the association between the genetic instruments and asthma, as well as the instruments and plasma proteins, within a meta-analysis framework with the intercept constrained to zero.<sup>[17]</sup> In contrast, the MR-Egger method does not constrain the intercept to pass through zero; instead, it provides a robust estimate of the causal effect after adjusting for horizontal pleiotropy.<sup>[18]</sup> The weighted median method calculates the median of the inverse-variance weighted ratio estimates, which is more resilient to outliers compared to IVW and MR-Egger. Lastly, the weighted mode method uses the mode of the inverse-variance weighted ratio estimates, offering greater power than MR-Egger, though it remains less powerful than the IVW and weighted median methods. We employed the false discovery rate (FDR) method for  $P$ -value correction;  $P_{\text{FDR}}$  below 0.05 was considered statistically significant, and  $P_{\text{FDR}}$  between 0.05 and 1 was considered suggestive statistically.<sup>[19]</sup>

### Colocalization analysis

Colocalization analysis aims to confirm the presence of shared causal genetic variants between the exposure and outcome. This analysis relied on a Bayesian model designed to evaluate five exclusivity hypotheses: (1) No correlation with any trait; (2) correlation restricted to trait 1; (3) correlation restricted to trait 2; (4) correlation with both traits driven by distinct causal variants; (5) correlation with both traits mediated by the same causal variant. Each hypothesis ( $H_0$ – $H_4$ ) yields a posterior probability from the test. We prioritized variants with a high colocalization posterior probability of hypothesis 4 ( $\text{PPH}_4 > 0.8$ ), and defined genes with a combined posterior probability ( $\text{PPH}_3 + \text{PPH}_4$ ) of 0.8 or higher as those with potential protein colocalization.<sup>[20]</sup> We conducted COLOC based on the positive MR results for proteins, examining SNPs within a  $\pm 1\text{MB}$  range upstream and downstream of the genes associated with these proteins and their colocalization with asthma.<sup>[21]</sup>

### Phenome-wide association study

PWAS is a method that explores associations between SNPs and a wide range of phenotypes across the entire phenome. This approach is particularly useful for investigating potential side effects associated with drug targets. In this study, we used IVW regression to systematically infer the causal effects of 2,272 plasma protein traits from the Finnish database, then tested all the significant associations ( $P < 0.05$ ) in an independent cohort. We utilized the positive MR results as

exposures and examined the outcomes,  $P_{\text{FDR}}$  value below 0.05 was considered statistically significant.<sup>[19,22]</sup>

### Transcriptome data

The potential causal effects of plasma protein levels on asthma were further evaluated using transcriptome data. The transcriptome dataset was obtained from the GEO database under accession number GSE167225. This dataset included transcriptome profiles along with patients' clinical information, comprising 10 normal samples and 14 asthma samples. We extracted gene expression levels from this dataset and performed differential expression analysis to identify genes that were differentially expressed between asthma and normal samples. Particular focus was placed on genes corresponding to proteins identified in the positive MR results. An absolute value of fold change (FC)  $> 2$  was considered to indicate significant expression,  $P < 0.05$  was considered to indicate a statistically significant difference. Statistical analysis was conducted using the Wald ratio test implemented in the R programming language.

### Single-cell type expression analysis

The target genes underlying the potential causal effects of plasma protein levels on asthma were further evaluated using single-cell RNA-seq data from GSE193816. We applied Harmony to integrate samples and performed downstream processes using Seurat. The genes were normalized and scaled using the NormalizeData and ScaleData. PCA was performed on the top 2,000 variable genes identified by FindVariableFeatures. We reduced the gene expression matrix to its first 20 principal components by FindClusters, then applied graph-based clustering with a resolution parameter of 0.3. The marker genes of each cell population were identified using “Wilcox” (Likelihood-ratio test) implemented in the FindAllMarkers function, with the following criteria: expression in  $\geq 10\%$  of cells within a cluster and an average log FC  $> 0.25$ . Differentially expressed genes between asthma and control subjects within each cell cluster were also identified using the Wilcoxon rank-sum test through FindAllMarkers, defined as genes with an absolute average log FC  $> 0.25$  and a  $P < 0.05$ .<sup>[23]</sup> We further visualized protein expression across clusters using the DotPlot function in the R programming language.

### Druggability evaluation through protein–protein interaction (PPI)

The potential targeted plasma proteins associated with asthma risk were analyzed using a PPI network.<sup>[24]</sup> Our goal was to explore the interactions between the targeted proteins identified through MR analysis and the current medication targets for asthma. We gathered information on current

drugs and their targets from the OpenTargets platform. We employed the search tool for the retrieval of interacting genes (STRING) to conduct the PPI analysis, using a threshold score of 0.4 for significance.

## RESULTS

### Plasma proteins related to allergic asthma

A total of 2,549 plasma proteins related to asthma were subjected to MR analysis using the IVW, MR-Egger, Wald ratio, weighted median, and weighted mode methods. Table S1 provides additional details on the selected SNPs. The results indicated that two plasma proteins were significantly associated with asthma risk ( $P_{\text{FDR}} < 0.05$ ), and one protein showed a suggestive statistical association with asthma risk ( $0.05 < P_{\text{FDR}} < 0.1$ ). Specifically, NPNT and NEGR1 were found to be statistically significantly associated with a noticeable protective effect against asthma. NPNT protective effect was estimated to be 0.768 (95% confidence interval [CI] = 0.671–0.879,  $P_{\text{FDR}} = 0.049$ ) using the IVW method, similar results were observed using weighted median [odds ratio [OR] (95%): 0.756 (0.654–0.873),  $P_{\text{FDR}} = 0.120$ ], using MR-Egger [OR (95%): 0.639 (0.475–0.859),  $P_{\text{FDR}} = 0.997$ ], and using weighted mode [OR (95%): 0.743(0.639–0.865),  $P_{\text{FDR}} = 0.910$ ]. NEGR1 showed a negative correlation with the risk of asthma as evidenced by IVW method (OR = 0.699, 95% CI = 0.584–0.836,  $P_{\text{FDR}} = 0.049$ ), and by Wald ratio method (OR = 0.668, 95% CI = 0.544–0.820,  $P_{\text{FDR}} = 0.068$ ), TNFAIP3 showed a suggestive association with asthma protective effect by Wald ratio method (OR = 0.647, 95% CI: 0.504–0.830). These results are summarized in Table 1.

### Colocalization and sensitivity analysis between asthma genes and plasma pQTLs

For the three plasma proteins NPNT, NEGR1, and TNFAIP3, we used the COLOC method to assess the possibility that the same SNP is responsible for both changing asthma risk and modulating the protein levels of the corresponding gene. Analyses were performed within a  $\pm 1$  MB window flanking each gene to explore potential associations with asthma. The results indicated that NPNT, NEGR1, and TNFAIP3 share a causal variant in this region (PPH4 > 0.8). NPNT

and NEGR1 showed strong evidence of colocalization with asthma associations, while TNFAIP3 exhibited a suggestive statistical association with a protective effect on asthma, as shown in Table 1. The funnel and scatter plots for NPNT and NEGR1 in this MR study are shown in Figure 2a-e. Leave-one-out analyses were conducted to evaluate the robustness of NPNT and NEGR1 [Figure 2c and f]. The consistency of the effect estimates after removing individual SNPs, with all remaining estimates lying on the same side of the null line, supports the robustness of the MR findings. No plots were generated for TNFAIP3 because only one SNP was available for analysis.

### Phenome-wide associations analysis of asthma for NPNT and NEGR1

PWAS was conducted to evaluate the potential effects of NPNT and NEGR1, which are associated with asthma, on other phenotypes [Figure 3]. We filtered the analysis to include 2,272 phenotypes from the Finnish database. Overall, no significant associations ( $P_{\text{FDR}} < 0.05$ ) were identified using the IVW method. However, the weighted median method revealed a significant causal association between NPNT and one asthma-related phenotype ( $P_{\text{FDR}} < 0.05$ ). Specifically, higher blood levels of NPNT were found to be beneficial for asthma-related acute respiratory infections (OR = 0.767, 95% CI: 0.680–0.864,  $P_{\text{FDR}} = 0.029$ ). A trend was also observed, indicating that higher blood NPNT levels may be advantageous for asthma-related infections (OR = 0.804, 95% CI: 0.723–0.894,  $P_{\text{FDR}} = 0.062$ ). Conversely, elevated blood levels of NEGR1 were associated with an increased risk of aphakia (OR = 6.218, 95% CI: 2.527–15.296,  $P_{\text{FDR}} = 0.078$ ). These results suggest a significant relationship between asthma and NPNT and NEGR1. Detailed information, including funnel plots, scatter plots, and leave-one-out test results from this MR-PWAS study, can be found in Table S2.

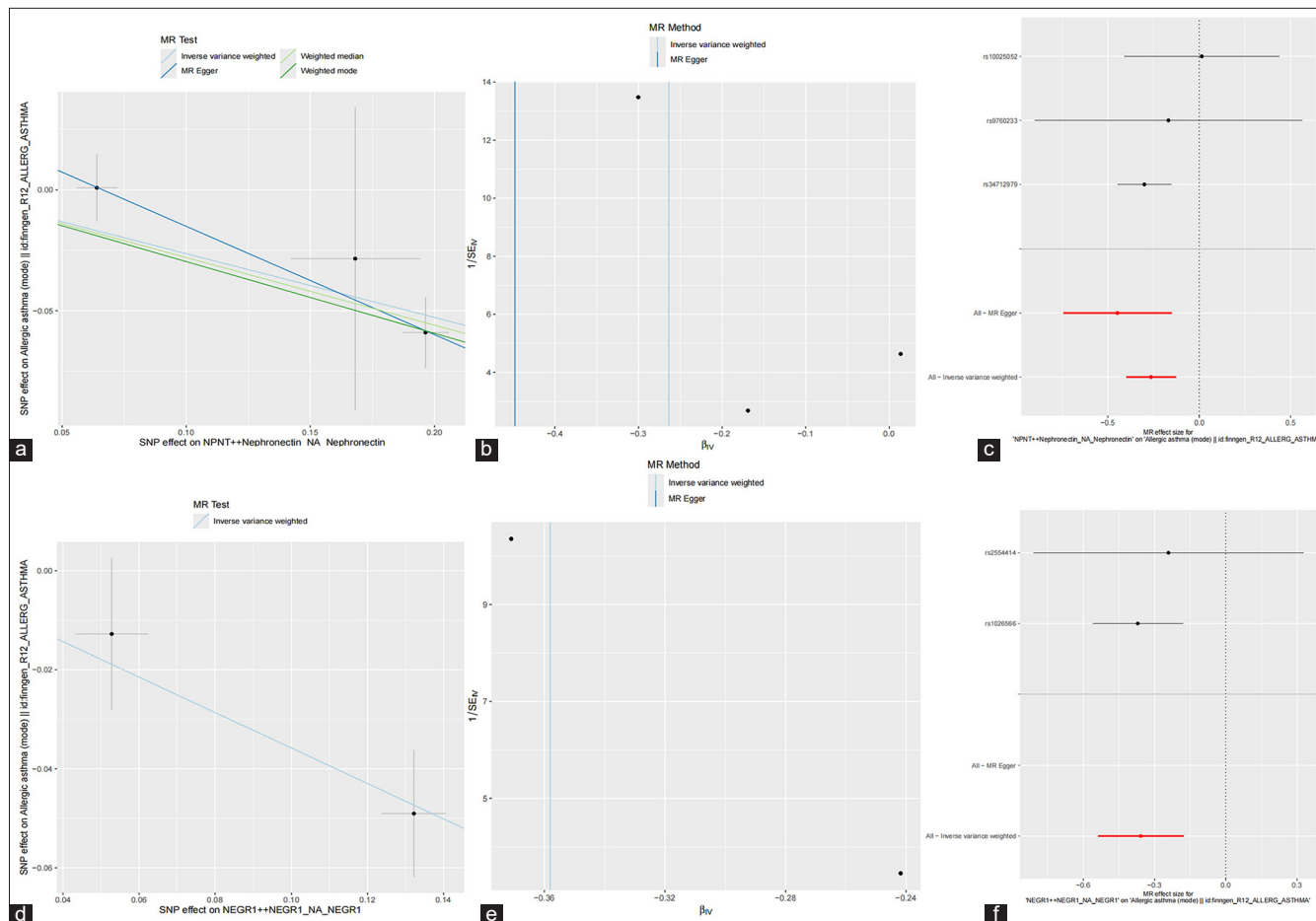
### The expression of NPNT and NEGR1 in transcriptome

The GSE167225 datasets from GEO. In asthma patients, the expression levels of NPNT (FC = -0.35,  $P = 0.809$ ) and NEGR1 (FC = -7.41,  $P = 0.293$ ) genes were decreased compared to healthy tissues. The expression of NEGR1 was significantly decreased in asthma, whereas the two proteins

**Table 1:** The exposure, method, OR, Beta, SE,  $P_{\text{FDR}}$  values and H4 for the Mendelian randomization analysis (MR).

Exposure	Method	OR(95% CI)	Beta	SE	$P_{\text{FDR}}$	PP4	PP4/(PP3+PP4)	Causal variant
NPNT	IVW	0.768(0.671 to 0.879)	-0.264	0.069	0.049	0.957	0.996	Yes
NEGR1	IVW	0.699(0.584 to 0.836)	-0.358	0.092	0.049	0.868	0.953	Yes
TNFAIP3	Wald ratio	0.647(0.504 to 0.830)	-0.436	0.127	0.080	0.810	0.941	Yes

This table shows the exposure, method, OR, Beta, SE,  $P_{\text{FDR}}$  values and H4 for the MR. For the two genes that were significant at  $P_{\text{FDR}}$  level in the asthma MR, the result of COLOC H4, which represent the Bayesian posterior probability that a genetic variant was shared by two traits. OR: odds ratio; CI: confidence interval; Beta:  $\beta$  regression coefficient; SE: Standard Error;  $P_{\text{FDR}}$ : p value False Discovery Rate.



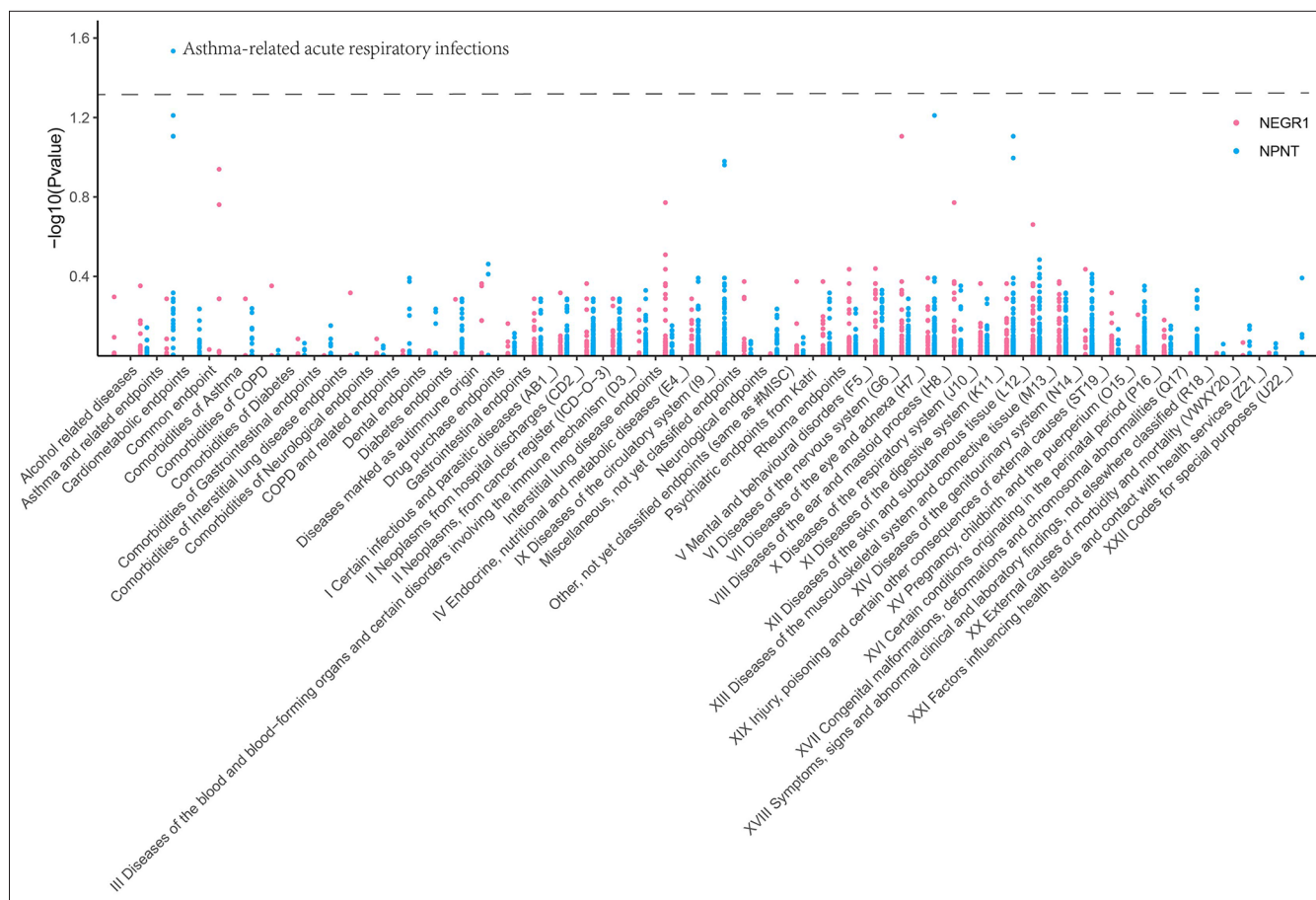
**Figure 2:** Sensitivity analysis of nephronectin (NPNT), neuronal growth regulator 1 (NEGR1) (a-c) NPNT was exposure, and asthma was outcome. (d-f) NEGR1 was exposure, and asthma was outcome. (a and d) Scatter plots for the *NPNT* and *NEGR1* genes. (b and e) Funnel plots for the *NPNT* and *NEGR1* genes. (c and f) The “leave-one-out” analyses for the *NPNT* and *NEGR1* genes.

showed no statistically significant difference. The result confirmed the MR conclusion of NPNT and NEGR1 at the transcriptome level [Figure 4].

### Cell-type specificity expression in the normal and asthma tissues

To investigate whether the genes encoding the 3 plasma proteins exhibit cell-type-specific enrichment in the normal and asthmatic lung tissues, we further performed single-cell analysis from GSE193816 datasets. 25 cell types were identified, including central memory T cell, CD8-positive alpha-beta T cell 1, club cell, goblet cell, intermediate monocyte cell, type II pneumocyte cell, smooth muscle cell, B cell, plasmacytoid dendritic cell, langerhans cell, helper T cell, suprabasal cell 1, CD8-positive alpha-beta T cell 2, lung ciliated cell 1, alpha-beta T cell, regulatory T cell, alveolar monocyte cell, myelocyte cell, CD4-positive alpha-beta T cell, CD16-positive natural killer cell, brush cell, lung ciliated cell cell 2, suprabasal cell 2, mast cell, plasmablast,

as showed in Figure 5a. Figure 5b-g showed the single-cell expression of the genes encoding the three plasma proteins (NPNT, NEGR1, and TNFAIP3) across all clusters in normal and asthmatic lung tissues. NPNT expression was higher in type II pneumocyte cell (FC = 1.26,  $P = 0.027$ ) in asthma tissue compared to normal tissue [Figure 5b-i]. The expression of NEGR1 was higher in smooth muscle cell (FC = 1.84,  $P = 0.016$ ), regulatory T cell (FC = 26.91,  $P = 0.005$ ), plasmacytoid dendritic cell (FC = 3.03,  $P = 0.020$ ), helper T cell (FC = 2.90,  $P = 0.040$ ) in asthma compared to normal tissue [Figure 5d-i]. TNFAIP3 was widely distributed in intermediate monocyte cell, helper T cell, smooth muscle cell, Langerhans cell, myelocyte cell, and many kinds of T cells, which included central memory T cell, regulatory T cell, CD8-positive alpha-beta T cell 1, CD8-positive alpha-beta T cell 2, CD4-positive alpha-beta T cell, and alpha-beta T cell. Moreover, the TNFAIP3 expression was lower in CD4-positive alpha-beta T cell (FC = 0.53,  $P = 0.013$ ), lung ciliated cell Cell 1 (FC = 0.51,  $P = 0.0001$ ), and smooth muscle cell (FC = 0.82,  $P = 0.011$ ), while higher in CD8-positive alpha-



**Figure 3:** Manhattan plot of phenome-wide Mendelian randomization results for blood nephronectin (NPNT) and neuronal growth regulator 1 (NEGR1). The data include 2,272 proteins phenotypes for NPNT and NEGR1.

beta T cell 1 (FC = 1.35,  $P = 0.005$ ), helper T cell (FC = 1.66,  $P = 0.003$ ), myelocyte cell (FC = 2.08,  $P = 0.025$ ) in asthma tissue compare to normal tissue [Figure 5f-i].

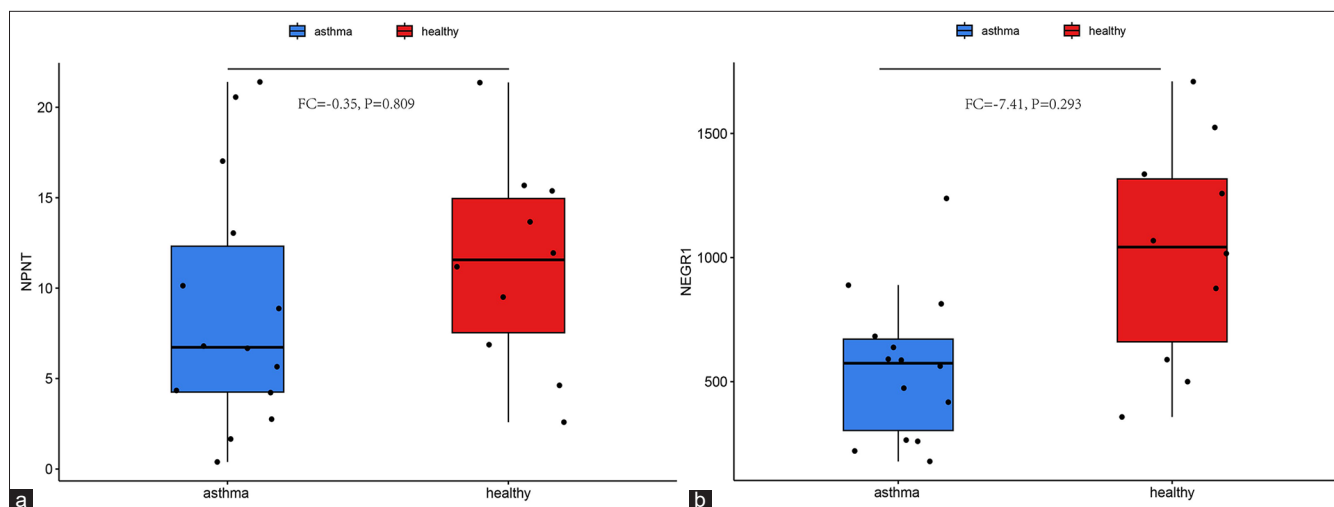
### The association of potential drug targets with asthma medications

The relationship between the prioritized proteins NPNT and NEGR1 and current asthma medication targets was analyzed through the PPI network. Among these interactions, the ITGA8-NPNT connection was identified as the most reliable by STRING. NPNT interacts with ITGA8, a marker for resident alveolar fibroblasts. Mesenchymal stromal cells (MSCs) exerted immunomodulatory effects to alleviate inflammation through interactions with components of both the innate and adaptive immune systems. This mechanism may contribute to the attenuation of asthma-related inflammation through the ITGA8-NPNT interaction in alveolar fibroblasts. In addition, the NEGR1-melanocortin-4 receptor (MC4R) interaction was also identified by STRING. The  $\alpha$ -Melanocortin stimulating hormone ( $\alpha$ -MSH) has been shown to reduce pro-inflammatory cytokines in several

pulmonary inflammatory disorders, including asthma.  $\alpha$ -MSH activates the MC4R, which interacts with NEGR1 [Figure 6].

### DISCUSSION

This study employed an integrated approach that combined MR, COLOC, PWAS analysis, transcriptome analysis, single-cell data analysis, and druggability evaluation to explore the causal effects of 2,923 plasma proteins on asthma risk. NPNT and NEGR1 were found to be statistically significantly associated with a noticeable protective effect against asthma. TNFAIP3 was statistically suggestive related to asthma protective effect in MR, all of which exhibited positive causal associations with asthma. Subsequent colocalization analysis suggested that NPNT and NEGR1 have greater potential to serve as drug targets for asthma treatment. PWAS analysis highlighted some potential side effects associated with therapies targeting NPNT or NEGR1. At the transcriptomic level, NPNT and NEGR1 were found to be downregulated in asthma patients compared to healthy individuals. Notably, NPNT expression was elevated in suprabasal cells, club cells,



**Figure 4:** (a) The gene expression of nephronectin in asthmatic and normal tissues in the GSE167225 dataset. (b) The gene expression of neuronal growth regulator 1 in asthmatic and normal tissues in the GSE167225 dataset.

type II pneumocytes, and goblet cells in asthmatic tissues compared to normal tissues. Similarly, NEGR1 expression was higher in lung ciliated cells in asthmatic tissues versus normal tissues. The inflammation associated with asthma was linked to the ITGA8-NPNT interaction in alveolar fibroblasts. In addition,  $\alpha$ -MSH activated the MC4R, which interacts with NEGR1. These findings suggest that NPNT and NEGR1 could be promising targets for current asthma medications.

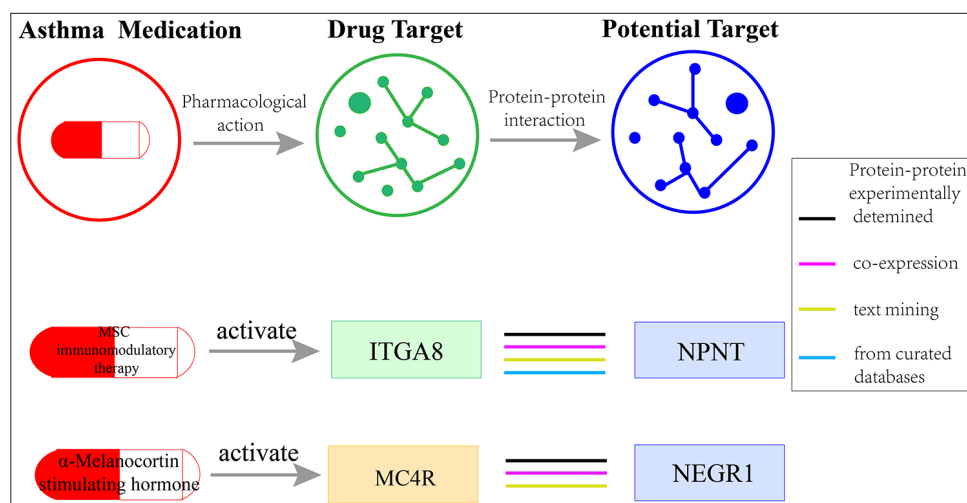
Our PPI network analysis identified predicted interactions involving NPNT-ITGA8 and NEGR1-MC4R, which may point to novel regulatory axes in respiratory infection susceptibility. While these associations are computationally inferred and require experimental confirmation, they are biologically plausible. NPNT is an extracellular matrix protein involved in tissue remodeling and integrin-mediated signaling, processes relevant to airway repair and immune cell recruitment during infection.<sup>[25]</sup> Similarly, NEGR1 has been implicated in cell adhesion and neuronal development, and emerging evidence suggests it may influence airway tone and inflammatory responses. Further functional studies are needed to determine whether these interactions directly modulate host defense against respiratory pathogens.

NPNT showed the strongest genetic associations with both altered lung function and COPD, encoding nephronectin.<sup>[26]</sup> This suggests that NPNT may be a lung function-related plasma protein, and it has been identified as a gene associated with the relative risk of COPD,<sup>[25,26]</sup> supporting our findings. NPNT is highly expressed in lung alveolar epithelial cells in both COVID-19-infected and uninfected samples.<sup>[27]</sup> NPNT is abundantly present in both fetal and adult lung tissue and alveolar epithelial cells and is involved in lung development and function.<sup>[28]</sup> NPNT

has been implicated in epithelial remodeling, to a potential major regulator of the progression of pulmonary fibrosis. Dependent on its interaction with ITGA3, NPNT exerts an inhibitory effect on the hyperactivation of large tumor suppressor kinase 1 (LATS1) and Mps one binder kinase activator 1 (MOB1), promotes the nuclear translocation of yes-associated protein 1 (YAP1), and prevents YAP1 from undergoing ubiquitination and subsequent degradation.<sup>[29]</sup> Our results identified that the ITGA8-NPNT interaction was reliable. Previous studies have indicated that ITGA8 is crucial for normal lung development and inhibiting inflammatory signaling,<sup>[30]</sup> MSCs utilize immunomodulatory therapy to attenuate inflammation through interactions with components of both the innate and adaptive immune systems.<sup>[31]</sup> In conclusion, the ITGA8-NPNT interaction in alveolar fibroblasts may offer therapeutic potential for alleviating asthma-related inflammation.

NEGR1 encodes the neuronal growth regulator 1 protein, it is considered related with fibro-adipogenic progenitor proliferation and immune cell infiltration in the early stage of driving diaphragm fibrosis during mechanical ventilation, reduced secretion of NEGR1 combined with leukocyte transendothelial migration activation resulted in the progressive loss of phrenic nerve terminals through inflammatory cell recruitment and apoptosis promotion during 24 h of mechanical ventilation in the diaphragm,<sup>[32]</sup> NEGR1-associated immune-metabolic cross-talk. A significant variant associated with COPD, identified as 1:71792134, is intragenic to the *NEGR1* gene.<sup>[33]</sup> The research has shown that seven SNPs are associated with obesity, but only rs517762 within the *NEGR1* gene remained significant after FDR correction. NEGR1 has also been implicated in both obesity and leptin dysfunction.<sup>[34]</sup> Our data suggest that NEGR1 may serve as a promising therapeutic target





**Figure 6:** Interaction between asthma medication targets and potential targets.

This study has several limitations. First, the cohorts used in our MR analysis consisted solely of individuals of the European descent, and there is a lack of information regarding non-European populations, existing single-ancestry bias. Second, the limited number of GWAS on asthma, along with constraints on cohort size, hindered our ability to conduct a replication study; these researches lack replication. Third, our analysis focused on plasma proteins as surrogates for airway biology; however, additional tissues could be taken into consideration, such as lung tissue or cells from bronchoalveolar lavage fluid. Fourth, although we applied strict inclusion criteria and statistical adjustments, residual heterogeneity within the cohort – such as different types of asthma, comorbidities, and prior antibiotic exposure – may still influence the associations observed. Future studies with more granular phenotypic data and larger sample sizes are needed to stratify analyses and validate our findings across clinically distinct subpopulations. Fifth, while MR provides evidence for causal relationships, it does not elucidate the underlying biological mechanisms. Functional studies – including *in vitro* assays, animal models, and detailed molecular profiling – are essential to understand how identified proteins such as NPNT, NEGR1, and TNFAIP3, mechanistically contribute to respiratory infection susceptibility. Experimental validation will help translate these genetic associations into actionable therapeutic targets. Future validation strategies could involve overexpression or knockout of NPNT and NEGR1 in mice and in relevant cell models. Such experimental validation would enhance our understanding of asthma pathophysiology in both *in vitro* and *in vivo* systems.

## CONCLUSION

This study employed an integrated approach that combined MR, COLOC, PWAS analysis, transcriptome analysis, single-

cell data analysis, and druggability evaluation to explore the causal effects of 2,923 plasma proteins on asthma risk. Ultimately, NPNT and NEGR1 could be promising targets for current asthma medications. NEGR1 was first identified in MR of asthma, and we further performed across-omics validation to verify the roles of NPNT, NEGR1 and TNFAIP3.

**Authors' contributions:** XC: Drafted and submitted the manuscript. YY: Conducted the research.

**Ethical approval:** Institutional Review Board approval is not required.

**Declaration of patient consent:** Patient's consent is not required as there are no patients in this study.

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