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Dissecting causal relationships between immune cells, plasma metabolites, and COPD: a mediating Mendelian randomization study

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Objective: This study employed Mendelian Randomization (MR) to investigate the causal relationships among immune cells, COPD, and potential metabolic mediators.

Methods: Utilizing summary data from genome-wide association studies, we analyzed 731 immune cell phenotypes, 1,400 plasma metabolites, and COPD. Bidirectional MR analysis was conducted to explore the causal links between immune cells and COPD, complemented by two-step mediation analysis and multivariable MR to identify potential mediating metabolites.

Results: Causal relationships were identified between 41 immune cell phenotypes and COPD, with 6 exhibiting reverse causality. Additionally, 21 metabolites were causally related to COPD. Through two-step MR and multivariable MR analyses, 8 cell phenotypes were found to have causal relationships with COPD mediated by 8 plasma metabolites (including one unidentified), with 1-methylnicotinamide levels showing the highest mediation proportion at 26.4%.

Conclusion: We have identified causal relationships between 8 immune cell phenotypes and COPD, mediated by 8 metabolites. These findings contribute to the screening of individuals at high risk for COPD and offer insights into early prevention and the precocious diagnosis of Pre-COPD.

KEYWORDS

Mendelian randomization, immune cells, plasma metabolites, COPD, mediation analysis

1 Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous disorder primarily characterized by airway pathologies (bronchitis, bronchiolitis) and/or alveolar abnormalities (emphysema), leading to chronic respiratory symptoms (dyspnea, cough, sputum production) and progressively worsening airflow limitation (1). Globally, COPD accounts for more than half of all chronic respiratory disease cases (2), gradually becoming the third leading cause of death worldwide (3). With the increasing prevalence of an aging population, both the incidence and mortality rates of COPD are on the rise annually (4), imposing a significant economic burden on society (5).

Immune cells possess multifaceted functions in maintaining homeostasis and facilitating repair after injury (6). The lungs may serve as a battleground for the interaction between various microbes and the host's innate and adaptive immune defenses (7). Consequently, the immune system could be a pivotal driving force in the pathogenesis of COPD, with immune responses being significantly associated with acute exacerbations of COPD (8). However, the detailed physiological mechanisms remain insufficiently explored (9). Most existing evidence, primarily from observational studies, indicates that compared to healthy controls, individuals with COPD have an increased presence of immune cells in lung tissue (10, 11) and an upregulated immune cell response (12). Cells such as CD68+ myeloid antigen-presenting cells, CD4+ T cells, and CD8 T cells are found to proliferate in the lungs of patients with COPD, potentially leading to persistent inflammation (13, 14). Certain immune cells exhibit a negative correlation with the frequency of COPD exacerbations (15), such as CD4 T cells and resting natural killer cells (16). Consequently, the causal relationship and underlying mechanisms between immune cells and COPD remain unclear. Metabolites, as intermediates of metabolic reactions, can influence disease progression (17) and serve as targets for therapeutic intervention (18). They have the potential to improve the diagnosis and treatment of COPD (19) and may play a synergistic role in its pathogenesis (20), possibly mediating important immunoregulatory functions (21). Compared to healthy controls, COPD patients exhibit reduced levels of the metabolites 1-methylnicotinamide creatinine, and lactate (17). Glutamylphenylalanine may serve as a biomarker for acute exacerbations of COPD (22), while sphingolipids are associated with pulmonary function (23). Therefore, we hypothesize a causal relationship between immune cells, metabolites, and COPD. Elucidating these associations and understanding the true causal relationships among immune cells, metabolites, and COPD could aid in the early identification, prevention, and management of COPD.

Mendelian Randomization (MR) represents a potential method for causal inference, designed to estimate the causal effects of exposure factors on outcomes, with the capability to control for potential confounding factors and circumvent reverse causation biases (24). Utilizing the methodology of MR, we are poised to conduct a bidirectional MR study concerning immune cells and COPD, concurrently undertaking two mediating analyses to dissect the causal relationships among immune cells, metabolites, and COPD.

2 Methods

2.1 Study design

Grounded in two-sample Mendelian Randomization, our study initially assessed the causal relationship between 731 immune cell phenotypes across 7 panels (B cell, cDC, TBNK, Treg, Myeloid cell, Maturation stages of T cell and Monocyte) and COPD. Proceeding with COPD as the exposure factor and employing the Inverse Variance Weighted (IVW) method to select immune cells as the outcome factor, we conducted reverse Mendelian Randomization to ascertain the presence of a reverse causal relationship. Utilizing both the two-step MR (TSMR) and multivariable MR approaches, with 1400 plasma metabolites serving as mediating factors, we aimed to elucidate the significant mediatory role that plasma metabolites may play in the causal pathway between immune cells and COPD (Figure 1).

2.2 Data sources

The genetic information pertinent to COPD was sourced from the GWAS database (https://gwas.mrcieu.ac.uk/), with the selected dataset bearing the identifier ebi-a-GCST90018807, encompassing 468,475 samples and 24,180,654 SNPs, all of which pertain to the European population. The genetic data related to 731 immune cell phenotypes were derived from a 2020 study (25), all pertaining to the European demographic, with the catalog identifiers ranging from ebi-a-GCST90001391 to ebi-a-GCST90002121. The GWAS data for 1,400 plasma metabolites, hailing from a 2023 study (18), are accessible from the GWAS database, with identifiers spanning from GCST90199621 to GCST90201020, all associated with the European population.

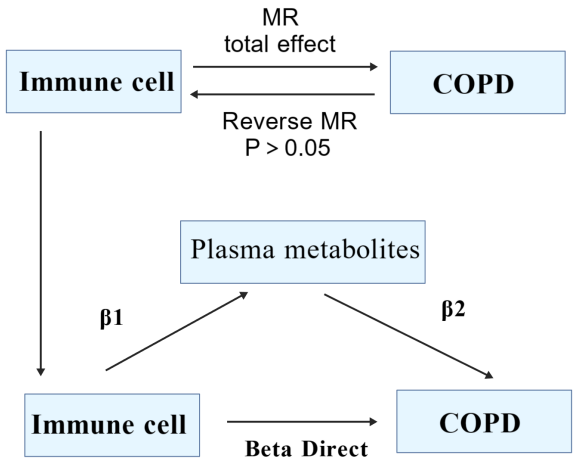

FIGURE 1
Research ideas.

2.3 Instrumental variable selection

The selection of instrumental variables necessitates adherence to several assumptions (26), to fulfill their relevance (27), we conducted an association analysis on 731 immune cell phenotypes and 1,400 plasma metabolites, uniformly applying a threshold of $P<1\times10^{-5}$ (28, 29). Subsequently, SNPs exhibiting linkage disequilibrium were filtered out using criteria of R^2<0.001 and Kb=10,000 (30), followed by the calculation of the F-statistic for the selected SNPs to eliminate weak instrumental variables. An F-statistic greater than 10 is considered indicative of the absence of weak instrumental variables (31, 32).

2.4 Statistical analysis

We employed five methodologies to assess causality: Inverse Variance Weighted (IVW), MR-Egger, Weighted Median, Simple Mode, and Weighted Mode methods, with IVW serving as the primary approach (33, 34). P<0.05 was indicative of a causal relationship (35), while the other four methods served as supplementary analyses (36). To evaluate the robustness of our results, we conducted sensitivity analysis using the "leave-one-out" approach, further examining pleiotropy and heterogeneity, P>0.05 suggesting the absence of both (37, 38). Utilizing the TSMR approach, we first calculated the total effect from immune cells to COPD, the effect of immune cells on metabolites (β1), and the effect of metabolites on COPD (β2), followed by the calculation of the mediating effect (β1*β2), with the direct effect being the total effect minus the mediating effect (39). All analyses were conducted using the R language (version 4.3.2), with the TwoSampleMR package at version 0.6.0.

3 Results

3.1 Genetic causality between immune cells and COPD

Through the selection of quantitative tools, we conducted an associative analysis, eliminating linkage disequilibrium and weak instrumental variables, thereby identifying 13,318 SNPs associated with immune cells, with the smallest F-value being 19.53. Preliminary investigations via the Inverse Variance Weighted (IVW) method revealed 41 immune cell phenotypes correlated with COPD, including but not limited to IgD+ CD38br %B cell, CD19 on IgD- CD38br, CD19 on PB/PC, and CD24 on memory B cell within the B cell category; TCRgd %T cell, HLA DR+ T cell%T cell, NKT %lymphocyte, and HLA DR+ NK AC within the TBNK category; CCR2 on plasmacytoid DC, CCR2 on CD62L+ plasmacytoid DC, CD80 on granulocyte, and CD62L on monocyte within the cDC category; and CD28+ CD45RA- CD8br %T cell, CD45RA+ CD28- CD8br %CD8br, CD25hi %T cell, and CD25++ CD8br %CD8br within the Treg category. In our study, we conducted a reverse Mendelian randomization analysis with COPD as the exposure factor and 41 immune cell phenotypes as the outcome factors. Our findings revealed that COPD does not exhibit a reverse causal relationship with 35 of the immune cell phenotypes (r-Pvalue > 0.05). However, a reverse causality was observed in six immune cell phenotypes (r-Pvalue < 0.05), specifically CD14+ CD16+ monocyte AC, CD4+ CD8dim % lymphocyte, CD4+ CD8dim %leukocyte, CD3- lymphocyte AC, CD3 on EM CD8br, and CD45 on Im MDSC. Furthermore, 21 immune cell phenotypes demonstrated a negative correlation with COPD, while 20 showed a positive correlation. Concurrently, tests for pleiotropy and heterogeneity yielded results (P> 0.05), with the direction of OR values being consistent, and leave-one-out sensitivity analysis confirmed the robustness of the MR findings (Table 1, Figure 2).

3.2 Genetic causality between metabolites and COPD

Through the selection of instrumental variables, we conducted an association analysis, eliminating linkage disequilibrium and weak instrumental variables, thereby identifying 29,302 SNPs associated with plasma metabolites, with the smallest F-statistic being 19.50. The IVW method preliminarily identified 21 plasma metabolites causally related to COPD, comprising 16 known metabolites and 5 unknown. Among the known metabolites, 7 were potentially associated with an increased risk of COPD, namely Stearidonate (18:4n3), Alpha-hydroxyisovalerate, Epiandrosterone sulfate, Cinnamoylglycine, 1-methylnicotinamide, the Arachidonate (20:4n6) to pyruvate ratio, and the Histidine to alanine ratio. Conversely, 9 metabolites were potentially inversely correlated with COPD risk, including 4-vinylphenol sulfate, 16a-hydroxy DHEA 3-sulfate, 1-palmitoyl-GPG (16:0), N-oleoylserine, Alpha-tocopherol, Taurochenodeoxycholate, the Adenosine 5'-diphosphate (ADP) to fructose ratio, the Uridine to cytidine ratio, and the Cysteinylglycine to glutamate ratio (Table 2, Figure 3).

3.3 Mediated Mendelian randomization analysis

Building upon the previously identified immune cells and plasma metabolites, we employed the TSMR approach to further compute mediation through Mendelian randomization. Utilizing the 35 selected immune cell phenotypes as exposure factors and the 21 plasma metabolites as outcome measures, we conducted a MR analysis from immune cell phenotypes to plasma metabolites. This analysis revealed causal relationships between 20 immune cell phenotypes and 14 plasma metabolites, yielding the effect size β1 from immune cell phenotypes to metabolites. Our research has uncovered that there is a negative correlation between CD62L- myeloid DC AC and 1-palmitoyl-GPG (16:0), a positive correlation between TCRgd %T cell and 1-methylnicotinamide, and a positive correlation between HLA DR+ T cell%T cell and Alpha-tocopherol levels, among other findings. Further analysis has revealed that a single immune cell phenotype can have causal relationships with

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TABLE 1 MR analysis of immune cells and COPD.

Exposure	Method	Nsnp	Beta	Se	P-val	Pleiotropy	Heterogeneity
IgD+ CD38br %B cell	IVW	27	0.037	0.015	0.014	0.780	0.789
Myeloid DC AC	IVW	23	0.037	0.012	0.001	0.605	0.733
CD62L- myeloid DC AC	IVW	17	0.043	0.017	0.013	0.731	0.363
CD62L- CD86+ myeloid DC %DC	IVW	19	0.023	0.011	0.042	0.156	0.762
CD25hi %T cell	IVW	20	-0.034	0.016	0.032	0.901	0.468
CD33dim HLA DR+ CD11b- % CD33dim HLA DR+	IVW	25	0.017	0.007	0.016	0.487	0.840
CD14+ CD16+ monocyte AC	IVW	21	-0.032	0.015	0.036	0.843	0.487
CD4+ CD8dim %lymphocyte	IVW	22	-0.058	0.022	0.007	0.716	0.101
CD4+ CD8dim %leukocyte	IVW	17	-0.059	0.027	0.033	0.867	0.053
TCRgd %T cell	IVW	19	-0.036	0.015	0.018	0.363	0.523
HLA DR+ T cell%T cell	IVW	33	-0.023	0.010	0.020	0.348	0.622
NKT %lymphocyte	IVW	36	0.041	0.015	0.006	0.225	0.180
CD3- lymphocyte AC	IVW	18	-0.057	0.022	0.009	0.954	0.947
HLA DR+ NK AC	IVW	19	-0.050	0.019	0.007	0.648	0.720
CD25++ CD8br %CD8br	IVW	23	0.042	0.020	0.039	0.792	0.523
CD28+ CD45RA- CD8br %T cell	IVW	27	0.014	0.007	0.045	0.488	0.355
CD45RA+ CD28- CD8br %CD8br	IVW	35	0.001	0.000	0.020	0.937	0.721
CD19 on CD20- CD38-	IVW	20	0.080	0.022	0.000	0.587	0.116
CD19 on IgD- CD38br	IVW	17	-0.046	0.020	0.019	0.778	0.452
CD19 on PB/PC	IVW	24	-0.062	0.020	0.002	0.101	0.258
CD24 on memory B cell	IVW	32	0.027	0.012	0.024	0.270	0.263
CD25 on transitional	IVW	22	0.033	0.016	0.042	0.579	0.927
CD27 on CD24+ CD27+	IVW	31	0.039	0.012	0.001	0.975	0.196
CD27 on unsw mem	IVW	31	0.036	0.015	0.014	0.521	0.340
CD27 on sw mem	IVW	30	0.032	0.014	0.017	0.265	0.306
IgD on IgD+ CD24-	IVW	30	-0.034	0.015	0.023	0.810	0.459
CD62L on monocyte	IVW	25	0.028	0.013	0.036	0.238	0.883
CD62L on granulocyte	IVW	18	-0.058	0.020	0.004	0.230	0.476
CD3 on naive CD8br	IVW	25	-0.035	0.012	0.004	0.948	0.972
CD3 on EM CD8br	IVW	20	-0.033	0.015	0.026	0.953	0.507
CD3 on CM CD8br	IVW	19	-0.046	0.017	0.008	0.549	0.956
CD127 on CD28+ CD45RA- CD8br	IVW	19	-0.030	0.013	0.021	0.237	0.618
CD127 on CD28- CD8br	IVW	20	-0.044	0.022	0.041	0.246	0.618
HLA DR on monocyte	IVW	18	-0.026	0.013	0.041	0.558	0.077
CCR2 on plasmacytoid DC	IVW	19	0.035	0.014	0.013	0.765	0.423
CCR2 on CD62L+ plasmacytoid DC	IVW	19	0.039	0.014	0.006	0.447	0.550
CD80 on granulocyte	IVW	33	-0.033	0.012	0.008	0.110	0.798
CD45 on Im MDSC	IVW	11	-0.036	0.013	0.007	0.271	0.391

(Continued)

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TABLE 1 Continued

Exposure	Method	Nsnp	Beta	Se	P-val	Pleiotropy	Heterogeneity
SSC-A on HLA DR+ T cell	IVW	23	-0.044	0.017	0.009	0.673	0.511
CD11b on CD66b++ myeloid cell	IVW	18	0.032	0.015	0.027	0.209	0.540
HLA DR on plasmacytoid DC	IVW	23	0.019	0.009	0.024	0.146	0.339

multiple metabolites. For instance, HLA DR+ NK AC not only exhibits a negative correlation with Cinnamoylglycine but also with the Uridine to cytidine ratio. Similarly, CD19 on PB/PC shows a positive correlation with Stearidonate (18:4n3) as well as with 4-vinylphenol sulfate. Moreover, CD24 on memory B cell not only negatively correlates with 1-palmitoyl-GPG (16:0) and the Adenosine 5'-diphosphate (ADP) to fructose ratio but also positively correlates with 1-methylnicotinamide, among others. When considering the 14 plasma metabolites as exposure factors and COPD as the outcome, an MR analysis was conducted along with an MR-PRESSO test ($p > 0.05$), indicating no pleiotropy and unbiased SNPs. This led to the determination of the effect size β2 from metabolites to COPD, and subsequently, the overall effect from immune cells to COPD was calculated (Figure 4, Table 3).

3.4 Mediation analysis

In our final analysis, we conducted a mediation analysis to elucidate the causal relationship between immune cell phenotypes and COPD, mediated by plasma metabolites. We discovered that 8 plasma metabolites mediated the relationship between 8 immune cell phenotypes and COPD ($P < 0.05$), among which 7 are known plasma metabolites and one remains unidentified. Notably, the CD24 on memory B cells was mediated by two distinct plasma metabolites. The mediation proportion of 1-methylnicotinamide was found to be the highest at 26.4% (P=0.013), followed by the unidentified metabolite X-19438 with a mediation proportion of 21.8% (P=0.004), Taurochenodeoxycholate at 18.8% (P=0.008), and Alpha-hydroxyisovalerate at 14.2% (P=0.036), among others (Figure 5, Table 4).

4 Discussion

In our MR study, the findings indicated a causal relationship between 41 immune cell phenotypes and COPD. However, a reverse MR analysis revealed that 35 immune cell phenotypes bore no causal relationship with COPD, while 6 did exhibit a causal connection. Further employing TSMR and MVMR for mediation

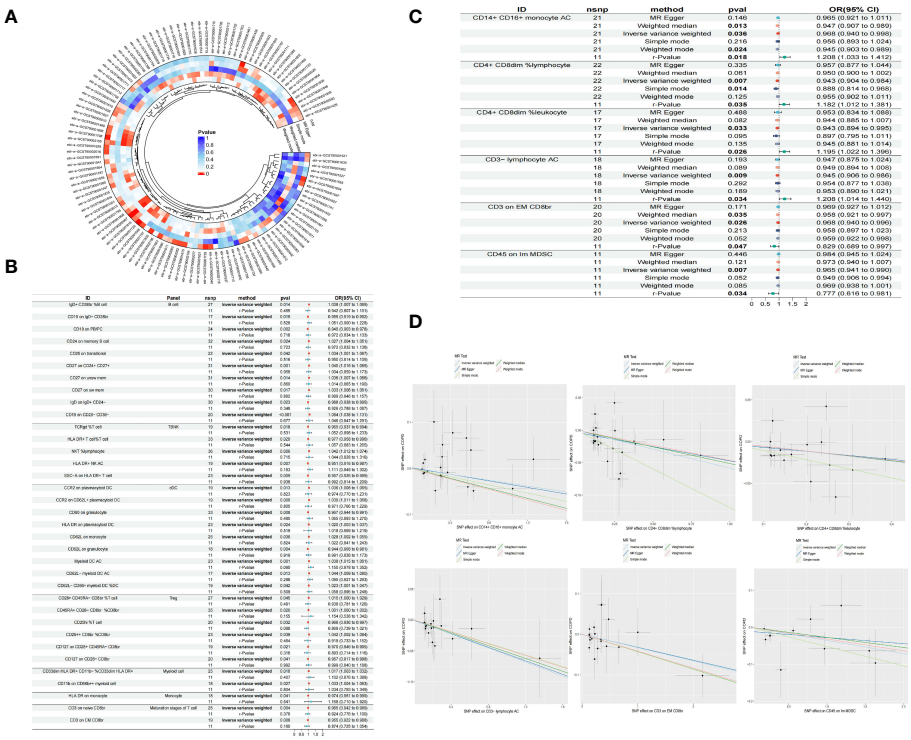

FIGURE 2
Circle plot of five Mendelian randomized methods (P<0.05) **(A)** Forest plot of causality between 35 immune cells and COPD (r-Pvalue is the result of reverse MR) **(B)** Forest plot of causality between six immune cells and COPD **(C)** Scatter plot of six immune cells reducing COPD risk **(D)**.

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TABLE 2 MR analysis of metabolites and COPD.

Exposure	Method	Nsnp	Beta	Se	P-val	Pleiotropy	Heterogeneity
Stearidonate (18:4n3) levels	IVW	27	0.097	0.034	0.004	0.751	0.304
Alpha-hydroxyisovalerate levels	IVW	22	0.106	0.036	0.003	0.591	0.055
Epiandrosterone sulfate levels	IVW	23	0.055	0.019	0.004	0.650	0.393
4-vinylphenol sulfate levels	IVW	25	-0.080	0.027	0.003	0.692	0.224
16a-hydroxy DHEA 3-sulfate levels	IVW	24	-0.061	0.021	0.005	0.667	0.407
Cinnamoylglycine levels	IVW	33	0.071	0.025	0.005	0.946	0.984
1-palmitoyl-GPG (16:0) levels	IVW	24	-0.078	0.029	0.008	0.462	0.583
N-oleoylserine levels	IVW	20	-0.075	0.027	0.006	0.568	0.984
Alpha-tocopherol levels	IVW	28	-0.086	0.029	0.003	0.845	0.656
Taurochenodeoxycholate levels	IVW	18	-0.124	0.040	0.002	0.563	0.521
1-methylnicotinamide levels	IVW	18	0.168	0.039	0.000	0.486	0.594
X-12100 levels	IVW	21	0.099	0.038	0.008	0.869	0.293
X-19438 levels	IVW	24	-0.081	0.029	0.005	0.335	0.861
X-23654 levels	IVW	39	0.062	0.023	0.006	0.131	0.944
X-24243 levels	IVW	20	0.094	0.034	0.006	0.645	0.834
X-24947 levels	IVW	27	0.047	0.018	0.008	0.714	0.446
Adenosine 5'-diphosphate (ADP) to fructose ratio	IVW	32	-0.079	0.020	0.000	0.409	0.622
Arachidonate (20:4n6) to pyruvate ratio	IVW	16	0.098	0.031	0.002	0.848	0.868
Uridine to cytidine ratio	IVW	22	-0.110	0.035	0.002	0.374	0.477
Cysteinylglycine to glutamate ratio	IVW	25	-0.082	0.031	0.009	0.228	0.569
Histidine to alanine ratio	IVW	32	0.076	0.029	0.009	0.080	0.280

analysis, we identified that 8 cell phenotypes could be causally linked to COPD through 8 plasma metabolites (including one unidentified), among which the mediation proportion of 1-methylnicotinamide levels was the highest at 26.4%.

Our research has corroborated the existence of a causal relationship between 41 immune cell phenotypes and COPD, aligning with previous studies that posit chronic inflammation leading to compromised immunity and immunosuppression as pivotal in the pathogenesis of COPD (40), a condition persistently present in the disease (41). It has been observed that, compared to healthy individuals, patients with COPD exhibit an increase in B cells and their products in the blood and lungs (42), alongside an upsurge in the expression of genes related to inflammation, B cell activation, and proliferation. This activation of B cells is associated with an autoimmune-mediated mechanism of COPD pathogenesis (43, 44). However, the association between B cells and COPD does not imply causality (45). Our study, however, confirms a causal relationship between specific B cell phenotypes and COPD, with an increase in memory B-cells being linked to impaired lung function and small airway dysfunction (46), consistent with our findings that CD24 on memory B cells increases the risk of COPD. Furthermore, the subgroups of peripheral blood TBNK lymphocytes in COPD patients show a certain correlation with COPD (47) and its severity (48), aligning with our MR results and suggesting a causal relationship. Regulatory T (Treg) cells play a crucial role in the immune system by suppressing excessive immune responses and maintaining immune balance. The relationship between Treg cells and lung function (49), the imbalance of Treg cells during COPD progression (50), and the potential of modulating Treg cells to improve COPD (51) and lung inflammation underscore their significance (52). Myeloid cells, capable of phagocytosing pathogens, initiating inflammatory responses, and presenting antigens to other immune cells, contribute to tissue repair and remodeling. Our analysis identified six immune cell phenotypes with a causal relationship to COPD in both directions, namely CD14+ CD16+ monocyte AC, CD4+ CD8dim %lymphocyte, CD4+ CD8dim %leukocyte, CD3- lymphocyte AC, CD3 on EM CD8br, and CD45 on Im MDS, all associated with a reduced risk of COPD. The CD14+ CD16+ monocyte AC, a monocyte subgroup expressing CD14 and CD16, plays a role in modulating inflammatory responses and promoting tissue repair, with monocytes being etiologically related to COPD (53) and influencing its pathogenesis and diagnosis (54, 55), serving as key drivers of lung inflammation and tissue remodeling (56). The CD4+

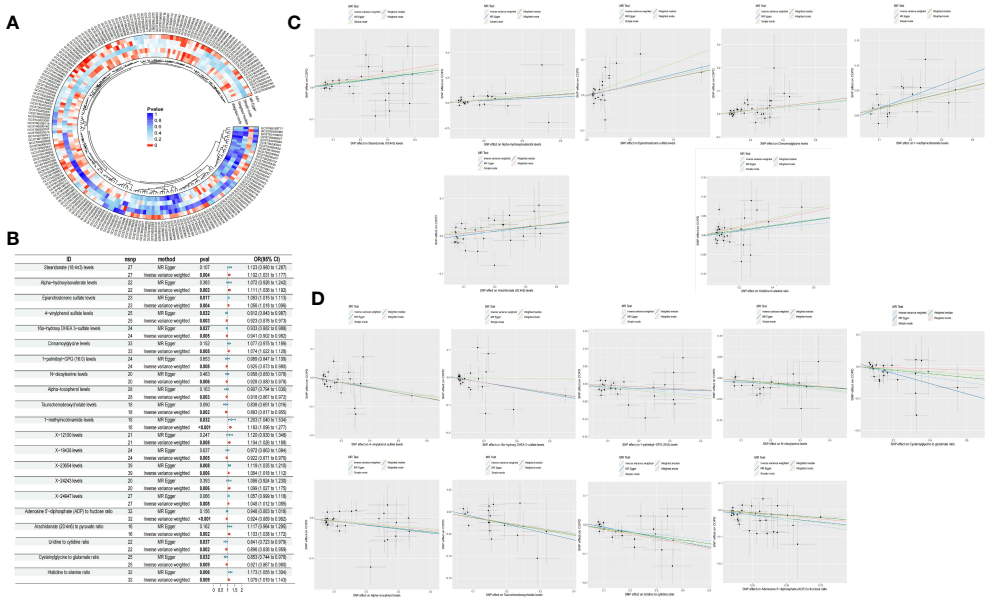
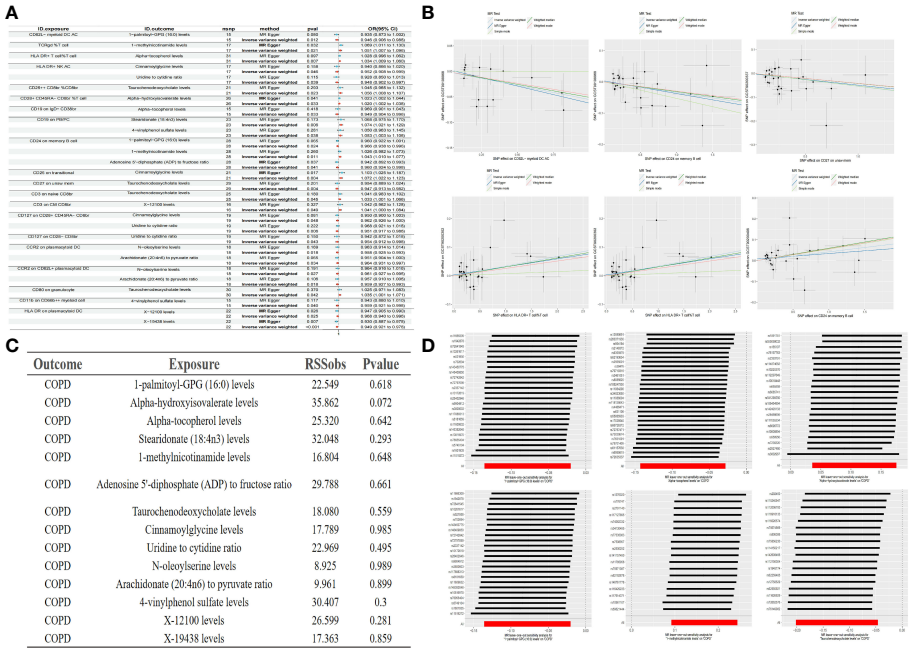


FIGURE 3
Circle plot of five Mendelian randomization methods (p <0.05) **(A)** Forest plot of causality of 21 metabolites and COPD **(B)** Scatter plot of 7 metabolites with increased COPD risk **(C)** Scatter plot of the 9 metabolites reducing the risk of COPD **(D)**.

CD8dim %lymphocyte, a unique lymphocyte, plays a role in regulating immune responses and maintaining immune balance, with CD4-regulated T cells controlling autoimmunity and thus managing lung inflammation in COPD (57, 58), while CD4 and CD8 are related to bronchiolar wall remodeling in COPD (59) and the reduction of terminal bronchioles (60).

Metabolites play a crucial role in the early identification of individuals at high risk and in the prevention of diseases (61). Clinically, they enable us to differentiate the disease characteristics of COPD (62), identify diagnostic biomarkers (63, 64), and evaluate the efficacy indicators of COPD treatments (65). Our study has discovered a causal relationship between CD24 on memory B cells



Outcome	Exposure	RSSobs	Pvalue
COPD	1-palmitoyl-GPG (16:0) levels	22.549	0.618
COPD	Alpha-hydroxyisovalerate levels	35.862	0.072
COPD	Alpha-tocopherol levels	25.320	0.642
COPD	Stearidonate (18:4n3) levels	32.048	0.293
COPD	1-methylnicotinamide levels	16.804	0.648
COPD	Adenosine 5'-diphosphate (ADP) to fructose ratio	29.788	0.661
COPD	Taurochenodeoxycholate levels	18.080	0.559
COPD	Cinnamoylglycine levels	17.789	0.985
COPD	Uridine to cytidine ratio	22.969	0.495
COPD	N-oleoylserine levels	8.925	0.989
COPD	Arachidonate (20:4n6) to pyruvate ratio	9.961	0.899
COPD	4-vinylphenol sulfate levels	30.407	0.3
COPD	X-12100 levels	26.599	0.281
COPD	X-19438 levels	17.363	0.859

FIGURE 4
Forest plot of immune cells and metabolites **(A)** Partial scatter plot of immune cells and metabolites **(B)** Test of MR-PRESSO of metabolites to COPD **(C)** Partial leave-one-out method sensitivity analysis of metabolites to COPD **(D)**.

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TABLE 3 MR analysis of immune cells and metabolites.

Exposure	Outcome	Method	Nsnp	Beta	Se	P-val	Pleiotropy	Heterogeneity
CD62L- myeloid DC AC	1-palmitoyl-GPG (16:0) levels	IVW	15	-0.055	0.022	0.012	0.676	0.764
TCRgd %T cell	1-methylnicotinamide levels	IVW	17	0.049	0.022	0.021	0.360	0.855
HLA DR+ T cell% T cell	Alpha-tocopherol levels	IVW	31	0.034	0.013	0.007	0.575	0.709
HLA DR+ NK AC	Cinnamoylglycine levels	IVW	17	-0.049	0.024	0.046	0.700	0.764
HLA DR+ NK AC	Uridine to cytidine ratio	IVW	17	-0.053	0.026	0.039	0.553	0.371
CD25++ CD8br %CD8br	Taurochenodeoxycholate levels	IVW	21	0.055	0.024	0.023	0.753	0.957
CD28+ CD45RA- CD8br %T cell	Alpha-hydroxyisovalerate levels	IVW	26	0.019	0.009	0.033	0.552	0.830
CD19 on IgD- CD38br	Alpha-tocopherol levels	IVW	15	-0.052	0.025	0.033	0.471	0.782
CD19 on PB/PC	Stearidonate (18:4n3) levels	IVW	23	0.071	0.026	0.006	0.888	0.244
CD19 on PB/PC	4-vinylphenol sulfate levels	IVW	23	0.052	0.025	0.038	0.938	0.531
CD24 on memory B cell	1-palmitoyl-GPG (16:0) levels	IVW	28	-0.034	0.015	0.024	0.673	0.723
CD24 on memory B cell	1-methylnicotinamide levels	IVW	28	0.042	0.016	0.011	0.323	0.175
CD24 on memory B cell	Adenosine 5'-diphosphate (ADP) to fructose ratio	IVW	28	-0.040	0.020	0.041	0.306	0.589
CD25 on transitional	Cinnamoylglycine levels	IVW	21	0.069	0.024	0.004	0.329	0.338
CD27 on unsw mem	Taurochenodeoxycholate levels	IVW	29	-0.054	0.019	0.004	0.828	0.486
CD3 on naive CD8br	Taurochenodeoxycholate levels	IVW	25	0.033	0.016	0.045	0.753	0.770
CD3 on CM CD8br	X-12100 levels	IVW	16	0.041	0.021	0.049	0.987	0.750
CD127 on CD28+ CD45RA- CD8br	Cinnamoylglycine levels	IVW	19	-0.039	0.020	0.048	0.525	0.155
CD127 on CD28+ CD45RA- CD8br	Uridine to cytidine ratio	IVW	19	-0.051	0.018	0.006	0.315	0.310
CD127 on CD28- CD8br	Uridine to cytidine ratio	IVW	19	-0.047	0.023	0.043	0.697	0.379
CCR2 on plasmacytoid DC	N-oleoylserine levels	IVW	18	-0.043	0.018	0.018	0.802	0.876
CCR2 on plasmacytoid DC	Arachidonate (20:4n6) to pyruvate ratio	IVW	18	-0.037	0.017	0.034	0.480	0.668
CCR2 on CD62L+ plasmacytoid DC	N-oleoylserine levels	IVW	18	-0.040	0.018	0.027	0.838	0.775
CCR2 on CD62L+ plasmacytoid DC	Arachidonate (20:4n6) to pyruvate ratio	IVW	18	-0.041	0.018	0.018	0.894	0.881
CD80 on granulocyte	Taurochenodeoxycholate levels	IVW	30	0.035	0.017	0.042	0.655	0.478
CD11b on CD66b+ + myeloid cell	4-vinylphenol sulfate levels	IVW	15	-0.042	0.020	0.040	0.558	0.594

(Continued)

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TABLE 3 Continued

Exposure	Outcome	Method	Nsnp	Beta	Se	P-val	Pleiotropy	Heterogeneity
HLA DR on plasmacytoid DC	X-12100 levels	IVW	22	-0.033	0.015	0.025	0.228	0.222
HLA DR on plasmacytoid DC	X-19438 levels	IVW	22	-0.052	0.015	0.001	0.282	0.696

and COPD, mediated by two intermediaries: 1-methylnicotinamide and 1-palmitoyl-GPG (16:0). There is a positive correlation between CD24 on memory B cells and COPD, where an increase in CD24 on memory B cells elevates the risk of COPD. Furthermore, CD24 on memory B cells is positively correlated with 1-methylnicotinamide, which, in turn, is positively associated with COPD. 1-methylnicotinamide, a primary metabolite found in all living organisms and involved in growth, development, or reproduction, possesses various immunomodulatory properties. It is linked to inflammatory responses in lung epithelial cells (66)and the activation of the NLRP3 inflammasome (67), a significant mediator in COPD inflammation (68). Through NLRP3, lung inflammation can be regulated (69, 70), indicating a certain correlation between 1-methylnicotinamide and COPD. Our research confirms a causal relationship between 1-methylnicotinamide and COPD, with CD24 on memory B cells influencing COPD risk through the mediation of 1-methylnicotinamide. Conversely, CD24 on memory B cells is negatively correlated with 1-palmitoyl-GPG (16:0), which is positively associated with COPD. Research on 1-palmitoyl-GPG (16:0) is limited, but it is generally considered part of lipid metabolism, related to vitamin D deficiency (71) and pulmonary hypertension (72). Vitamin D may be a risk factor for COPD (73), though specific studies on COPD are lacking. Lipid metabolism plays a key role in the adaptive immune response to chronic inflammation (74) and is associated with lung inflammation in mice (75), with COPD patients exhibiting higher lipid expression (76). Our MR analysis concludes a causal relationship between CD24 on memory B cells and COPD through 1-palmitoyl-GPG (16:0).

Taurochenodeoxycholate could potentially serve as an intermediary in the causal relationship between CD27 on unsw mem and COPD, demonstrating a negative correlation with both CD27 on unsw mem and COPD. Taurochenodeoxycholate, a bile acid functionally related to chenodeoxycholic acid, is involved in inflammatory responses (77), immune cell regulation (78), endoplasmic reticulum stress inhibition (79), and is associated with pulmonary fibrosis (80). However, research specifically targeting its role in COPD is scarce. MR analysis suggests that CD27 on unsw mem may have a causal relationship with COPD through the mediation of taurochenodeoxycholate. Alpha-tocopherol, the most active form of Vitamin E, has been observed in studies to reduce the risk of COPD in women (81), exhibiting anti-inflammatory and antioxidant properties that improve bronchial epithelial thickening, alveolar destruction, and lung function (82). Consistent with our MR analysis, alpha-tocopherol is negatively correlated with the risk of COPD, mediating a causal relationship between COPD and the percentage of HLA DR+ T cells among T cells. Alpha-hydroxyisovalerate, initially identified in studies related to human aging and early development (83), is associated with the severity of bronchiolitis (84) and, consistent with our MR analysis, positively correlated with the risk of COPD. It mediates a causal relationship between COPD and the phenotype of CD28+ CD45RA- CD8 bright %T cells. Cinnamoylglycine, with limited research related to COPD, has been found through MR analysis to be positively correlated with COPD. CD25 on

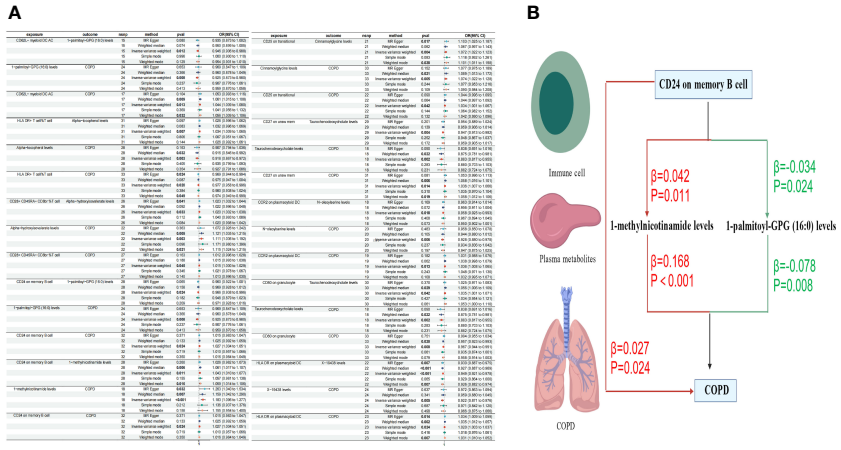

FIGURE 5
Forest plots of immune cells, plasma metabolites, and COPD **(A)** Plasma metabolites mediate the causal relationship between immune cells and COPD (red is a risk factor, green is a protective factor) **(B)**.

TABLE 4 Mendelian randomization analyses of the causal effects between immune cells, plasma metabolites and COPD.

Immune cell	Metabolite	Outcome	Mediated effect	Mediated proportion	Beta Direct	P-val
CD62L- myeloid DC AC	1-palmitoyl-GPG (16:0) levels	COPD	0.00431	10%	0.039	0.041
HLA DR+ T cell%T cell	Alpha-tocopherol levels	COPD	-0.0029	12.4%	-0.020	0.012
CD28+ CD45RA- CD8br %T cell	Alpha-hydroxyisovalerate levels	COPD	0.00205	14.2%	0.012	0.036
CD24 on memory B cell	1-palmitoyl-GPG (16:0) levels	COPD	0.00267	10%	0.024	0.039
CD24 on memory B cell	1-methylnicotinamide levels	COPD	0.0070	26.4%	0.020	0.013
CD25 on transitional	Cinnamoylglycine levels	COPD	0.00491	14.8%	0.028	0.039
CD27 on unsw mem	Taurochenodeoxycholate levels	COPD	0.00671	18.8%	0.029	0.008
CCR2 on plasmacytoid DC	N-oleoylserine levels	COPD	0.00321	9.09%	0.032	0.040
HLA DR on plasmacytoid DC	X-19438 levels	COPD	0.00424	21.8%	0.015	0.004

transitional cells has a causal relationship with COPD mediated by cinnamoylglycine. N-oleoylserine, a secondary metabolite functionally related to oleic acid and with scant research in the context of lung inflammation (85), has been found through MR analysis to be negatively correlated with COPD, suggesting a protective factor.

5 Conclusion

This study represents a comprehensive assessment of the causal relationships between immune cell phenotypes, plasma metabolites, and COPD. We have identified 8 immune cell phenotypes that exhibit a causal relationship with COPD, mediated by 8 metabolites. These findings illuminate the significance of the underlying mechanisms between immune cells, metabolites, and COPD. They contribute to the screening of individuals at high risk for COPD and offer insights into early prevention and the preemptive diagnosis of Pre-COPD conditions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

ZC: Writing – review & editing, Writing – original draft, Conceptualization. TW: Writing – original draft, Data curation. YF: Writing – original draft, Data curation. FS: Writing – original draft, Supervision, Data curation. HD: Writing – original draft, Data curation. LZ: Writing – original draft, Data curation. LS: Writing – review & editing, Writing – original draft, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Publisher's note

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