Journal of Inflammation Research

Dovepress
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ORIGINAL RESEARCH

# Causal Correlations Between Plasma Metabolites, Inflammatory Proteins, and Chronic Obstructive Pulmonary Disease: A Mendelian Randomization and Bioinformatics-Based Investigation

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**Background:** An increasing number of studies have demonstrated a strong correlation between metabolism, inflammation, and chronic obstructive pulmonary disease (COPD). However, it remains unclear if there is a causal relationship between these factors. This study employed the Mendelian randomization (MR) approach to investigate the associations between these factors and explore the mediating roles of key inflammatory proteins.

**Methods:** MR was used to assess the causal associations between plasma metabolites, inflammatory proteins, and COPD. Sensitivity analyses were performed to verify the robustness of the findings. Mediation analysis was conducted to explore the roles of inflammatory proteins in the metabolism-COPD pathway. We constructed protein-protein interaction (PPI) network and explored the potential mechanism through gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Single-cell sequencing and transcriptome datasets were used for auxiliary validation. Finally, experimental validation was performed using human lung tissue.

**Results:** This study identified 63 metabolites, 10 metabolite ratios, and 48 inflammatory proteins that were associated with COPD, all of which exhibited potential causal relationships. Furthermore, three proteins were identified as mediators in the metabolite-to-COPD pathway. PPI network, GO and KEGG enrichment analysis revealed the biological pathways in which they were involved. Validation of the expression of these three intermediary proteins in lung tissue demonstrated that NRXN3 was expressed in pulmonary endothelial cells and exerted a protective effect against COPD development.

**Conclusion:** The MR analysis revealed causal associations among metabolism, inflammation, and COPD. These findings offer novel insights into metabolism-inflammation-COPD mechanisms, suggesting that interventions targeting metabolic processes may represent a promising strategy for preventing the onset or progression of COPD.

**Keywords:** Mendelian randomization, chronic obstructive pulmonary disease, inflammation, metabolism, single-cell RNA-seq, transcriptomics

## Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory respiratory condition characterized by high morbidity and mortality rates, posing significant social and economic burdens.[1,2] In 2019, the World Health Organization identified COPD as the third leading cause of global mortality, highlighting the critical need to explore its pathogenesis.

In recent decades, extensive research has been conducted on the pathogenesis of COPD. With the emergence of metabolomics, researchers have increasingly focused on metabolic changes associated with COPD.[3] Previous studies have identified significant metabolic abnormalities in patients with COPD. For example, Fujii et al reported that the lipid

Received: 20 December 2024
Accepted: 14 March 2025
Published: 18 March 2025

composition of alveolar macrophages (AMs) changes in a severity-dependent manner in COPD.[4] These altered lipids include sphingolipids, cholesterol, phospholipids, and fatty acids, suggesting a role of lipid metabolism in the development and progression of COPD. Furthermore, AMs in individuals with COPD utilize aerobic glycolysis to rapidly produce cytokines while employing mitochondrial respiration to sustain inflammatory responses. These cells adapt their metabolic characteristics to local environments, undergoing metabolic reprogramming.[5] Chronic inflammatory stimuli and the release of specific inflammatory factors have been suggested to influence these metabolic processes. Such metabolic changes enhance the survival and functioning of lung immune cells, enabling adaptation to the chronic inflammatory environment.[6] Additionally, metabolites such as prostaglandins and leukotrienes, generated through these processes, mediate airway inflammation.[7] Alterations in metabolic processes and metabolite accumulation are thought to play critical roles in COPD pathogenesis; however, further research is needed to clarify their precise roles and regulatory mechanisms.

COPD is also characterized by abnormal changes in plasma inflammatory proteins. Its pathogenesis involves immune imbalance and inflammation mediated by various cytokines, inflammatory mediators, and inflammatory cells, including neutrophils and macrophages.[8] Neutrophils are pivotal in the inflammatory response via key cytokines, including interleukin (IL)-8 and leukotriene B4,[9] while monocyte-macrophage cytokines (CCL2 and CCL3) also play significant roles.[10] Circulating leukocyte counts and levels of C-reactive protein, IL-6, tumor necrosis factor (TNF), and fibrinogen are considered reliable markers of systemic inflammation.[11,12] Numerous studies have demonstrated the involvement of inflammatory cells, factors, and related proteins in the occurrence and progression of COPD; however, further comprehensive research is required to elucidate the specific mechanisms involved.[13]

The metabolic regulation of inflammatory factors and inflammation-related proteins in COPD, as well as their potential influence on metabolites, remains incompletely understood. Although some studies have reported that alterations in metabolic processes can stimulate the release of specific inflammatory factors, further investigation is required. Recent advancements in bioinformatics have enabled the use of Mendelian randomization (MR) to mitigate confounding factors from a genetic perspective, providing a more direct and accurate assessment of the association between exposure factors and outcomes. This is because genetic variations are randomly allocated at birth and are generally unaffected by confounding factors such as the environment, behavior, or socioeconomic status. Moreover, genetic variations precede the onset of diseases, which more clearly determine causal direction. This study aimed to investigate the causal relationships between various metabolites, inflammatory proteins, and COPD using MR. Additionally, it sought to explore the mediating roles of key inflammatory proteins between crucial metabolites and COPD while identifying relevant immunometabolic pathways.

# Materials and Methods

## Study Design

This study employed MR analysis to explore the causal relationship between plasma metabolites, inflammatory proteins, and COPD. Further details are provided in Figure 1. The MR method relies on three assumptions: (1) correlation: the genetic tools are strongly linked to the exposure; (2) independence: genetic variation is unrelated to confounding factors; and (3) exclusion restriction: genetic variations influence the outcome exclusively via the exposure, not through alternative mechanisms.[14]

## Data Sources

The genome-wide association study (GWAS) Catalog (https://www.ebi.ac.uk/gwas/) provides summary statistics for plasma metabolomics, accessible via the study accession numbers GCST90199621-GCST90201020. This dataset included 1,091 plasma metabolites and 309 metabolite ratios derived from a cohort of 8,299 individuals of European descent.

The protein quantitative trait loci were identified using the GWAS data on the levels of circulating protein expression from deCODE Genetics, which included 35,559 individuals of Icelandic descent and encompassed 4,907 proteins. We selected 5,886 genes related to inflammation and immunity from the human-related gene set (H, C1–C8) found on the

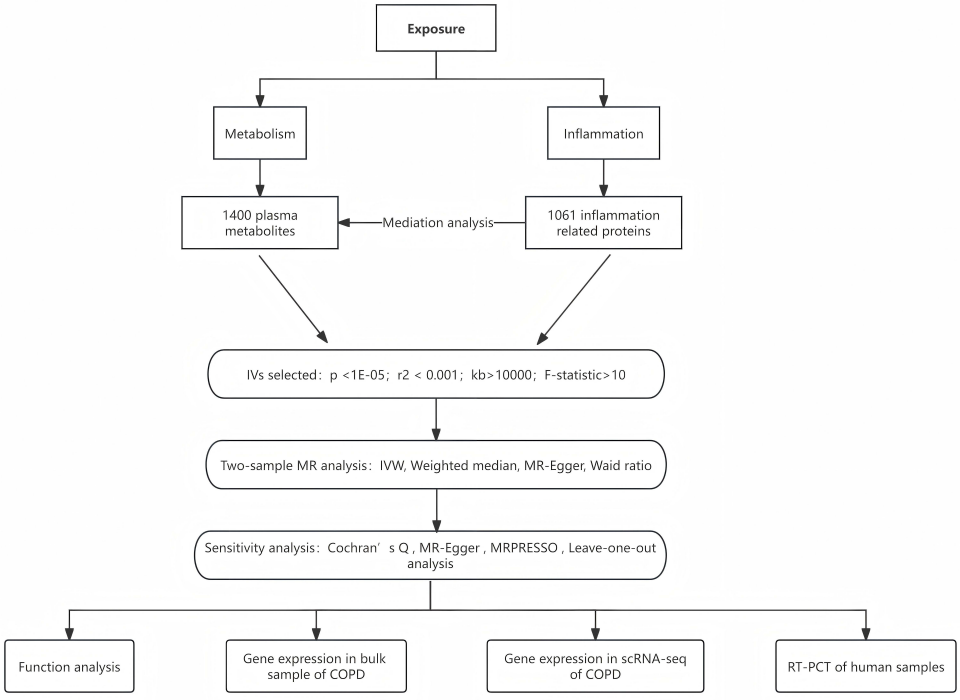



**Figure 1** Flow chart of the study.

Gene Set Enrichment Analysis website (https://www.gsea-msigdb.org/gsea) using "inflammation" and "immunity" as keywords (Table S1). The proteins obtained from these two steps were crossed, resulting in 925 proteins related to inflammation and immunity.[15]

Genetic information on COPD was retrieved from the FinnGen database, which included 18,266 European cases and 311,286 European controls, totaling 20,169,090 single nucleotide polymorphisms (SNPs). Genetic data for COPD are available at https://r9.finngen.fi/. For detailed information about the data, please refer to Table S2.

## Selection of Instrumental Variables

(1) For COPD and inflammatory proteins as exposures, a genome-wide significance threshold of $p < 5\times10^{-8}$ was applied to select potential instrumental variables (IVs) for each exposure trait. When metabolite data was used as the exposure factor, the p-value threshold was reduced to $1\times10^{-5}$ to include sufficient number of SNPs.[16] (2) SNPs significantly associated with the outcome variable ($p < 0.05$) were excluded. (3) To mitigate linkage disequilibrium effects, a clustering process was performed ($r^2 < 0.001$, window size = 10,000 kb) using the "clump_data" function in the "TwoSampleMR" software package.[17] (4) The F-statistic was used to assess IV exposure correlations. The F value for each SNP was calculated to determine the presence of weak IV bias. If F was > 10, the correlation was considered strong enough to avoid weak IV bias.[18] (5) IVs strongly correlated with the outcome were removed using Steiger filtering (Tables S3 and S4).

## MR Analysis and Sensitivity Analysis

These analyses were performed using the "Two-Sample MR" and "MRPRESSO" packages in R 4.1.0. The primary approach used inverse variance weighting to estimate the odds ratio and its 95% confidence interval (CI). Subsequently, to ensure a comprehensive analysis, we employed MR-Egger regression, the weighted median method, and the Wald ratio method.[19] To assess SNP heterogeneity, the Cochran's Q test was utilized and considered $p < 0.05$ as an indication of heterogeneity. Horizontal pleiotropy was assessed using the MR-Egger and MRPRESSO methods, and the data showing horizontal pleiotropy were excluded to ensure the credibility of the results.[19] We applied the Steiger test to demonstrate that the correlation between genetic variations and the exposure variable was significantly stronger than that

between genetic variations and the outcome variable, thereby mitigating concerns regarding reverse causality. To address multiple testing, the Benjamini-Hochberg method, incorporating the false discovery rate, was applied; however, all results with a raw p-value of less than 0.05 were accepted to capture as many potential positive results as possible.[20] Finally, to evaluate the influence of each SNP on metabolites or inflammatory proteins, a leave-one-out analysis was performed to remove relevant SNPs individually and calculate the combined effect of the remaining SNPs.[21]

## Mediation Analysis

Mediation analysis was used to explore the mediating role of inflammatory proteins in the process of metabolic influences on COPD development. This analysis focused on COPD-related inflammatory proteins. First, (A) represents the causal relationship between plasma metabolites and inflammatory proteins and was evaluated using a two-sample MR approach. Second, (B) represents the causal relationship between inflammatory proteins and COPD after correcting for the effect of plasma metabolites using multivariable MR. Two-step MR was used to calculate the mediating effect: mediating effect = β (A) × β (B). The total effect of metabolites on COPD was obtained from the first two MR Steps, and the direct effect was calculated as "total effect-mediation effect." The mediating proportion was calculated using the formula: mediating proportion = (mediating effect / total effect) × 100%. The delta method was used to estimate the 95% CI of the mediation effect and proportion of mediation. When exposure was causally associated with the outcome, mediation was causally associated with the outcome, and when exposure was causally associated with the mediator, the identified inflammatory proteins were considered potential mediators in the pathway from plasma metabolites to COPD. The identified inflammatory proteins were considered strong mediators when they fulfilled the above conditions and had mediating effects that were significantly different from zero.[22]

## Protein-Protein Interaction Network Construction and Functional Enrichment Analysis

The protein-protein interaction (PPI) network was introduced into the STRING database (https://www.string-db.org/), and the interaction results were imported into Cytoscape to build a PPI network map.[23] To identify the biological functions and signaling pathways of positive proteins, we employed the "clusterProfiler" package for enrichment analysis of GO terms and KEGG pathways using a p-value < 0.05 as the cutoff criterion.[24] Finally, identified proteins with mediating roles were imported into the GeneMANIA database for gene function prediction. Subsequently, proteins with shared functions were identified and a PPI network diagram was drawn for visualization.[25]

## Single-Cell RNA Sequencing Data Analysis

We obtained single-cell RNA sequencing (scRNA-seq) data from patients with COPD (GSE173896) from the GEO database (https://www.ncbi.nlm.nih.gov/geo/), which included lung tissue samples from nine patients with COPD, four smokers without COPD, and three never-smokers.[26] The publicly available datasets used in this study received ethical approval. The "Seurat" R package was used for scRNA-seq data analysis.[27] First, the number of cell features (nFeature_RNA) was set between 500 and 8,000, and the proportion of mitochondrial genes (percentage mt) was set below 20% to select high-quality cells. Dead and double cells (doublets) were excluded, and the DoubletFinder tool was used to further eliminate interference from bimodal cells. Subsequently, the data were log-normalized using the Seurat's normalized data function. Each sample was log-normalized and scaled based on the number of genes and the ratio of mitochondrial gene expression to ensure comparability and consistency.[28] For dimensionality reduction analysis, principal component analysis was performed in R using the Seurat package. To minimize differences caused by batch effects, a harmony algorithm was used to integrate the scRNA-seq data. Cluster analysis of single cells was performed using Seurat's FindNeighbours and FindCluster functions. Cell clustering results after dimension reduction were visualized using t-distributed random neighborhood embedding (t-SNE) and uniform manifold approximation and projection plots, generated using the RunTSNE and RunUMAP functions, respectively.[29] Clustering results were annotated and grouped based on the expression of specific tissue compartment marker genes. Finally, the expression patterns of these genes in various cell types were visualized using t-SNE and bubble plots.

## Bulk RNA Data Analysis

We used the keywords "chronic obstructive pulmonary disease (COPD)" from the GEO database to download all datasets related to COPD. We selected the GSE76925 dataset, which included 111 patients with COPD and 40 controls, and the GSE38974 dataset for validation, which included 23 patients and nine controls.[30,31] Differentially expressed genes in COPD were identified using the limma R package, setting $p < 0.05$ and $|\text{fold change (FC)}| > 0.1$ as the selection criteria.[32] Finally, the rank-sum test was used to compare gene expression differences between COPD and control samples.

## Human Sample Collection

All lung tissue samples were voluntarily donated. This study was conducted in accordance with the tenets of the Declaration of Helsinki, and written informed consent was obtained from all participants. Lung tissues from control individuals and patients with COPD were collected at Wuxi People's Hospital during surgery for lung nodules between November 2024 and December 2024. To control experimental error, we selected lung tissues that were laterally more than above 5 cm away from the benign nodules, confirmed using postoperative pathology assessment. All tissue samples were obtained from pathologically-confirmed non-diseased areas to ensure their representation of the normal or COPD lung tissue status. After obtaining the lung tissue in the operating room, it was frozen in liquid nitrogen and stored at −80°C. All samples were processed immediately within 6 hours after collection to ensure sample quality.[33] All the patients had complete clinical information, including age, sex, smoking history, lung function, and comorbidities. All patients with COPD met the 2019 GOLD criteria for COPD diagnosis, and the medical history of each participant is presented in Table 1.

**Table 1** Clinical Information of All Participants

| | Gender | Age | Smoking History (pack-years) | FEV1 (%) Predicted | FEV1/FVC (%) | Complication |
|---|---|---|---|---|---|---|
| Control individual 1 | Male | 71 | – | 91 | 88.1 | Pulmonary Nodule |
| Control individual 2 | Male | 37 | – | 109.4 | 90.3 | Pulmonary Nodule |
| Control individual 3 | Male | 45 | – | 102.3 | 88.9 | Pulmonary Nodule |
| Control individual 4 | Female | 64 | – | 83.5 | 84.4 | Pulmonary Nodule |
| Control individual 5 | Female | 60 | – | 95 | 87.3 | Pulmonary Nodule |
| Control individual 6 | Female | 36 | – | 82.4 | 78.89 | Pulmonary Nodule |
| Patient with COPD 1 | Male | 62 | 20 | 25.9 | 28.4 | Pulmonary Nodule, Hypertension |
| Patient with COPD 2 | Female | 64 | 20 | 19.3 | 31.7 | Pulmonary Nodule, Hypertension |
| Patient with COPD 3 | Male | 65 | 30 | 24 | 44.9 | Pulmonary Nodule, Diabetes Mellitus |
| Patient with COPD 4 | Male | 53 | 20 | 26 | 33.7 | Pulmonary Nodule |
| Patient with COPD 5 | Male | 72 | 40 | 27 | 49.7 | Pulmonary Nodule, Hypertension |
| Patient with COPD 6 | Male | 77 | 30 | 38.1 | 31.8 | Pulmonary Nodule, Hypertension |
| | | | **Control Individuals** | | **Patients with COPD** | |
| Number | | | 6 | | 6 | |
| Male, n (%) | | | 50 | | 83.3 | |

(*Continued*)


https://doi.org/10.2147/JIR.S513526

**Table 1** (Continued).

| | Gender | Age | Smoking History (pack-years) | FEV1 (%) Predicted | FEV1/FVC (%) | Complication |
|---|---|---|---|---|---|---|
| Age(years) | | | 52.20±19.15 | | 65.6±4.28 | |
| Smoking (pack-years) | | | 0 | | 26.67±8.16 | |
| FEV1% pred | | | 93.93±10.59 | | 26.72±7.17 | |
| FEV1/FVC (%) | | | 86.32±4.14 | | 36.7±8.52 | |

**Note**: Data are presented as means ± SD, unless otherwise stated.
**Abbreviations**: COPD, chronic obstructive pulmonary disease; FEV1, Forced Expiratory Volume in the first second; FVC, Forced Vital Capacity.

## RNA Extraction and Real-Time Polymerase Chain Reaction

Total RNA was isolated using RNAiso Plus (Takara; Shiga, Japan; 9108/9109), and 1 μg was reverse-transcribed into cDNA using HiScript III RT SuperMix for qPCR (+gDNA wiper) (Vazyme; Nanjing, China; R323-01). Real-time polymerase chain reaction (RT-PCR) was performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme; Nanjing, China; Q711-02) and an ABI 9600 RT-PCR detection system (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase was used as the internal control for mRNA expression.[34] Fold changes in gene expression were calculated using the 2-ΔΔCT method. Primer sequences are detailed in Table 2.[35]

# Results

## Causal Effects of Metabolites on COPD

Using MR, we analyzed 1,400 metabolites and metabolite ratios for causal associations with COPD. A total of 63 metabolites and 10 metabolite ratios were identified as having potential causal relationships with COPD (Figure 2A, C and Table S5). Neither the MR-Egger test nor the Cochran's Q test indicated significant horizontal pleiotropy or heterogeneity. These findings were validated through the leave-one-out analysis (Tables S6–S8). Furthermore, the Steiger test was used to prevent the potential influence of reverse causality (Table S9).

## Causal Effects of Inflammatory Proteins on COPD

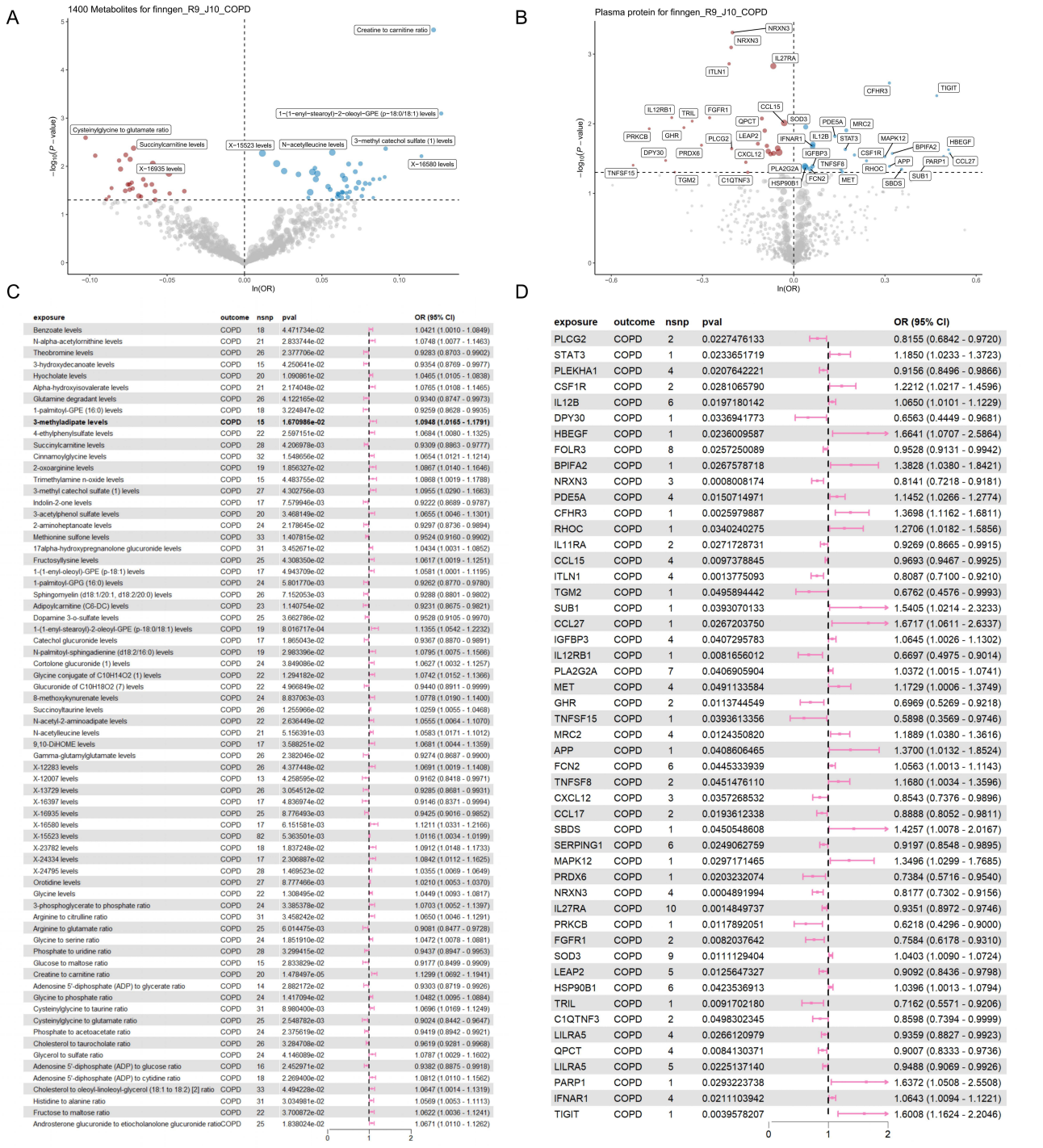
From the 925 inflammatory immune-related proteins, 48 proteins associated with COPD pathogenesis were screened (Figure 2B, D and Table S10). MR-Egger regression results revealed no horizontal pleiotropy, and the Cochran's Q test indicated no heterogeneity among IVs. The stability of the results was verified using the leave-one-out method (Tables S11–S13).

## Mediation Analysis of Potential Inflammatory Proteins

To explore the potential mechanisms underlying COPD development and progression, we performed mediation analysis to identify inflammatory and immune-related proteins that mediate the causal pathway from plasma metabolites to COPD. Four mediating relationships were identified (Tables S14–S18). The results indicate that CFHR3 may mediate the pathway from 1-methylnicotinamide levels to COPD (β = 0.054, 95% CI [−0.003, 0.11]), with a mediating proportion of 31.91% (95% CI: −1.79%, 65.61%), suggesting a positive mediating effect. Conversely, CFHR3 exhibited a negative mediating effect in the

**Table 2** Primer Sequences for NRXN3

| Genes | Sequence (5′ to 3′) | |
|---|---|---|
| NXNR3 | h-NRXN3-F1 | CATTGCAGTCGAGCTTGTCAAGG |
| | h-NRXN3-R1 | CCGAGTGATGACGACATTGTGC |




| exposure | outcome | nsnp | pval | OR (95% CI) |
|---|---|---|---|---|
| Benzoate levels | COPD | 18 | 4.471734e-02 | 1.0421 (1.0010 - 1.0849) |
| N-alpha-acetylornithine levels | COPD | 21 | 2.833744e-02 | 1.0748 (1.0077 - 1.1463) |
| Theobromine levels | COPD | 26 | 2.377706e-02 | 0.9283 (0.8703 - 0.9902) |
| 3-hydroxydecanoate levels | COPD | 15 | 4.250641e-02 | 0.9354 (0.8769 - 0.9977) |
| Hyocholate levels | COPD | 20 | 1.090861e-02 | 1.0465 (1.0105 - 1.0838) |
| Alpha-hydroxyisovalerate levels | COPD | 21 | 2.174048e-02 | 1.0765 (1.0108 - 1.1465) |
| Glutamine degradant levels | COPD | 26 | 4.122165e-02 | 0.9340 (0.8747 - 0.9973) |
| 1-palmitoyl-GPE (16:0) levels | COPD | 18 | 3.224847e-02 | 0.9259 (0.8628 - 0.9935) |
| **3-methyladipate levels** | **COPD** | **15** | **1.670986e-02** | **1.0948 (1.0165 - 1.1791)** |
| 4-ethylphenylsulfate levels | COPD | 22 | 2.597151e-02 | 1.0684 (1.0080 - 1.1325) |
| Succinylcarnitine levels | COPD | 28 | 4.206978e-03 | 0.9309 (0.8863 - 0.9777) |
| Cinnamoylglycine levels | COPD | 32 | 1.548656e-02 | 1.0654 (1.0121 - 1.1214) |
| 2-oxoarginine levels | COPD | 19 | 1.856327e-02 | 1.0887 (1.0140 - 1.1646) |
| Trimethylamine n-oxide levels | COPD | 15 | 4.483755e-02 | 1.0868 (1.0019 - 1.1788) |
| 3-methyl catechol sulfate (1) levels | COPD | 27 | 4.302756e-03 | 1.0955 (1.0290 - 1.1663) |
| Indolin-2-one levels | COPD | 17 | 7.579946e-03 | 0.9222 (0.8689 - 0.9787) |
| 3-acetylphenol sulfate levels | COPD | 20 | 3.468149e-02 | 1.0655 (1.0046 - 1.1301) |
| 2-aminoheptanoate levels | COPD | 24 | 2.178645e-02 | 0.9297 (0.8736 - 0.9894) |
| Methionine sulfone levels | COPD | 33 | 1.407815e-02 | 0.9524 (0.9160 - 0.9902) |
| 17alpha-hydroxypregnanolone glucuronide levels | COPD | 31 | 3.452671e-02 | 1.0434 (1.0031 - 1.0852) |
| Fructosyllysine levels | COPD | 25 | 4.308350e-02 | 1.0617 (1.0019 - 1.1251) |
| 1-(1-enyl-oleoyl)-GPE (p-18:1) levels | COPD | 17 | 4.943709e-02 | 1.0581 (1.0001 - 1.1195) |
| 1-palmitoyl-GPG (16:0) levels | COPD | 24 | 5.801770e-03 | 0.9262 (0.8770 - 0.9780) |
| Sphingomyelin (d18:1/20:1, d18:2/20:0) levels | COPD | 26 | 7.152053e-03 | 0.9288 (0.8801 - 0.9802) |
| Adipoylcarnitine (C6-DC) levels | COPD | 23 | 1.140754e-02 | 0.9231 (0.8675 - 0.9821) |
| Dopamine 3-o-sulfate levels | COPD | 25 | 3.662786e-02 | 0.9528 (0.9105 - 0.9970) |
| 1-(1-enyl-stearoyl)-2-oleoyl-GPE (p-18:0/18:1) levels | COPD | 19 | 8.016717e-04 | 1.1355 (1.0542 - 1.2232) |
| Catechol glucuronide levels | COPD | 17 | 1.865043e-02 | 0.9367 (0.8870 - 0.9891) |
| N-palmitoyl-sphingadienine (d18:2/16:0) levels | COPD | 19 | 2.983396e-02 | 1.0795 (1.0075 - 1.1566) |
| Cortolone glucuronide (1) levels | COPD | 24 | 3.849086e-02 | 1.0627 (1.0032 - 1.1257) |
| Glycine conjugate of C10H14O2 (1) levels | COPD | 22 | 1.294182e-02 | 1.0742 (1.0152 - 1.1366) |
| Glucuronide of C10H18O2 (7) levels | COPD | 22 | 4.966849e-02 | 0.9440 (0.8911 - 0.9999) |
| 8-methoxykynurenate levels | COPD | 24 | 8.837063e-03 | 1.0778 (1.0190 - 1.1400) |
| Succinoyltaurine levels | COPD | 26 | 1.255966e-02 | 1.0259 (1.0055 - 1.0468) |
| N-acetyl-2-aminoadipate levels | COPD | 22 | 2.636449e-02 | 1.0555 (1.0064 - 1.1070) |
| N-acetylleucine levels | COPD | 21 | 5.156391e-03 | 1.0583 (1.0171 - 1.1012) |
| 9,10-DiHOME levels | COPD | 17 | 3.588251e-02 | 1.0681 (1.0044 - 1.1359) |
| Gamma-glutamylglutamate levels | COPD | 26 | 2.382046e-02 | 0.9274 (0.8687 - 0.9900) |
| X-12283 levels | COPD | 26 | 4.377448e-02 | 1.0691 (1.0019 - 1.1408) |
| X-12007 levels | COPD | 13 | 4.258595e-02 | 0.9162 (0.8418 - 0.9971) |
| X-13729 levels | COPD | 26 | 3.054512e-02 | 0.9285 (0.8681 - 0.9931) |
| X-16397 levels | COPD | 17 | 4.836974e-02 | 0.9146 (0.8371 - 0.9994) |
| X-16935 levels | COPD | 25 | 8.776493e-03 | 0.9425 (0.9016 - 0.9852) |
| X-16580 levels | COPD | 17 | 6.151581e-03 | 1.1211 (1.0331 - 1.2166) |
| X-15523 levels | COPD | 82 | 5.363501e-03 | 1.0116 (1.0034 - 1.0199) |
| X-23782 levels | COPD | 18 | 1.837249e-02 | 1.0912 (1.0148 - 1.1733) |
| X-24334 levels | COPD | 17 | 2.306887e-02 | 1.0842 (1.0112 - 1.1625) |
| X-24795 levels | COPD | 28 | 1.469523e-02 | 1.0355 (1.0069 - 1.0649) |
| Orotidine levels | COPD | 27 | 8.777466e-03 | 1.0210 (1.0053 - 1.0370) |
| Glycine levels | COPD | 22 | 1.308495e-02 | 1.0449 (1.0093 - 1.0817) |
| 3-phosphoglycerate to phosphate ratio | COPD | 24 | 3.385378e-02 | 1.0703 (1.0052 - 1.1397) |
| Arginine to citrulline ratio | COPD | 31 | 3.458242e-02 | 1.0650 (1.0046 - 1.1291) |
| Arginine to glutamate ratio | COPD | 25 | 6.014475e-03 | 0.9081 (0.8477 - 0.9728) |
| Glycine to serine ratio | COPD | 24 | 1.851910e-02 | 1.0472 (1.0078 - 1.0881) |
| Phosphate to uridine ratio | COPD | 28 | 3.299415e-02 | 0.9437 (0.8947 - 0.9953) |
| Glucose to maltose ratio | COPD | 15 | 2.833829e-02 | 0.9177 (0.8499 - 0.9909) |
| Creatine to carnitine ratio | COPD | 20 | 1.478497e-05 | 1.1299 (1.0692 - 1.1941) |
| Adenosine 5'-diphosphate (ADP) to glycerate ratio | COPD | 14 | 2.882172e-02 | 0.9303 (0.8719 - 0.9926) |
| Glycine to phosphate ratio | COPD | 24 | 1.417094e-02 | 1.0482 (1.0095 - 1.0884) |
| Cysteinylglycine to taurine ratio | COPD | 31 | 8.980400e-03 | 1.0696 (1.0169 - 1.1249) |
| Cysteinylglycine to glutamate ratio | COPD | 25 | 2.548782e-03 | 0.9024 (0.8442 - 0.9647) |
| Phosphate to acetoacetate ratio | COPD | 24 | 2.375619e-02 | 0.9419 (0.8942 - 0.9921) |
| Cholesterol to taurocholate ratio | COPD | 26 | 3.284708e-02 | 0.9619 (0.9281 - 0.9968) |
| Glycerol to sulfate ratio | COPD | 24 | 4.146089e-02 | 1.0787 (1.0029 - 1.1602) |
| Adenosine 5'-diphosphate (ADP) to glucose ratio | COPD | 16 | 2.452971e-02 | 0.9382 (0.8875 - 0.9918) |
| Adenosine 5'-diphosphate (ADP) to cytidine ratio | COPD | 18 | 2.269400e-02 | 1.0812 (1.0110 - 1.1562) |
| Cholesterol to oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] ratio | COPD | 33 | 4.494228e-02 | 1.0647 (1.0014 - 1.1319) |
| Histidine to alanine ratio | COPD | 31 | 3.034981e-02 | 1.0569 (1.0053 - 1.1113) |
| Fructose to maltose ratio | COPD | 22 | 3.700872e-02 | 1.0622 (1.0036 - 1.1241) |
| Androsterone glucuronide to etiocholanolone glucuronide ratio | COPD | 25 | 1.838024e-02 | 1.0671 (1.0110 - 1.1262) |

| exposure | outcome | nsnp | pval | OR (95% CI) |
|---|---|---|---|---|
| PLCG2 | COPD | 2 | 0.0227476133 | 0.8155 (0.6842 - 0.9720) |
| STAT3 | COPD | 1 | 0.0233651719 | 1.1850 (1.0233 - 1.3723) |
| PLEKHA1 | COPD | 4 | 0.0207642221 | 0.9156 (0.8496 - 0.9866) |
| CSF1R | COPD | 2 | 0.0281065790 | 1.2212 (1.0217 - 1.4596) |
| IL12B | COPD | 6 | 0.0197180142 | 1.0650 (1.0101 - 1.1229) |
| DPY30 | COPD | 1 | 0.0336941773 | 0.6563 (0.4449 - 0.9681) |
| HBEGF | COPD | 1 | 0.0236009587 | 1.6641 (1.0707 - 2.5864) |
| FOLR3 | COPD | 8 | 0.0257250089 | 0.9528 (0.9131 - 0.9942) |
| BPIFA2 | COPD | 1 | 0.0267578718 | 1.3828 (1.0380 - 1.8421) |
| NRXN3 | COPD | 3 | 0.0008008174 | 0.8141 (0.7218 - 0.9181) |
| PDE5A | COPD | 4 | 0.0150714971 | 1.1452 (1.0266 - 1.2774) |
| CFHR3 | COPD | 1 | 0.0025979887 | 1.3698 (1.1162 - 1.6811) |
| RHOC | COPD | 1 | 0.0340240275 | 1.2706 (1.0182 - 1.5856) |
| IL11RA | COPD | 2 | 0.0271728731 | 0.9269 (0.8665 - 0.9915) |
| CCL15 | COPD | 4 | 0.0097378845 | 0.9693 (0.9467 - 0.9925) |
| ITLN1 | COPD | 4 | 0.0013775093 | 0.8087 (0.7100 - 0.9210) |
| TGM2 | COPD | 1 | 0.0495894442 | 0.6762 (0.4576 - 0.9993) |
| SUB1 | COPD | 1 | 0.0393070133 | 1.5405 (1.0214 - 2.3233) |
| CCL27 | COPD | 1 | 0.0267203750 | 1.6717 (1.0611 - 2.6337) |
| IGFBP3 | COPD | 4 | 0.0407295783 | 1.0645 (1.0026 - 1.1302) |
| IL12RB1 | COPD | 1 | 0.0081656012 | 0.6697 (0.4975 - 0.9014) |
| PLA2G2A | COPD | 7 | 0.0406905904 | 1.0372 (1.0015 - 1.0741) |
| MET | COPD | 4 | 0.0491133584 | 1.1729 (1.0006 - 1.3749) |
| GHR | COPD | 2 | 0.0113744549 | 0.6969 (0.5269 - 0.9218) |
| TNFSF15 | COPD | 1 | 0.0393613356 | 0.5898 (0.3569 - 0.9746) |
| MRC2 | COPD | 4 | 0.0124350820 | 1.1889 (1.0380 - 1.3616) |
| APP | COPD | 1 | 0.0408606465 | 1.3700 (1.0132 - 1.8524) |
| FCN2 | COPD | 6 | 0.0445333939 | 1.0563 (1.0013 - 1.1143) |
| TNFSF8 | COPD | 2 | 0.0451476110 | 1.1680 (1.0034 - 1.3596) |
| CXCL12 | COPD | 3 | 0.0357268532 | 0.8543 (0.7376 - 0.9896) |
| CCL17 | COPD | 2 | 0.0193612338 | 0.8888 (0.8052 - 0.9811) |
| SBDS | COPD | 1 | 0.0450548608 | 1.4257 (1.0078 - 2.0167) |
| SERPING1 | COPD | 6 | 0.0249062759 | 0.9197 (0.8548 - 0.9895) |
| MAPK12 | COPD | 1 | 0.0297171465 | 1.3496 (1.0299 - 1.7685) |
| PRDX6 | COPD | 1 | 0.0203232074 | 0.7384 (0.5716 - 0.9540) |
| NRXN3 | COPD | 4 | 0.0004891994 | 0.8177 (0.7302 - 0.9156) |
| IL27RA | COPD | 10 | 0.0014849737 | 0.9351 (0.8972 - 0.9746) |
| PRKCB | COPD | 1 | 0.0117892051 | 0.6218 (0.4296 - 0.9000) |
| FGFR1 | COPD | 2 | 0.0082037642 | 0.7584 (0.6178 - 0.9310) |
| SOD3 | COPD | 9 | 0.0111129404 | 1.0403 (1.0090 - 1.0724) |
| LEAP2 | COPD | 5 | 0.0125647327 | 0.9092 (0.8436 - 0.9798) |
| HSP90B1 | COPD | 6 | 0.0423536913 | 1.0396 (1.0013 - 1.0794) |
| TRIL | COPD | 1 | 0.0091702180 | 0.7162 (0.5571 - 0.9206) |
| C1QTNF3 | COPD | 2 | 0.0498302345 | 0.8598 (0.7394 - 0.9999) |
| LILRA5 | COPD | 4 | 0.0266120979 | 0.9359 (0.8827 - 0.9923) |
| QPCT | COPD | 4 | 0.0084130371 | 0.9007 (0.8333 - 0.9736) |
| LILRA5 | COPD | 5 | 0.0225137140 | 0.9488 (0.9069 - 0.9926) |
| PARP1 | COPD | 1 | 0.0293223738 | 1.6372 (1.0508 - 2.5508) |
| IFNAR1 | COPD | 4 | 0.0211103942 | 1.0643 (1.0094 - 1.1221) |
| TIGIT | COPD | 1 | 0.0039578207 | 1.6008 (1.1624 - 2.2046) |

**Figure 2** MR analysis illustrating the causal association of metabolites and inflammatory-related proteins with COPD. (**A**) The volcano plot displays the causal association between 63 metabolites, 10 metabolite ratios, and COPD; (**B**) The volcano plot displays the causal association between 48 inflammatory-related proteins and COPD; (**C**) The forest plot presents the causal association between 63 metabolites, 10 metabolite ratios, and COPD; and (**D**) The forest plot presents the causal association between 48 inflammatory-related proteins and COPD.

pathway from Uridine to cytidine ratio to COPD (β= −0.053, 95% CI [−0.111, 0.005]), with a mediating proportion of 17.69% (95% CI: 37.03%, −1.65%). In addition, NRXN3 played a positive mediating role in the Arachidonate (20:4n6) to pyruvate ratio pathway to COPD (β= 0.034, 95% CI [−0.002, 0.071]), with a mediating ratio of 29.80% (95% CI: −2.06%, 61.65%). ITLN1 negatively mediated the phosphate to N-acetylneuraminate ratio pathway to COPD (β= −0.034, 95% CI [−0.069, 0.002]), with

**Table 3** Inflammatory-Related Proteins as Intermediates in Causal Effects of Metabolites on COPD

| Exposure | $\beta_{e-i}$ | Intermediate | $\beta_{i-o}$ | Outcome | $\beta_{e-o}$ | β | Intermediate Ratio (%) |
|---|---|---|---|---|---|---|---|
| 1-methylnicotinamide levels | 0.17 | CFHR3 | 0.315 | COPD | 0.168 | 0.054 | 31.91 |
| Arachidonate (20:4n6) to pyruvate ratio | −0.166 | NRXN3 | −0.206 | COPD | 0.115 | 0.034 | 29.795 |
| Phosphate to N-acetylneuraminate ratio | 0.158 | ITLN1 | −0.212 | COPD | −0.199 | −0.034 | 16.91 |
| Uridine to cytidine ratio | −0.168 | CFHR3 | 0.315 | COPD | −0.3 | −0.053 | 17.691 |

a mediating proportion of 16.91% (95% CI: 34.75%, −0.93%). The results are presented in Tables 3 and S19. The causal relationship between plasma metabolites and inflammatory and immune-related proteins in the mediating pathway is shown in Figure S1.

## Construction of the PPI Network and GO/KEGG Analysis

We focused on 48 positive genes and used the STRING database to construct a PPI network comprised of 32 nodes and 65 edges (Figure 3A). Subsequently, the three mediating proteins, ITLN1, NRXN3, and CFHR3, were imported into the GeneMANIA database to predict gene functions. The interaction results were visualized in Cytoscape and a PPI network comprising 23 nodes and 226 edges was constructed (Figure 3B). Functional prediction suggested that these proteins play important roles in humoral immune response, complement activation, intraspecies biological processes, and inter-organism interactions. GO and KEGG pathway enrichment analyses, conducted using the "Cluster Analyzer" package in R, further explored the potential biological functions and mechanisms of the 48 positive genes. A total of 687 GO entries revealed 607 biological processes (BP), 53 molecular functions (MF), and 27 cellular components (CC). In terms of BP, core genes were enriched in cytokine-mediated signaling pathways, positive regulation of the mitogen-activated protein kinase cascade, and positive regulation of IL−10 production. For MF, core genes were involved in receptor-ligand activity, cytokine receptor binding, and cytokine activity. Regarding CC localization, the core genes were predominantly enriched in the external side of the plasma membrane and the collagen-containing extracellular matrix (Figure 3C). KEGG pathway analysis identified 62 pathways, including Cytokine−Cytokine receptor interaction, as key areas of involvement (Figure 3D). Additional details can be found in Tables S20 and S21.

## Single-Cell Analysis Results

We obtained a single-cell sequencing dataset (GSE173896) from the GEO database, which comprised lung tissue samples from nine patients with COPD, four healthy smokers, and three never-smokers. After data processing, cells were successfully divided into 13 subsets: B cells, basophils, endothelial cells, epithelial cells, fibroblasts, macrophages, mast cells, monocytes, neutrophils, T/NK cells, plasmacytoid dendritic cells, pericytes/smooth muscle cells, and proliferating immune cells (Figure 4A). Expression analysis showed that ITLN1 and CFHR3 were minimally expressed in lung tissues, whereas NRXN3 was highly expressed (Figure 4B–D), predominantly in endothelial and epithelial cells (Figure 4E). Notably, NRXN3 expression was downregulated in COPD lung tissues, with this trend appearing to correlate with smoking. Expression trends of NRXN3 in disease states was validated using transcriptome data and PCR technology (below). Additionally, endothelial cells were grouped and annotated based on the expression patterns of related marker genes. NRXN3 was most densely distributed in VE−capillary B cells, followed by VE−arteria, VE −capillary A, VE−vein, and VE-lymphatic cells (Figure 4F).

## Bulk RNA Analysis Results

We selected GSE 76925 and GSE38974 datasets, with GSE38974 as the training set, and used the limma package to conduct differential gene expression analysis between healthy individuals and patients with COPD. The threshold was set as $p < 0.05$ and $LogFC > 0.1$. A significant down-regulation of NRXN3 was observed in patients with

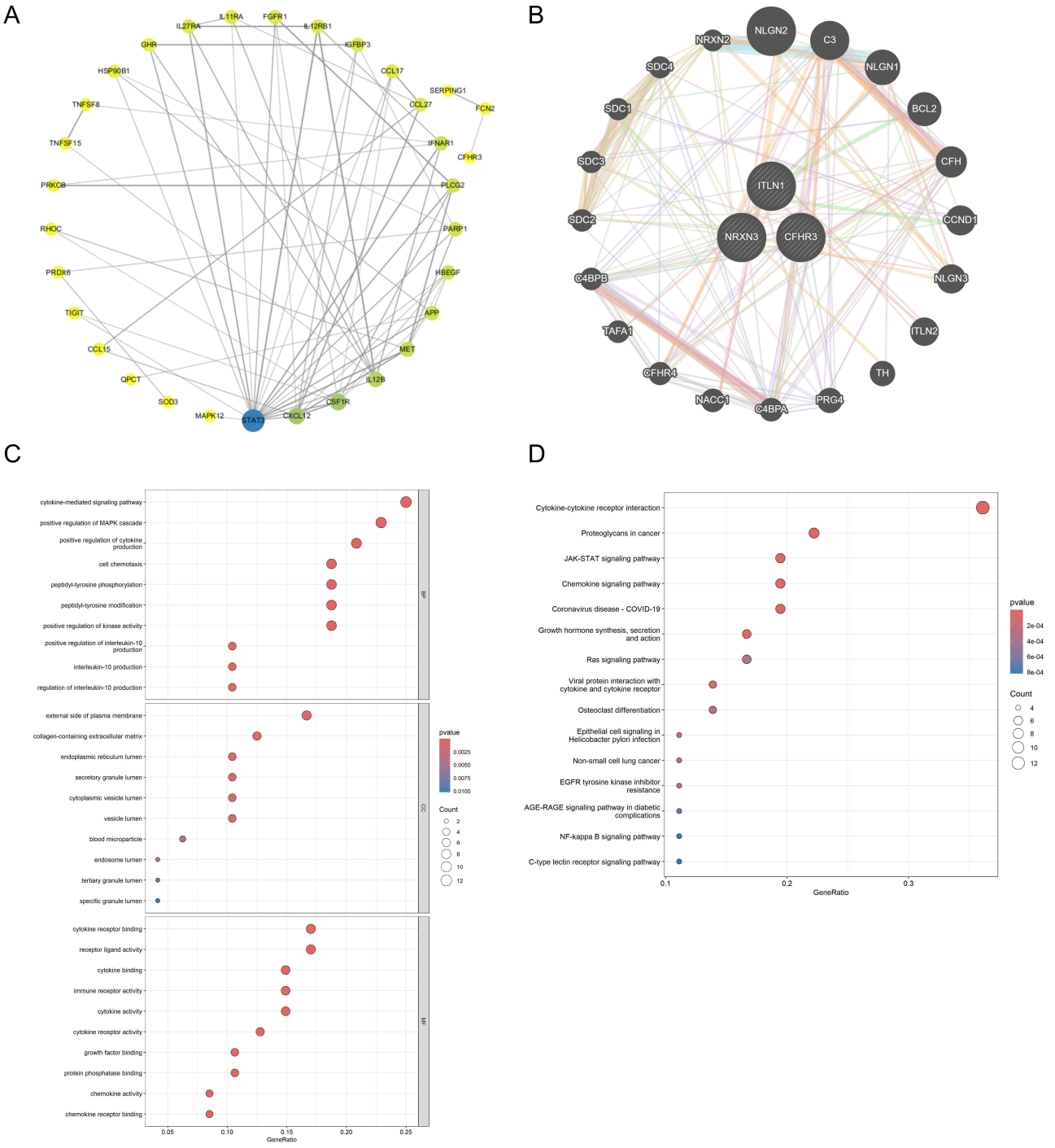



**Figure 3** Construction of PPI network and GO and KEGG analyses. (**A**) PPI network of positive proteins. (**B**) PPI network of intermediates. (**C**) Results of GO enrichment analysis. (**D**) Results of KEGG enrichment analysis.

COPD (Table S22). We performed a Wilcoxon rank-sum test to visualize gene expression differences between patients with COPD and healthy controls across both datasets. NRXN3 was significantly downregulated in COPD lung tissue, aligning with the MR (Figure 5).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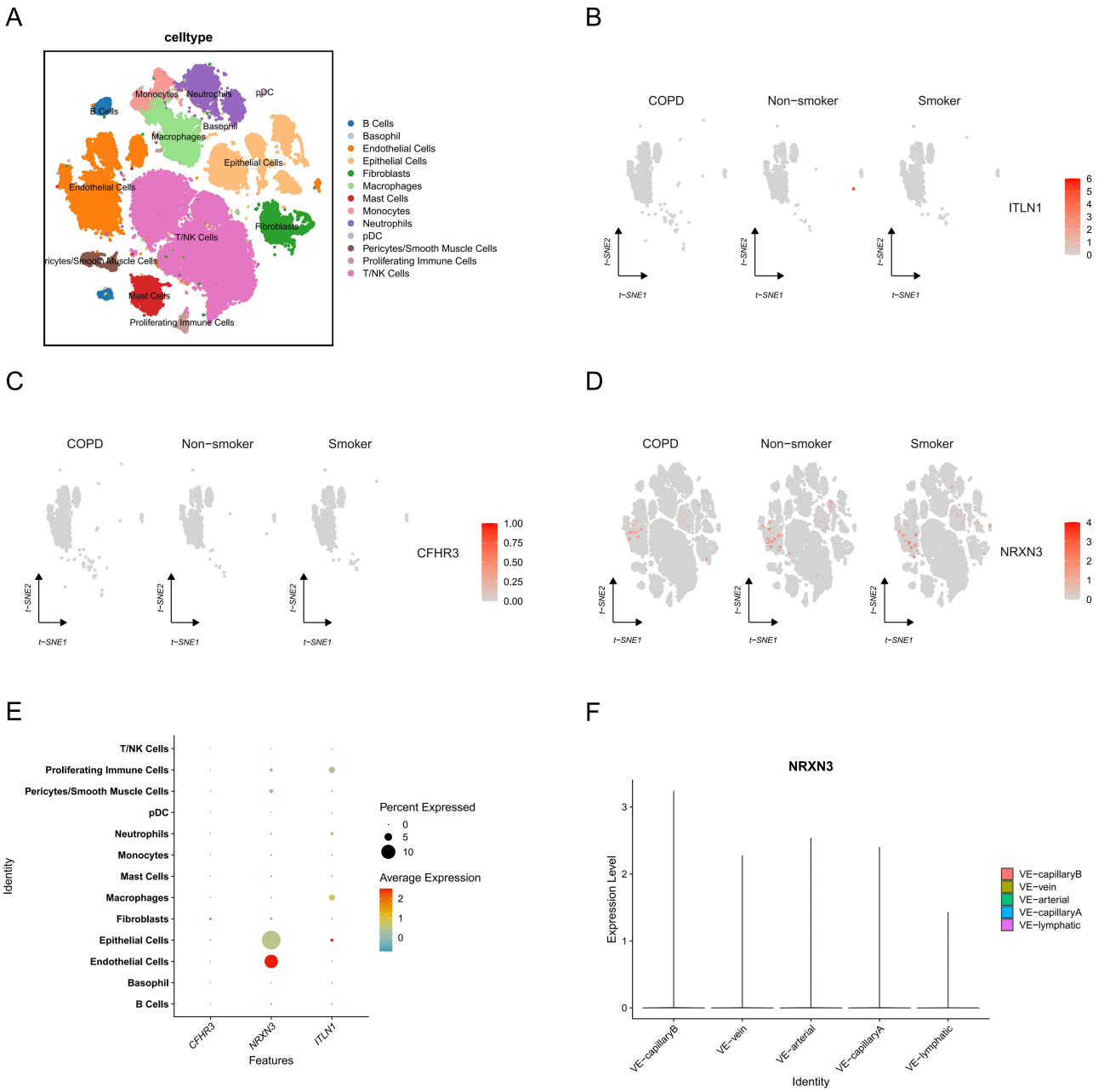


**Figure 4** Validation of inflammatory mediator gene expression in single-cell sequencing datasets. (**A**) t-Distributed Stochastic Neighbor Embedding (t-SNE) clustering map of human lung tissues in the GSE173896 database; (**B**) Expression of ITLN1 in each cell type; (**C**) Expression of CFHR3 in each cell type; (**D** and **E**) Expression of NRXN3 in each cell type; and (**F**) Expression of NRXN3 in each endothelial cell type.

## Validation of NRXN3 by RT- PCR in Human Tissues

RT-PCR analysis of lung tissues from healthy individuals and patients with COPD confirmed a significant reduction in NXNR3 expression in COPD lung tissues, consistent with data analysis findings (Figure 6).

# Discussion

In this study, we used MR to explore the causal relationships between circulating metabolites, inflammatory proteins, and COPD, alongside the mediating role of inflammatory proteins in the relationship between metabolism and COPD. We identified 63 metabolites, 10 metabolite ratios, and 48 inflammatory proteins with potential causal associations with COPD. In addition, we identified three proteins (NRXN3, CFHR3, and ITLN1) that play mediating roles in the

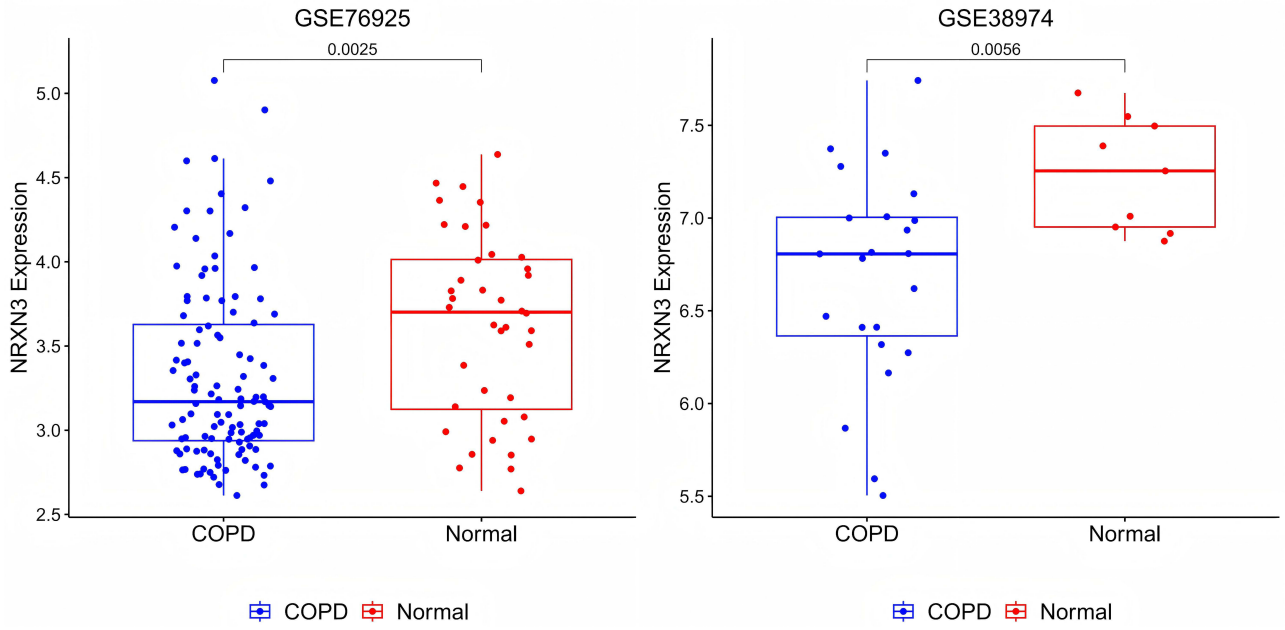


**Figure 5** Validation of the NRXN3 expression trend in the GSE76925 and GSE38974 datasets.

metabolite-to-COPD pathway, along with four mediating pathways. Expression analysis using a single-cell sequencing dataset, a transcriptome dataset, and PCR confirmed that only NRXN3 was highly expressed in lung tissues. We speculate that NRXN3 may be more directly involved in the pathogenesis and development of COPD, while the other two proteins (CFHR3 and ITLN1) may indirectly contribute to COPD pathogenesis by regulating systemic inflammation.

We found that most of the inflammatory proteins were enriched in the production and regulation of inflammatory factors and cytokine-related signaling pathways, further indicating that inflammatory cytokines play a role in the occurrence and progression of COPD, with numerous inflammatory proteins acting as regulatory factors. For example, several genes identified in this study were enriched in multiple GO terms related to IL-10, a multi-cell-derived, multifunctional cytokine that regulates cell growth and differentiation while promoting inflammation and immune responses. It is both an inflammatory and anti-inflammatory factor, as well as an immunosuppressive agent.[36] As an anti-inflammatory cytokine, IL-10 may exert its regulatory function by binding to a specific cell surface receptor (IL-10R), inhibiting the nuclear translocation of nuclear factor kappa B (NF-κB) and its DNA binding activity, thereby blocking pro-inflammatory cytokines and suppressing Nf-κb expression.[37] Xiong et al reported significantly increased IL-10 levels in patients with acute exacerbation of COPD compared to those in the normal control and stable groups, suggesting IL-10's involvement in the inflammatory processes of COPD.[38] IL-10 is widely recognized to play a key role in COPD pathogenesis and airway protection.

Most of the metabolites and metabolite ratios identified in this study were associated with glucose and lipid metabolism, both critical in COPD pathogenesis. Glucose, fatty acids, and amino acids are the three primary energy sources for cellular activities, providing energy through metabolic pathways such as glycolysis, fatty acid oxidation, and glutamine metabolism, even within the lungs. The metabolism of lung cells also impacts cellular functions. AMs play crucial roles in the development and progression of COPD, with several studies reporting significant alterations in the levels of various metabolites in patients with COPD. These changes may contribute to COPD development by regulating AM functions, including polarization, production of inflammatory mediators, and apoptosis.[39]

Recent studies have found that M1 phenotype polarization increases glucose uptake and relies on aerobic glycolysis to produce adenosine triphosphate, although the tricarboxylic acid (TCA) cycle is impaired. In contrast, M2 macrophages maintain an intact TCA cycle, with enhanced oxidative phosphorylation and fatty acid oxidation during polarization.[40] AMs employ different energy metabolism models during activation to elicit immune responses. Succinate, an intermediate of the TCA cycle produced by succinate dehydrogenase or the mitochondrial electron transport chain complex II,


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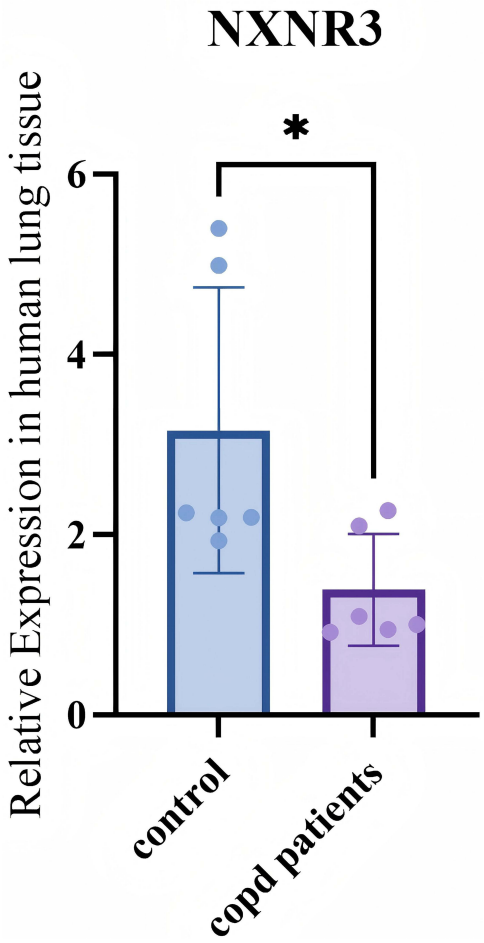


**Figure 6** Validation of NRXN3 expression in human tissues using RT-PCR. Lung tissues of humans were collected from patients with pulmonary nodules (without COPD; n = 6) or those with COPD (n = 6). The mRNA levels of NXNR3 were detected using qRT-PCR in human lung tissues. Data are presented as means ± SEM (n=3). *P < 0.05, compared with the control group.

can be released into the environment or blood upon cell damage. Succinate is a pro-inflammatory metabolite that stabilizes hypoxia-inducible factor 1α (HIF-1α) by inhibiting prolyl hydroxylase activity, enhancing reactive oxygen species (ROS) and reactive nitrogen species (RNS) production, essential for maintaining the M1 macrophage phenotype.[41] Stabilization of HIF-1α also significantly enhances glycolytic pathway activity, and high peroxide levels promote inflammatory stress-related factor expression.[42] As endogenous antioxidant enzyme levels decrease and pro-inflammatory cytokine production increases, changes in cytoskeletal dynamics impair AM phagocytosis in COPD, promoting disease development. α-Ketoglutarate (α-KG), a key TCA cycle metabolite, induces macrophage polarization to the M2 phenotype. As a substrate for prolyl hydroxylase, α-KG destabilizes HIF-1 and suppresses M1 polarization marker expression while promoting M2-type signature gene expression via fatty acid oxidation and histone demethylase JmjD3-dependent epigenetic regulation. Inhibition of α-KG synthetase glutaminase reduces M2 polarization, promotes M1 polarization, affects inflammatory factor release, and drives COPD progression.[43]

Triglycerides (TGs), composed of long-chain fatty acids and glycerol, are the most abundant lipids in the human body and the primary energy source. However, excessive TG levels can alter AM cell function, increase blood viscosity, and exacerbate COPD progression. This may be related to local hypoxia, which induces the expression of hypoxia-related genes such as HIF-1 in macrophages, making them more dependent on glycolysis and promoting peroxide production.[44] Other TG-rich lipids, such as palmitate or very-low-density lipoprotein, can activate macrophages by upregulating C16:0 ceramide levels within macrophages, enhancing pro-inflammatory responses.[45] In addition, palmitate activates the NLRP3 inflammasome and promotes the secretion of IL-1 and IL-18 via adenine monophosphate-activated protein

kinase and autophagy signaling, enhancing M1-type characteristics.[46] Cholesterol metabolism also affects macrophage-induced inflammatory responses. 25-hydroxycholesterol (25-HC), an intermediate in cholesterol metabolism catalyzed by cholesterol-25-hydroxylase (CH25H), inhibits further cholesterol biosynthesis in a negative feedback loop. CH25H gene deletion increases inflammasome activation, likely due to cholesterol accumulation in macrophage mitochondria, leading to mitochondrial stress. Elevated levels of oxidized cholesterol products, such as 25-HC and 27-hydroxycholesterol, have been observed in the airways of patients with COPD. Phospholipids are major components of cell membranes and include sphingomyelin and glycerophospholipids. Ceramide, a sphingomyelin component, is involved in various cellular functions, including growth, differentiation, and apoptosis. Studies have shown that ceramide induces apoptosis of lung epithelial cells in emphysema and contributes to alveolar structure destruction. Cigarette smoke exposure increases ceramide levels, which accumulates in macrophages and causes mitochondrial damage and PINK1-mediated necrosis, promotes lung parenchyma (epithelial and endothelial) cell apoptosis, induces AM accumulation in emphysema models, and impairs AM phagocytosis.[47] These findings are consistent with those in the present study.

In this study, we identified the mediating roles of CFHR3, ITLN1, and NRXN3. CFHR3 encodes a secreted protein belonging to the complement factor H-related protein family. It binds to heparin and may be involved in the regulation of the complement cascade.[48] The lungs of patients with COPD are characterized by persistent inflammation, with increased levels of various complement factors (C3a, C4a, and C5a) observed in the sputum and plasma of patients with "stable" COPD. In addition, acute exacerbations lead to further elevations in sputum C3a and C5a compared to their baseline levels during "unstable" disease, with these levels appearing to correlate directly with the severity and duration of the condition. Acute and chronic infections activate the complement system, exacerbating lung damage by mediating inflammatory responses and promoting the development of COPD.[49] While 1-methylnicotinamide is positively correlated with CFHR3, no relevant studies have investigated this association. 1-methylnicotinamide is a primary metabolite present in all organisms and is involved in growth, development, and reproduction, with various immunomodulatory properties. It has been associated with inflammatory responses in lung epithelial cells and NLRP3 inflammasome activation,[50] which contributes to COPD progression by promoting the production and release of inflammatory factors (IL-1β and IL-18) and to increasing oxidative stress levels.

ITLN1, a lectin that specifically recognizes microbial carbohydrate chains in a calcium-dependent manner, agglutinates bacteria via its carbohydrate-binding ability, playing a key role in innate immunity. Studies have shown that ITLN1 mediates IL-13-induced monocyte chemoattractant proteins (MCP-1 and MCP-3) in lung epithelial cells, promoting allergic airway inflammation. Thus, ITLN-1 is closely associated with type 2 inflammation both in vitro and in vivo, contributing to asthma and COPD pathogenesis.[51] Our MR analysis indicated a negative correlation between ITLN1 and COPD, supporting its role as an adipokine with anti-inflammatory effects and suggesting multiple regulatory pathways. The phosphate to N-acetylneuraminate ratio showed positive and negative correlations with COPD, but the specific regulatory mechanisms remain underexplored, warranting further investigation.

NRXN3 is a synaptic adhesion molecule belonging to the neurotoxin gene family and is primarily involved in regulating neurotransmitter release. However, research has shown that this gene also plays a role in cell adhesion, migration, invasion, and signaling pathways.[52] Single-cell sequencing revealed that NRXN3 is mainly expressed in endothelial cells and involved in adhesion between endothelial cells. Endothelial injury has been implicated in the pathogenesis of COPD and emphysema, and endothelial dysfunction has also been observed in patients with COPD. Following infection and inflammation, endothelial microparticles (EMPs) and circulating endothelial cells are detected in the peripheral blood as endothelial cells shed from the pulmonary microvasculature. EMPs are significantly elevated in patients with COPD and emphysema.[53] Peinado et al and Dinh-Xuan et al reported endothelial dysfunction and structural abnormalities of pulmonary vessels in the lung tissues of patients with early and late-stage COPD, respectively. Peinado et al also found increased intimal thickening of the small pulmonary arteries in smokers and patients with COPD patients compared with that in non-smokers, suggesting that endothelial dysfunction is induced by cigarette smoke.[54] This is consistent with our single-cell sequencing results. These mechanisms are primarily associated with vascular inflammation, lipid peroxidation, and oxidative stress. Inflammation and cigarette smoke stimulate the activation of vascular endothelial cells, release pro-inflammatory cytokines such as TNF-α, promote the development of emphysema, and damage the extracellular matrix of the alveolar wall. Furthermore, cigarette smoke can increase the interaction between

neutrophils and endothelial cells, promoting the release of additional inflammatory factors. Oxidative stress plays a key role in the pathogenesis of the pulmonary component of COPD. Cigarette smoke activates inflammatory cells, including alveolar macrophages, to release reactive oxygen and nitrogen species, activates endothelial cells, promotes microthrombosis, and triggers further release of ROS and RNS, which increase lipid peroxidation in endothelial cells.[55] This activates the receptor for advanced glycation end products, promotes endothelial dysfunction and injury, and induces cellular senescence.[56] Notably, our MR analysis revealed that the arachidonate-to-pyruvate ratio (20:4n6) negatively correlated with NRXN3 and positively correlated with COPD. In the cytoplasm, pyruvate is produced from glucose via glycolysis. Under aerobic conditions, pyruvate enters the mitochondria and, through the action of the pyruvate dehydrogenase complex, undergoes oxidative decarboxylation to form acetyl-coenzyme A, which then enters the TCA cycle. Pyruvate is the raw material for arachidonic acid synthesis; therefore, pyruvate indirectly influences arachidonic acid synthesis and its role in physiological and pathological processes by providing energy and converting it into arachidonic acid precursors. Studies have shown that arachidonic acid generates lipid hydroperoxide (LOOH) via lipid oxygenase catalysis. LOOH can decompose into various lipid peroxidation products, including malondialdehyde. Simultaneously, LOOH can further attack proteins, nucleic acids, and unsaturated compounds with double bonds via epoxidation, producing more free radicals and causing oxidative damage and apoptosis in cells.[57,58] In conclusion, arachidonic acid damages pulmonary vascular endothelial cells by increasing oxidative stress and promoting the development of COPD. NRNX3 plays a protective role in this process by acting as an adhesion protein between endothelial cells.

This study has several limitations. First, because we used summary statistics rather than raw data, we were unable to analyze the various stages of COPD onset, limiting the comprehensiveness of our study of the underlying mechanisms. Additionally, the data used in this study were obtained from individuals of European ancestry, which may introduce ethnic bias. Second, due to the unavailability of large publicly accessible datasets specifically related to lung tissue, we focused only on the causal relationship between peripheral blood protein levels, metabolites, and COPD. Finally, to identify as many potential positive results as possible, multiple testing corrections were not performed, and false-positive results may exist.

## Conclusion

In summary, we identified 63 metabolites, 10 metabolite ratios, 48 inflammatory proteins, and three mediator proteins with causal links to COPD, alongside four metabolism-inflammation-COPD pathways. Interventions targeting metabolic processes may become a key strategy for preventing the onset or progression of COPD in the future. However, the mechanisms related to metabolism, immunity, and COPD warrant further investigation.

## Abbreviations

COPD, chronic obstructive pulmonary disease; MR, Mendelian randomization; PPI, protein-protein interaction; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; AMs, alveolar macrophages; IL, interleukin; TNF, tumor necrosis factor; SNPs, single nucleotide polymorphisms; IVs, instrumental variables; CI, confidence interval; scRNA-seq, single-cell RNA sequencing; t-SNE, t-distributed random neighborhood embedding; FC, fold change; RT-PCR, real-time polymerase chain reaction; BP, biological processes; MF, molecular functions; CC, cellular components; NF-κB, nuclear factor kappa B; TCA, tricarboxylic acid; HIF-1α, hypoxia-inducible factor 1α; ROS, reactive oxygen species; RNS, reactive nitrogen species; α-KG, α-Ketoglutarate; TG, triglycerides; 25-HC, 25-hydroxycholesterol; CH25H, cholesterol-25-hydroxylase; EMPs, endothelial microparticles; LOOH, lipid hydroperoxide.

## Data Sharing Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Table S2.

## Ethical Approval and Consent to Participate

All lung tissue samples in the study were voluntarily donated by participants who provided written informed consent, in accordance with the principles outlined in the Declaration of Helsinki. The experiment was approved by the Ethics Committee of Wuxi People's Hospital, affiliated with Nanjing Medical University.

## Acknowledgments

Thanks to the public databases for providing us with data, and thanks to the developers of R software and R packages for their contributions and convenience.

## Author Contributions

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

## Funding

This study was supported by the Cohort and Clinical Research Program of Wuxi Medical Center, Nanjing Medical University (WMCC202315), the project of Major Program of Wuxi Medical Center, Nanjing Medical University (WMCM202309), and Respiratory medicine development discipline (FZXK-3).

## Disclosure

The authors declare that they have no competing interests.

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