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ORIGINAL RESEARCH

Causal Effects of Gut Microbiota and Metabolites on Chronic Obstructive Pulmonary Disease: A Bidirectional Two Sample Mendelian Randomization Study

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Background: Recent evidence suggests that the gut microbiome and metabolites are intricately involved in Chronic Obstructive Pulmonary Disease (COPD) pathogenesis, yet the precise causal relationships remain unclear due to confounding factors and reverse causation. This study employs bidirectional two-sample Mendelian Randomization (MR) to clarify these connections.

Methods: Summary data from publicly available Genome-Wide Association Studies (GWAS) concerning the gut microbiome, metabolites, and COPD were compiled. The selection of genetic instrumental variables (Single Nucleotide Polymorphisms, or SNPs) for MR analysis was conducted meticulously, primarily utilizing the Inverse Variance Weighting (IVW) method, supplemented by MR-Egger regression and the Weighted Median (WM) approach. The evaluation of heterogeneity and horizontal pleiotropy was performed using Cochran's Q test, the MR-Egger intercept test, and the MR-PRESSO global test. Sensitivity analyses, including leave-one-out tests, were conducted to verify the robustness of our results. And the mediation effect of gut microbiota-mediated changes in metabolites on the causal relationship with COPD was analyzed.

Results: Our study identified nine significant gut microbiota taxa and thirteen known metabolites implicated in COPD pathogenesis. Moreover, associations between the onset of COPD and the abundance of five bacterial taxa, as well as the concentration of three known metabolites, were established. These findings consistently withstood sensitivity analyses, reinforcing their credibility. Additionally, our results revealed that gut microbiota contribute to the development of COPD by mediating changes in metabolites.

Conclusion: Our bidirectional Two-Sample Mendelian Randomization analysis has revealed reciprocal causal relationships between the abundance of gut microbiota and metabolite concentrations in the context of COPD. This research holds promise for identifying biomarkers for early COPD diagnosis and monitoring disease progression, thereby opening new pathways for prevention and treatment. Further investigation into the underlying mechanisms is essential to improve our understanding of COPD onset.

Keywords: chronic obstructive pulmonary disease, gut microbiota, metabolites, Mendelian randomization, causal effect

Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous lung disorder characterized by chronic respiratory symptoms such as dyspnea, cough, sputum production, and/or exacerbations. These symptoms result from abnormalities in the airways (bronchitis, bronchiolitis) and/or alveoli (emphysema), leading to persistent and typically progressive airflow obstruction.[1] This disease ranks as the fourth leading cause of mortality globally, claiming the lives of over three million patients annually.[2] The multifaceted nature of COPD underscores its impact, representing a substantial economic and clinical burden.[3,4]

The intricate interplay within the gut microbiome, which comprises an intricate assembly housing over 1000 microbial entities within the digestive tracts of humans and animals,[5] orchestrates a complex impact on diseases spanning multiple bodily systems. A disruption in this intricate microbial community can induce alterations in pulmonary bacterial

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composition, giving rise to the "Gut-Lung axis".[6] This phenomenon enables the entry of microbial metabolites into the bloodstream, establishing a linkage between gut and lung, thereby fomenting inflammation at both local and systemic levels. The sustained nature of this inflammatory response may precipitate subacute or chronic manifestations.[7] Importantly, a growing body of research highlights that the changes in gut microbiota and metabolites play an important role in the pathogenesis of COPD.[8,9] By comparing the gut microbiome and metabolomic profiles of twenty-eight COPD patients and twenty-nine healthy individuals, Bowerman et al[10] identified one hundred and forty-six bacterial species that differed between the two groups, six types of gut bacteria were found to be enriched in individuals with COPD at the bacterial family level, and sixteen metabolites from lipids, amino acids, or foreign sources that were considered significantly different. Although the association between gut microbiota and COPD risk is well-documented, clarifying the causation of these associations remains a challenge.

Mendelian randomization (MR) represents a critical statistical approach for discerning causality within scientific investigations.[11] This method entails the selection of single nucleotide polymorphisms (SNPs) associated with a given exposure, functioning as instrumental variables (IVs), thereby enabling the estimation of causal relationships between exposure and outcome while mitigating confounding influences.[12] Recent research endeavors have explored the establishment of a causal nexus between gut microbiota and the development of COPD through the application of two-sample MR analysis (TSMR).[13,14] Regrettably, these investigations have hitherto overlooked the examination of reverse causal effects originating from COPD on gut microbiota and metabolites. The present study undertakes a comprehensive bidirectionalTSMR analysis, elucidating the intricate causal dynamics governing the relationships between gut microbiota, metabolites, and COPD.

Materials and Methods

The methodology implementation process is illustrated in Figure 1.

Study Design

This study utilized bidirectional TSMR analysis and mediation analysis to investigate the causal relationships between 211 gut microbiota and 452 blood metabolites with COPD. Additionally, it explored the potential role of gut microbiota in mediating this relationship through blood metabolites. SNPs were retrieved and used as IVs from genome-wide association studies (GWAS) summary-level data. The MR study should satisfy three assumptions: (1) the selected IV should be related to the exposure factor; (2) IVs were not associated with other confounding factors; (3) IVs can only affect the outcome through exposure factors (Figure 1).[11]

Data Sources

The source of the gut microbiome data was the MiBioGen Consortium's GWAS dataset,[15] which included genome-wide genotype and fecal microbiome data from 18340 individuals (24 cohorts). Quantitative trait loci mapping of the microbiome included 211 taxa present in at least 10% of the samples, including 9 phyla, 16 classes, 20 orders, 35 families and 131 genera. Metabolites were derived from the IEU open GWAS project. Shin et al [16] analyzed and identified 529 metabolites in plasma or serum samples from a total of 7824 subjects across two independent cohorts, using liquid chromatography and gas chromatography coupled with tandem mass spectrometry. They further identified 486 metabolites that were present in both cohorts and suitable for genetic analysis, including 309 known metabolites and 177 unknown metabolites. Approximately 2.1 million SNPs were analyzed through genome-wide association scanning, and GWAS summary data for 452 of these metabolites (275 known and 177 unknown) were archived in the IEU OpenGWAS project. The GWAS summary data for COPD were derived from the Finngen Biobank, Round 9[17]. Disease diagnoses were based on the ICD-10 coding system, including chronic obstructive bronchitis (code J44) and emphysema (code J43). Out of 392,423 individuals, 18,788 patients were diagnosed with one of these two disease types. After genotype quality control (QC), 522 individuals who did not meet the criteria were excluded. The study also included 311,286 control subjects.

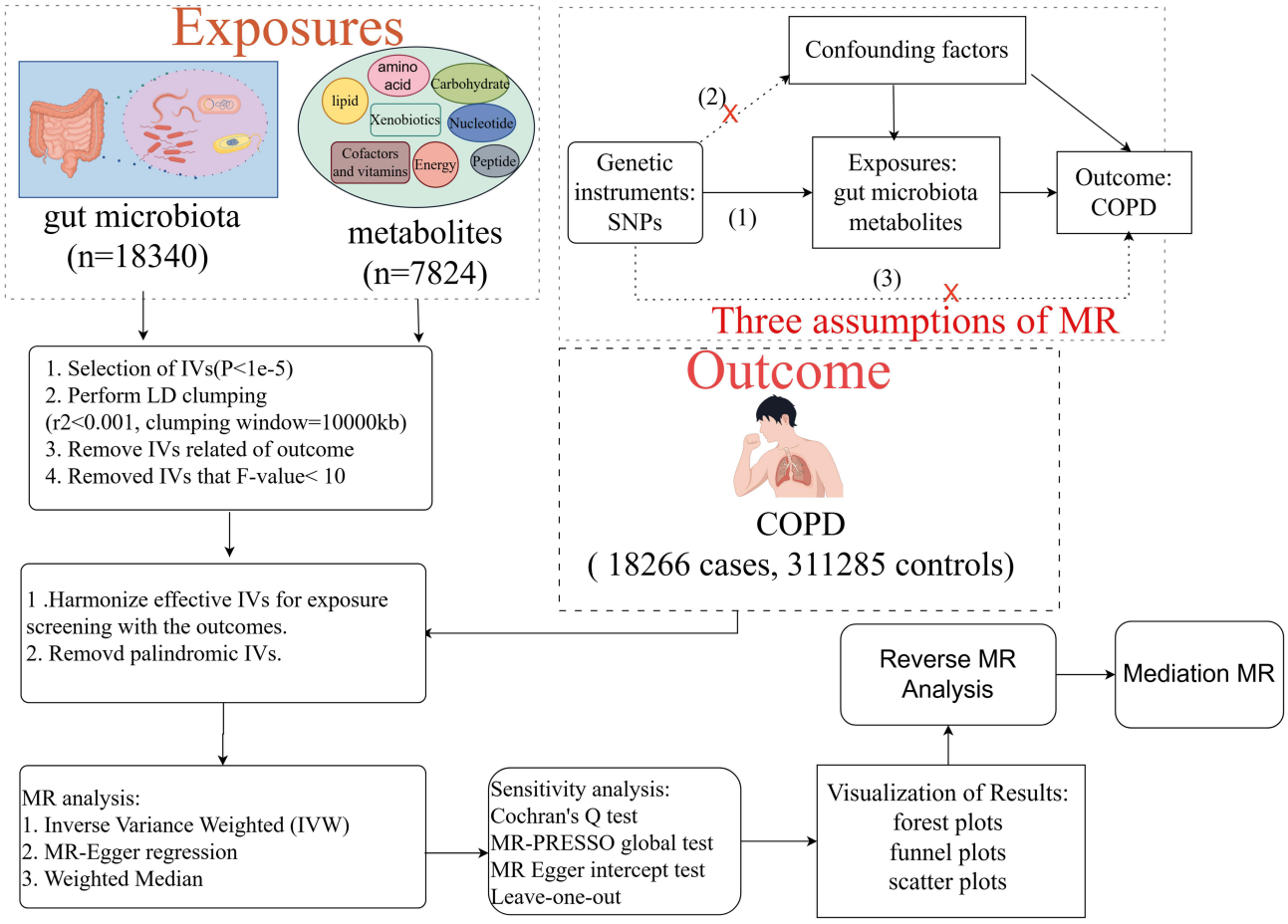


Figure 1 Schematic flow chart of this study. IVs, instrumental variables.
Abbreviations: MR, Mendelian randomization; COPD, chronic obstructive pulmonary disease; SNPs, single nucleotide polymorphisms; PRESSO, Pleiotropy Residual Sum and Outlier (By Figdraw).

The sample overlap rate between gut microbiota dataset and COPD dataset was 0.16%. The metabolite database consisted of cohorts from the UK and Germany, and no sample overlap was observed with the COPD database (Supplementary Table 1).

Instrumental Variable Selection

In the selection of IVs strongly associated with the designated exposure, stringent criteria were applied, with SNPs attaining a significance threshold of $P < 1.0*10^{-5}$ being considered. Following this, a comprehensive approach to mitigate the influence of SNP correlation and ensure the independence of genetic correlation was executed through linkage disequilibrium (LD) clumping ($r^2 < 0.001$; clumping window = 10,000 kb). The robustness of the selected SNPs was further scrutinized employing the F-statistic.[18] The F-statistic, expressed as $F = R^2 * (n - k - 1) / ((1 - R^2) * k)$, where "$R^2$" signifies the explained genetic variation, indicating the proportion of genetic variation in the exposure explicated by IVs. In this context, "n" denotes the sample size, and "k" represents the count of SNPs or IVs employed in MR analysis. SNPs with an F-value below 10 were identified as weak and subsequently excluded, in conjunction with palindromic SNPs. Additionally, any IVs absent in the outcome database underwent systematic removal, with no recourse to proxy SNPs.

MR Statistical Analysis

The investigation into the intricate interplay between intestinal flora, metabolites, and COPD entailed the systematic application of three distinct MR analytical methodologies. These methodologies comprised the Random Effect Inverse Variance Weighted (IVW), MR-Egger regression, and Weighted Median (WM) approaches. Serving as the primary

analytical modality, the IVW method amalgamated the Wald ratio of each IV in a manner akin to meta-analysis, with the robustness of results contingent upon the satisfaction of MR assumptions by each IV and the absence of horizontal pleiotropy.[19] To ensure a comprehensive evaluation of potential causal effects, the WM method and MR Egger regression method were implemented as complementary strategies to the IVW approach. The MR Egger regression method, designed to accommodate pleiotropic effects, required that the association between these effects and genetic exposure factors remains independent to achieve a more precise causal estimation[20,21] Moreover, the MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) methodology were applied to scrutinize the presence of pleiotropic bias, with outcomes mirroring IVW results subsequent to the exclusion of outliers.[22]

To bolster the integrity of our findings, a systematic series of sensitivity analyses was conducted. Initially, Cochran's Q test was harnessed to identify potential heterogeneity among IVs, with statistical significance set at $P < 0.05$ signifying the presence of such heterogeneity. Simultaneously, the MR-PRESSO global test and MR Egger intercept test were employed to scrutinize the potential for horizontal pleiotropy. In instances where an anomalous SNP was identified by the MR-PRESSO global test, said SNP was systematically excluded, and the MR Analysis was subsequently reiterated. A P-value below 0.05 denoted the presence of horizontal pleiotropy within the IVs. Furthermore, a leave-one-out sensitivity analysis was implemented to appraise whether the observed outcomes were disproportionately influenced by a single SNP. Finally, to ensure that the obtained results were not influenced by confounding factors, the "FastTraitR" package in R was used to extensively search for instrumental variables in the Catalog GWAS database. If SNPs associated with smoking were identified, they were removed, and the analysis was repeated.

One of the three methods exhibited a significance level with a $P < 0.05$ indicated a potential relationship between microbiome or metabolite and COPD. The IVW method yielded statistically significant results with no pleiotropy and heterogeneity, and the predicted effects were consistently in the same direction across all three analyses. Even when other analytical methods did not reach statistical significance ($P > 0.05$), these findings are still considered positive. In our study, we applied the Bonferroni correction to establish adjusted thresholds for each taxonomic level of gut microbiota and metabolites. These thresholds were defined as $P = 0.05/n$, where "n" represents the number of independent bacterial taxa or metabolite types.

Reverse MR Analysis

We selected SNPs associated with COPD as IV for reverse MR Analysis to verify the presence of a reverse causal effect between COPD and gut microbiota richness, as well as metabolites concentrations.

Two-Step MR and Mediation Analysis

Finally, the gut microbiota identified as significant in the previous MR analysis were used as exposures, and the metabolites identified in the previous MR analysis were used as outcomes for further MR analysis. The mediation effect and the proportion of mediation between gut microbiota-mediated changes in metabolites and the causal relationship with COPD were then calculated.

Statistical Analysis

All statistical analyses were performed using "TwoSampleMR" (version 0.5.7) packets in R (version 4.3.1). For binary outcomes, odds ratios (OR) with 95% confidence intervals (CI) were used; for continuous outcomes, regression coefficients (beta values) with 95% CI were reported.

Results

Selection of IVs

In the context of gut microbiota analysis, Supplementary Table 2 provides detailed information on the selected IVs, each demonstrating robustness with F-statistics ex-ceeding 10 (range: 16.91–88.42, median: 21.06). Notably, all selected IVs exhibit inde-pendence from outcomes ($P > 1*10-5$).

Regarding metabolite analysis (Supplementary Table 3), the F-statistic range for participating IVs spans from 17.64 to 2912.88, with a median of 21.42. Importantly, the associated P-values with outcomes surpass 1*10-5, denoting an absence of significant correlations.

In our reverse MR study focused on COPD, 103 identified SNPs, demonstrating genetic associations in the GWAS dataset, underwent meticulous adjustment for link-age disequilibrium. The F-statistic range for these IVs extends from 19.51 to 294.05, with a median of 21.78. Comprehensive details on the IVs employed in the reverse MR analysis are available in Supplementary Tables 4 and 5.

MR Analysis

MR Results of Gut Microbiota on COPD

We identified twenty-seven bacterial taxa that potentially exhibit a causal relationship with COPD through the three analysis methods of IVW (Figure 2, Supplementary Table 6), MR-Egger regression and WM, of which sixteen bacterial taxa showed positive results by IVW analysis (Supplementary Table 7). According to further sensitivity analysis, MR-Egger intercept test revealed evidence of horizontal pleiotropy in two bacterial taxa (family Bacteroidia, $P < 0.05$; order Bacteroidales, $P < 0.05$) (Supplementary Table 7), while MR-PRESSO global test did not identify any outliers (Supplementary Table 7). The Cochran's Q test indicated no significant heterogeneity ($P > 0.05$) (Supplementary Table 7), and leave-one-out method did not identify any single SNP causing bias in genetic prediction results (Figure S1). Subsequently, by cross-validating the three analytical methods, we observed inconsistent prediction trends for four bacterial taxa at the genus level: genus Butyricimonas (IVM: OR = 1.130, 95% CI = 1.021 - 1.250, $P = 0.018$; MR-Egger: OR = 0.943, 95% CI = 0.658–1.351, $P = 0.754$; WM: OR = 1.204, 95% CI = 1.047–1.385, $P = 0.009$), genus Butyricicoccus (IVW: OR = 0.848, 95% CI = 0.738–0.976, $P = 0.021$; MR-Egger: OR = 1.009, 95% CI = 0.844–1.431, $P = 0.510$; WM: OR = 0.926, 95% CI = 0.773–1.110, $P = 0.408$), genus Prevotella 9 (IVW: OR = 0.913, 95% CI = 0.843–0.988, $P = 0.023$; MR-Egger: OR = 1.014, 95% CI = 0.806–1.277, $P = 0.904$; WM: OR = 0.933, 95% CI = 0.838–1.038, $P = 0.202$) and genus Butyrivibrio (IVW: OR = 1.058, 95% CI = 1.008–1.112, $P = 0.024$; MR-Egger: OR = 0.995, 95% CI = 0.801–1.235, $P = 0.962$; WM: OR = 1.042, 95% CI = 0.974–1.116, $P = 0.232$) (Supplementary Table 7).

After removing the seven unrobust features, nine bacterial groups were considered to have a causal association with COPD (Figure 2). Genus Holdemanella (IVW: OR = 1.141, 95% CI = 1.055–1.235, $P = 9.68*10^{-4}$) and genus Marvinbryantia (IVW: OR = 1.154, 95% CI = 1.026–1.298, $P = 0.017$) are associated with an increased risk of COPD. On the contrary, seven bacterial taxa including genus Collinsella (IVW: OR = 0.828, 95% CI = 0.718–0.955,

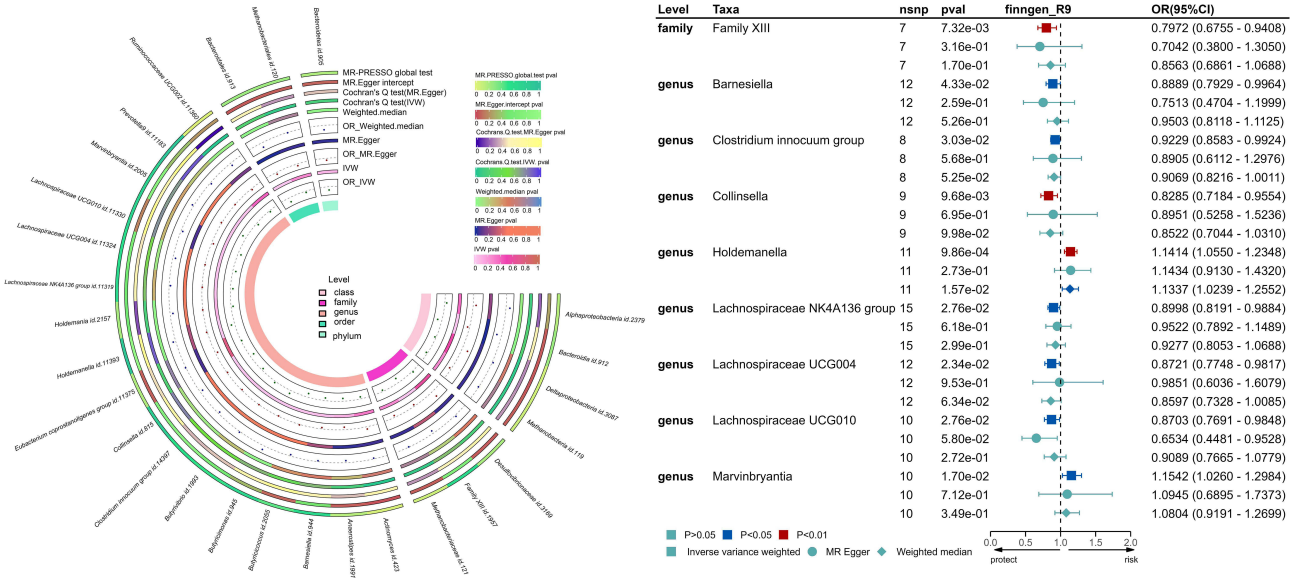


Figure 2 Potential relationship and causal relationship of gut microbiota on COPD. Significant P-values after multiple-testing correction (phylum $P = 5.56*10^{-3}$ (0.05/9), class $P = 3.13*10^{-3}$(0.05/16), order $P = 2.50*10^{-3}$(0.05/30), family $P = 1.43*10^{-3}$ (0.05/35) and genus $P = 3.82*10^{-4}$ (0.05/131).

Abbreviations: OR, odds ratio; IVW, Inverse Variance Weighted; WM, weighted median; MR-PRESSO, MR Pleiotropy Residual Sum and Outlier; CI, confidence interval.

$P = 0.010$), genus Barnesiella (IVW: OR = 0.889, 95% CI = 0.793–0.996, $P = 0.043$), genus Clostridium innocuum group (IVW: OR = 0.923, 95% CI = 0.858–0.992), $P = 0.030$), genus Lachnospiraceae UCG004 (IVW: OR = 0.872, 95% CI = 0.775–0.982, $P = 0.023$), genus Lachnospiraceae UCG010 (IVW: OR = 0.870, 95% CI = 0.769–0.985, $P = 0.028$), genus Lachnospiraceae NK4A136 group (IVW: OR = 0.900, 95% CI = 0.819–0.988, $P = 0.028$)) and family Family XIII (IVW: OR = 0.797, 95% CI = 0.675–0.941, $P = 0.007$) had a negative effect on the incidence of COPD. All results were considered nominally significant after correction for multiple testing.

MR Results of Metabolites on COPD

We initially identified twenty-five known metabolites and thirteen unknown metabolites that potentially have a potential relationship with COPD (Figure 3, Supplementary Table 8). Among these, the IVW method screened fifteen known metabolites and six unknown metabolites, which will be the focus of our analysis (Supplementary Table 9). The Cochran's Q test for IVW did not provide significant evidence of heterogeneity (Supplementary Table 9). Additionally, the MR-PRESSO global test indicated no evidence of horizontal Pleiotropy ($P > 0.05$) (Supplementary Table 9). However, the MR-Egger intercept test indicated potential horizontal pleiotropy for an unknown metabolite (X-12040, $P < 0.05$) and a nucleotide metabolite (pseudouridine, $P < 0.05$) (Supplementary Table 9). No abnormal SNP was detected in the Leave-one-out test (Figure S2). Subsequently, through further cross-validation of the three methods, we excluded two metabolites (taurodeoxycholate (IVW: OR = 0.852, 95% CI = 0.727–0.997, $P = 0.046$; MR-Egger: OR = 1.005, 95% CI = 0.569–1.775, $P = 0.987$; WM: OR = 0.977, 95% CI = 0.811–1.177, $P = 0.811$) and X-10500 (IVW: OR = 0.555, 95% CI = 0.348–0.884, $P = 0.013$; MR-Egger: OR = 1.243, 95% CI = 0.309–5.000, $P = 0.762$; WM: OR = 0.573, 95% CI = 0.275–1.191, $P = 0.136$)) (Supplementary Table 9).

Among the identified know metabolites (Figure 3), three amino acids (5-oxoproline (IVW: OR = 2.083 95% CI = 1.328–3.266, $P = 0.001$), creatine (IVW: OR = 1.415, 95% CI = 1.066–1.880, $P = 0.016$) and phenyllactate (PLA) (IVW: OR = 1.472, 95% CI = 1.031–2.104, $P = 0.034$)) and a lipid metabolite (1-heptadecanoylglycerophosphocholine (IVW:

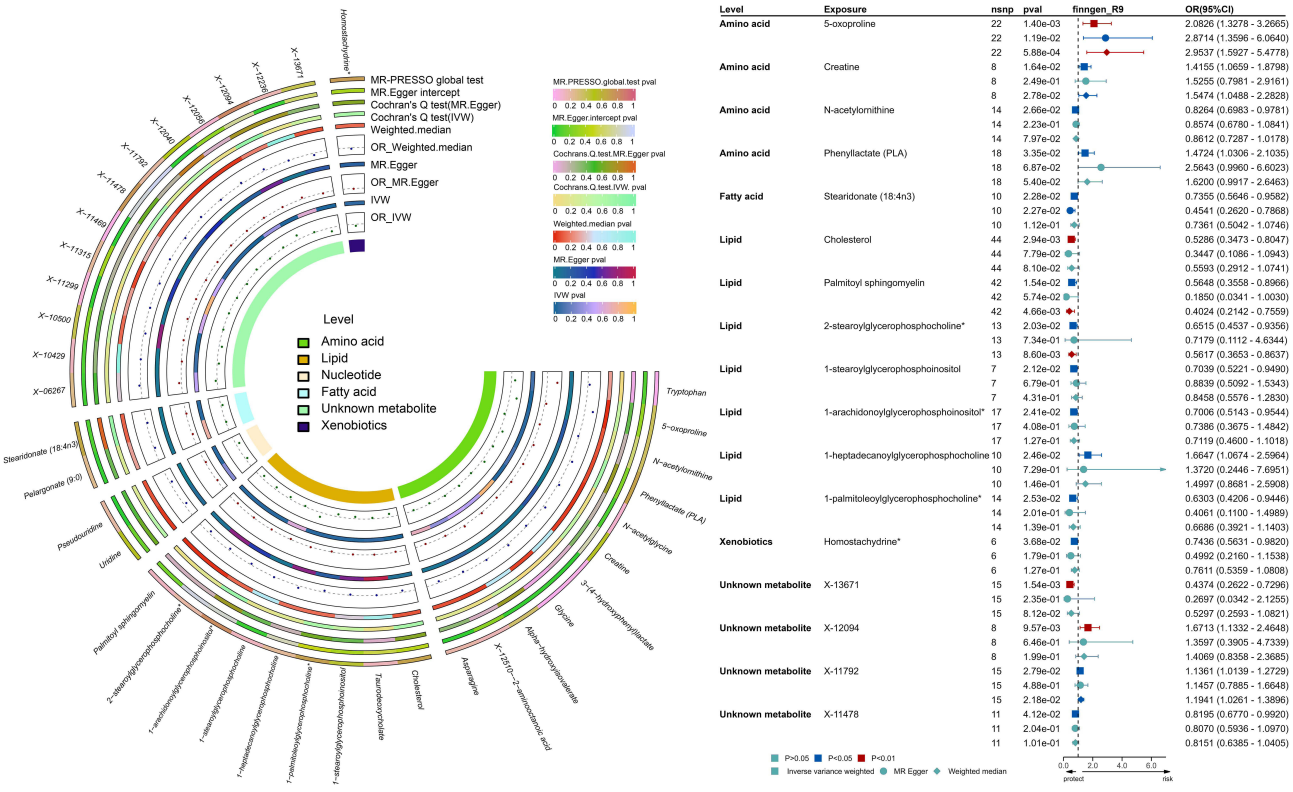


Figure 3 Potential relationship and causal relationship of metabolites on COPD. Significant P-values after multiple-testing correction $P = 1.10*10^{-4}$ (0.05/452).
Abbreviations: OR, odds ratio; IVW, Inverse Variance Weighted; WM, weighted median; MR-PRESSO, MR Pleiotropy Residual Sum and Outlier; CI, confidence interval.

OR = 1.665, 95% CI = 1.067–2.596, $P = 0.025$)) exhibited an association with increasing risk of COPD. Six lipids including cholesterol (IVW: OR = 0.529, 95% CI = 0.347–0.805, $P = 0.003$), 1-stearoylglycerophosphoinositol (IVW: OR = 0.704, 95% CI = 0.522–0.949, $P = 0.021$), 1-palmitoleoylglycerophosphocholine (IVW: OR = 0.630, 95% CI = 0.421–0.945, $P = 0.025$), 1-arachidonoylglycerophosphoinositol (IVW: OR = 0.701, 95% CI = 0.514–0.954, $P = 0.024$), 2-stearoylglycerophosphocholine (IVW: OR = 0.651, 95% CI = 0.454–0.936, $P = 0.02$) and palmitoyl sphingomyelin (IVW: OR = 0.565, 95% CI = 0.356–0.897, $P = 0.015$) have a negative effect on the incidence of COPD. Additionally, our study revealed that N-acetylornithine (IVW: OR = 0.826, 95% CI = 0.698–0.978, $P = 0.027$), stearidonate (18:4n3) (IVW: OR = 0.735, 95% CI = 0.565–0.958, $P = 0.023$) and homostachydrine (IVW: OR = 0.744, 95% CI = 0.563–0.982, $P = 0.037$) were significantly associated with reduced risk of COPD. In addition to this, we identified four unknown metabolites with causal effects on COPD. These associations did not withstand Bonferroni correction and were considered nominally significant.

Reverse MR Analysis

MR Results of COPD on Gut Microbiota

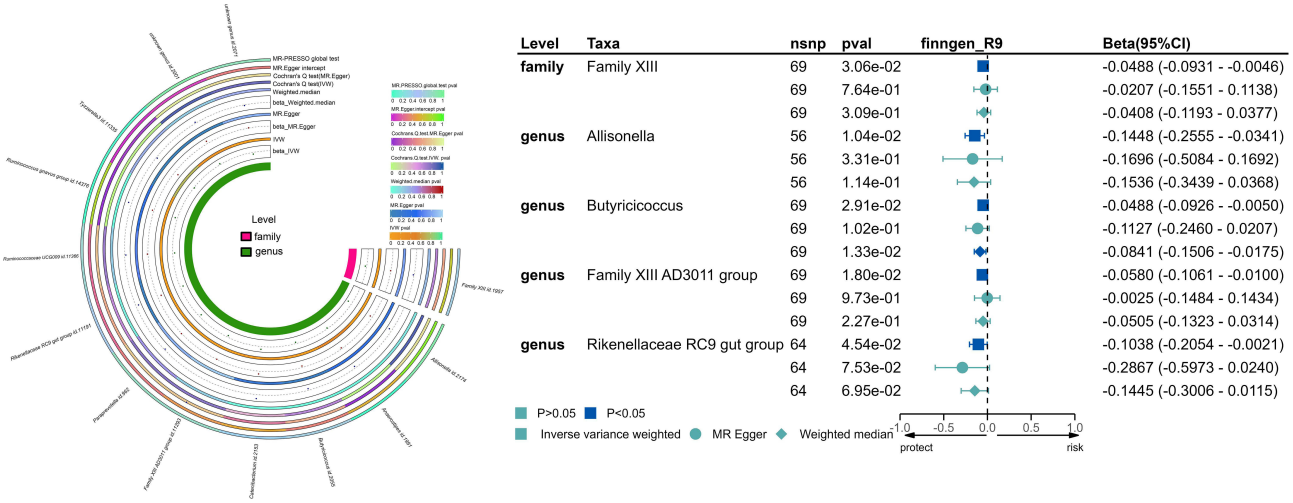
The reverse MR results revealed a potentially causal effect of COPD on the abundance of thirteen intestinal microbiome taxa (Figure 4, Supplementary Table 10), eight bacteria identified through IVW (Supplementary Table 11), and sensitive analysis did not reveal potential level multiplicity or heterogeneity (Supplementary Table 11). And no abnormalities were detected in the Leave-one-out test either (Figure S3). Finally, three bacteria species at the genus level (Paraprevotella (IVW: Beta = −0.084, 95% CI = −0.152 - −0.016, $P = 0.015$; MR-Egger: Beta = 0.021, 95% CI = −0.188–0.229, $P = 0.845$; WM: Beta = −0.043, 95% CI = −0.160–0.075, $P = 0.475$), Ruminococcaceae UCG009 (IVW: Beta = −0.069, 95% CI = −0.135 - −0.003, $P = 0.040$; MR-Egger: Beta = 0.050, 95% CI = −0.151–0.252, P = 0.624; WM: Beta = −0.069, 95% CI = −0.182–0.044, $P = 0.233$) and unknown genus id.2071 (IVW: Beta = −0.062, 95% CI = −0.113 - −0.011, $P = 0.017$; MR-Egger: Beta = 0.016, 95% CI = −0.140–0.172, $P = 0.841$; WM: Beta = −0.036, 95% CI = −0.115–0.043, $P = 0.343$)), which displayed different genetic prediction directions, were excluded based on cross-validation using three MR methods.

COPD showed negative correlation with family Family XIII (IVW: Beta = −0.049, 95% CI = −0.093 - −0.005, $P = 0.031$), genus Allisonella (IVW: Beta = −0.145, 95% CI = −0.256 - −0.034, $P = 0.010$), genus Butyricicoccus (IVW: Beta = −0.049, 95% CI = −0.093 - −0.005, $P = 0.029$), genus Family XIII AD3011 group (IVW: Beta = −0.058, 95% CI = −0.106 - −0.010, $P = 0.018$) and genus Rikenellaceae RC9 gut group (IVW: Beta = −0.104, 95% CI = −0.205 - −0.002, $P = 0.045$) (Figure 4). Although these associations did not withstand Bonferroni correction and were considered nominally significant.

MR Results of COPD on Metabolites

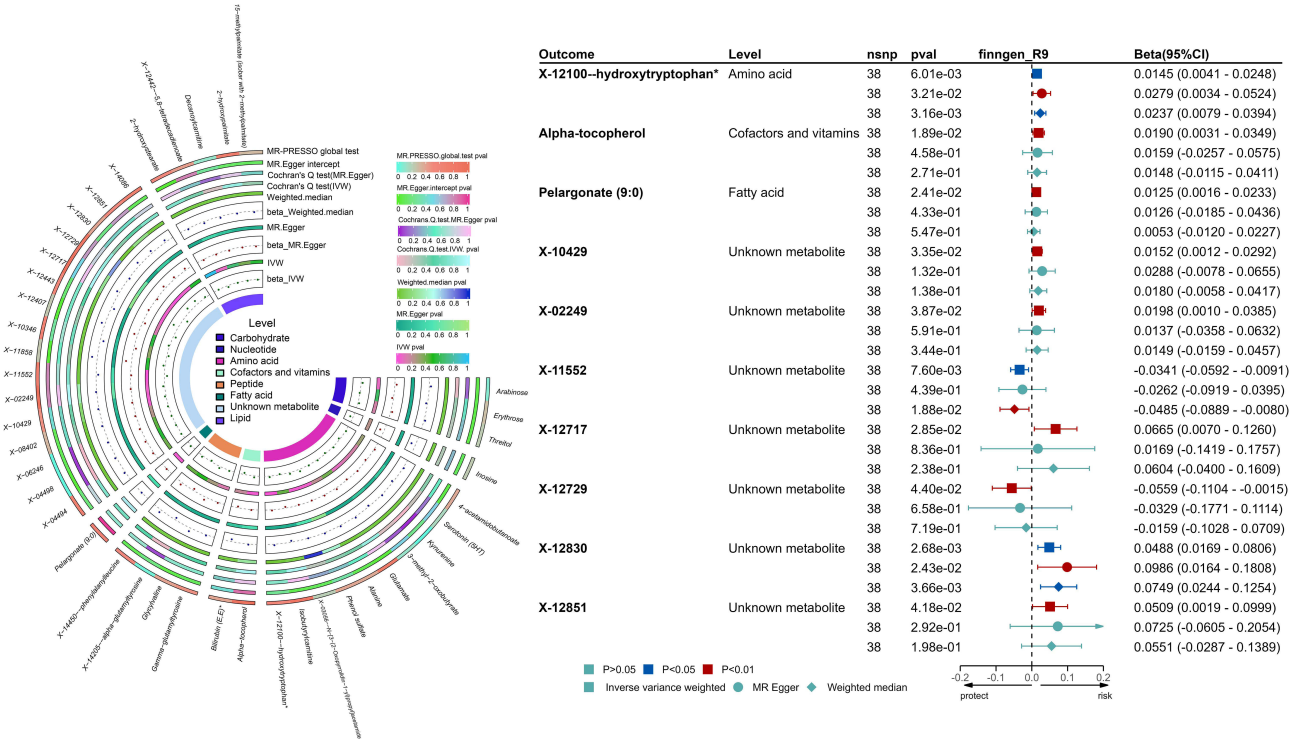
The potential causal effects of COPD on sixteen unknown metabolites and twenty-six known metabolites were preliminarily identified (Figure 5, Supplementary Table 12). The IVW method yielded significant results for a total of five known metabolites and seven unknown metabolites (Supplementary Table 13). Subsequently, MR-Egger intercept test and MR-PRESSO global test revealed no evidence of pleiotropy ($P > 0.05$) (Supplementary Table 13), and Cochran's Q test indicated no significant heterogeneity ($P > 0.05$) (Supplementary Table 13). Detailed results from the Leave-one-out test are presented in Figure S4. No single SNP was found to cause abnormal outcomes. Finally, cross-validation using the three MR methods revealed inconsistent directions in the genetic prediction of the two metabolite results (phenol sulfate (IVW: Beta = 0.028, 95% CI = 0.001–0.054, $P = 0.038$; MR-Egger: Beta = −0.007, 95% CI = −0.078–0.063, $P = 0.838$; WM: Beta = 0.018, 95% CI = −0.025–0.061, $P = 0.413$) and X-03056–N-[3-(2-Oxopyrrolidin-1-yl)propyl] acetamide (IVW: Beta = 0.017, 95% CI = 0.002 =0.032, $P = 0.023$; MR-Egger: Beta = −0.013, 95% CI = −0.050–0.025, $P = 0.511$; WM: Beta = −0.0005, 95% CI = −0.021–0.020, P = 0.966)).

Through sensitivity analysis and quality controls, we finally obtained reliable findings. Focusing on the results of three known metabolites, we found a positive correlation between COPD with Alpha-tocopherol (IVW: Beta = 0.019, 95% CI = 0.003–0.035, $P = 0.019$), Pelargonate (9:0) (IVW: Beta = 0.012, 95% CI = 0.002–0.023, $P = 0.024$) and X-12100–hydroxytryptophan (IVW: Beta = 0.014, 95% CI = 0.004–0.025, $P = 0.006$) (Figure 5). Additionally, we similarly confirmed the effect of COPD on the concentrations of seven unknown metabolites (Figure 5). All of the above findings are considered nominally significant.



Level	Taxa	nsnp	pval	finngen_R9	Beta(95%CI)
family	Family XIII	69	3.06e-02		-0.0488 (-0.0931 - -0.0046)
		69	7.64e-01		-0.0207 (-0.1551 - 0.1138)
		69	3.09e-01		-0.0408 (-0.1193 - 0.0377)
genus	Allisonella	56	1.04e-02		-0.1448 (-0.2555 - -0.0341)
		56	3.31e-01		-0.1696 (-0.5084 - 0.1692)
		56	1.14e-01		-0.1536 (-0.3439 - 0.0368)
genus	Butyricicoccus	69	2.91e-02		-0.0488 (-0.0926 - -0.0050)
		69	1.02e-01		-0.1127 (-0.2460 - 0.0207)
		69	1.33e-02		-0.0841 (-0.1506 - -0.0175)
genus	Family XIII AD3011 group	69	1.80e-02		-0.0580 (-0.1061 - -0.0100)
		69	9.73e-01		-0.0025 (-0.1484 - 0.1434)
		69	2.27e-01		-0.0505 (-0.1323 - 0.0314)
genus	Rikenellaceae RC9 gut group	64	4.54e-02		-0.1038 (-0.2054 - -0.0021)
		64	7.53e-02		-0.2867 (-0.5973 - 0.0240)
		64	6.95e-02		-0.1445 (-0.3006 - 0.0115)

Figure 4 Potential relationship and causal relationship of COPD on gut microbiota. Significant P-values after multiple-testing correction (phylum $P = 5.56*10^{-3}$ (0.05/9), class $P = 3.13*10^{-3}$ (0.05/16), order $P = 2.50*10^{-3}$ (0.05/30), family $P = 1.43*10^{-3}$ (0.05/35) and genus $P = 3.82*10^{-4}$ (0.05/131).
Abbreviations: OR, odds ratio; IVW, Inverse Variance Weighted; WM, weighted median; MR-PRESSO, MR Pleiotropy Residual Sum and Outlier; CI, confidence interval; IVW, Inverse Variance Weighted; WM, weighted median; MR-PRESSO, MR Pleiotropy Residual Sum and Outlier; CI, confidence interval.



Outcome	Level	nsnp	pval	finngen_R9	Beta(95%CI)
X-12100--hydroxytryptophan*	Amino acid	38	6.01e-03		0.0145 (0.0041 - 0.0248)
		38	3.21e-02		0.0279 (0.0034 - 0.0524)
		38	3.16e-03		0.0237 (0.0079 - 0.0394)
Alpha-tocopherol	Cofactors and vitamins	38	1.89e-02		0.0190 (0.0031 - 0.0349)
		38	4.58e-01		0.0159 (-0.0257 - 0.0575)
		38	2.71e-01		0.0148 (-0.0115 - 0.0411)
Pelargonate (9:0)	Fatty acid	38	2.41e-02		0.0125 (0.0016 - 0.0233)
		38	4.33e-01		0.0126 (-0.0185 - 0.0436)
		38	5.47e-01		0.0053 (-0.0120 - 0.0227)
X-10429	Unknown metabolite	38	3.35e-02		0.0152 (0.0012 - 0.0292)
		38	1.32e-01		0.0288 (-0.0078 - 0.0655)
		38	1.38e-01		0.0180 (-0.0058 - 0.0417)
X-02249	Unknown metabolite	38	3.87e-02		0.0198 (0.0010 - 0.0385)
		38	5.91e-01		0.0137 (-0.0358 - 0.0632)
		38	3.44e-01		0.0149 (-0.0159 - 0.0457)
X-11552	Unknown metabolite	38	7.60e-03		-0.0341 (-0.0592 - -0.0091)
		38	4.39e-01		-0.0262 (-0.0919 - 0.0395)
		38	1.88e-02		-0.0485 (-0.0889 - -0.0080)
X-12717	Unknown metabolite	38	2.85e-02		0.0665 (0.0070 - 0.1260)
		38	8.36e-01		0.0169 (-0.1419 - 0.1757)
		38	2.38e-01		0.0604 (-0.0400 - 0.1609)
X-12729	Unknown metabolite	38	4.40e-02		-0.0559 (-0.1104 - -0.0015)
		38	6.58e-01		-0.0329 (-0.1771 - 0.1114)
		38	7.19e-01		-0.0159 (-0.1028 - 0.0709)
X-12830	Unknown metabolite	38	2.68e-03		0.0488 (0.0169 - 0.0806)
		38	2.43e-02		0.0986 (0.0164 - 0.1808)
		38	3.66e-03		0.0749 (0.0244 - 0.1254)
X-12851	Unknown metabolite	38	4.18e-02		0.0509 (0.0019 - 0.0999)
		38	2.92e-01		0.0725 (-0.0605 - 0.2054)
		38	1.98e-01		0.0551 (-0.0287 - 0.1389)

Figure 5 Potential relationship and causal relationship of COPD on metabolites. Significant P-values after multiple-testing correction $P = 1.10*10^{-4}$ (0.05/452).
Abbreviations: IVW, Inverse Variance Weighted; WM, weighted median; MR-PRESSO, MR Pleiotropy Residual Sum and Outlier; CI, confidence interval.

Two-Step MR and Mediation Analysis

Based on our findings, we identified 17 metabolites as potential mediators and conducted a comprehensive MR analysis to examine the influence of gut microbiota on their concentrations. The analysis revealed that the family Family XIII is associated with lower levels of X-12094 (IVW: Beta = −0.085, 95% CI = −0.166 - −0.005, $P = 0.037$), while the genus Collinsella is linked to a reduction in X-13671 levels (IVW: Beta = −0.066, 95% CI = −0.125 - −0.007, $P = 0.029$). Additionally, the genus Lachnospiraceae NK4A136 group was found to increase X-12094 levels (IVW: Beta = 0.058

Table 1 Mediation MR Analysis Results

Exposure	Mediation	Mediation Effect	95% CI		Proportion
Family XIII	X-12094	−0.044	−0.099	0.345	19%
Genus Marvinbryantia	1-palmitoleoylglycerophosphocholine*	0.035	−0.013	0.439	24%
Genus Marvinbryantia	X-13671	0.046	−0.009	0.558	32%

95% CI = 0.003–0.114, $P = 0.028$), and the genus Marvinbryantia was associated with reductions in both X-13671 (IVW: Beta = −0.056, 95% CI = −0.110 - −0.001, $P = 0.047$) and 1-palmitoleoylglycerophosphocholine levels (IVW: Beta = −0.075, 95% CI = −0.147 - −0.003, $P = 0.040$) (Supplementary Table 14). Further sensitivity analysis did not find obvious horizontal pleiotropy and heterogeneity, and leave-one-out analysis did not find the mediation of single SNP.

In conclusion, our research identified three metabolites, including two unknowns and one lipid, that mediate the connection between gut microbiota and COPD. The level of 1-palmitoleoylglycerophosphocholine mediates the effect of the genus Marvinbryantia on COPD (mediation effect: 0.0347, 95% CI = −0.0127–0.4392, mediation proportion: 24.2%). We also found that the level of the X-13671 metabolite mediates the association between the genus Marvinbryantia and COPD (mediation effect: 0.0460, 95% CI = −0.0095–0.5577, mediation proportion: 32.1%). The metabolite X-12094 mediates the effect of the family Family XIII on COPD (mediation effect: −0.044, 95% CI = −0.0993–0.3445, mediation proportion: 19.4%) (Table 1).

Discussion

Our study meticulously executed an expansive and detailed genetic analysis aimed at elucidating the causal implications of gut microbiota and metabolites in the pathogenesis of COPD. Building upon prior research findings, which underscored the contributory role of altered gut microbiota in COPD through the modulation of metabolites and the initiation of inflammatory responses.[23,24] Our investigation successfully delineated specific causal relationships. We identified correlations between nine distinct gut microbiome taxa and thirteen established known metabolites closely associated with COPD. Moreover, we discerned various potential features with plausible effects, thereby augmenting our comprehension of the intricate interplay between gut microbiota, metabolites, and the onset of COPD.

The respiratory and gastrointestinal epithelia share structural similarities and the same embryonic origin. Previous epidemiological studies have found a higher prevalence of gut diseases in patients with chronic lung diseases. Smoking may contribute to this process, as it is the primary cause of COPD, with over 80% of COPD patients having a history of smoking.[25] Smoke exposure increases the number of lung dendritic cells (DCs), affecting their homing and thereby disrupting normal immune responses. IL-6 and TGF-β induce Th17-polarized immune responses outside the organs, while IL-13 stimulates cross-organ responses of NK cells and macrophages, driving interactions between the lung and gut.[25] Additionally, toxic substances in cigarette smoke can induce gut microbiota dysbiosis through various mechanisms. Smoking also increases intestinal pH, leading to gut microbiota imbalance. Some studies have observed an increase in Bacteroidetes and a decrease in Firmicutes and Proteobacteria in smokers.[26] Dysbiosis of the gut microbiota may contribute to the development of gastrointestinal diseases such as inflammatory bowel disease (IBD).[27] This suggests that smoking may cause gastrointestinal diseases by altering the gut microbial environment, leading to dysbiosis. Furthermore, lung-gut crosstalk may promote the development of COPD. However, further research is needed to validate these findings.

The genus Holdemanella, classified under the phylum Firmicutes, encompasses species such as Holdemanella biformis.[28] This particular genus has garnered attention for its potential health implications, as it has been observed to elicit anti-tumor and anti-inflammatory effects through the release of both short-chain fatty acids (SCFAs) and long-chain fatty acids (LCFAs),[29,30] thereby suggesting a plausible contribution to human health.[31] However, it differs from our findings. It is worth mentioning that previous studies have also found the pathogenic role of Holderman in certain diseases.[32,33] These discrepancies underscore the complexity of microbiome research, emphasizing that categorizing microbes as solely beneficial or harmful may oversimplify the intricate dynamics. The impact of microbes on the human body is multifaceted and influenced by genetic factors, dietary patterns, and lifestyle choices.[34] Furthermore, it is essential to recognize that different bacterial species within

the same genus may exhibit diverse effects on disease, adding an additional layer of complexity to our understanding of microbial contributions to health and disease.

Within the framework of the present study, the genus Marvinbryantia demonstrated a nominally significant positive association with COPD. Noteworthy is the reduced abundance of genus Marvinbryantia observed in individuals affected by conditions such as Alzheimer's disease, insulin resistance, or type 2 diabetes mellitus.[35,36] However, current evidence falls short of definitively establishing a correlation between Marvinbryantia abundance and COPD risk. Noteworthy findings from a recent investigation indicate that exposure to nicotine correlates with an augmented abundance of Marvinbryantia,[37] thereby introducing novel perspectives on potential pathogenic mechanisms linked to Marvinbryantia in the context of COPD.

Within the scope of our investigation, we identified seven protective causal associations linked to COPD, including family Family XIII, genus Collinsella, genus Lachnospiraceae UCG004, genus Lachnospiraceae UCG010, genus Lachnospiraceae NK4A136 group, genus Clostridium innocuum group and genus Barnesiella. Among them, the genus Lachnospiraceae UCG004, genusLachnospiraceae UCG010 and genus Lachnospiraceae NK4A136 group all falling under the family Lachnospiraceae, exhibit the capacity to produce SCFAs like butyrate and acetate, known for their anti-inflammatory effects through immune regulation.[38] Our findings corroborate with a preceding study that reported a diminished abundance of Lachnospiraceae in the intestinal microbiota of COPD patients compared to their healthy counterparts.[10] Additionally, the genus Clostridium innocuum group, recognized for its role in SCFAs production.[39] Notably, the genus Barnesiella, associated with positive health outcomes, exhibited a higher abundance in the gut microbiota of healthy individuals based on a meta-analysis of 3040 public datasets and 16S rRNA analyses of individuals affected by intestinal diseases.[40] Furthermore, genus Barnesiella demonstrated efficacy in preventing and treating vancomycin-resistant Enterococcus faecium colonization,[41] as well as enhancing immunomodulatory therapy in certain types of cancer.[42]

Previous scholarly investigations have elucidated genus Collinsella's capability to biosynthesize ursodeoxycholic acid (UDCA),[43] This bioactive compound has been shown to exert inhibitory effects on the binding of SARS-CoV-2 to angiotensin-converting enzyme 2 (ACE2), suggesting noteworthy therapeutic implications. Moreover, UDCA has demonstrated its efficacy in suppressing inflammatory factors, showcasing antioxidant and anti-apoptotic properties, and enhancing alveolar fluid clearance, particularly in the context of acute respiratory distress syndrome (ARDS).[43] Within the intricate milieu of the intestinal microbiota, genus Collinsella assumes a prominent role alongside Bifidobacterium dominance and may contribute to influencing lipid metabolism, thereby potentially impacting concentrations of fasting triglycerides, total cholesterol, and high-density lipoprotein cholesterol.[44]

We also found a negative causal effect of COPD on the richness of family Family XIII, genus Allisonella, genus Butyricicoccus, genus Family XIII AD3011 group and genus Rikenellaceae RC9 gut group. In the COPD milieu, the presence of systemic inflammatory mediators may potentially contribute to the compromised health of the gastrointestinal tract. Concurrently, chronic hypoxia has been implicated in the impairment of gastrointestinal epithelial integrity.[45] Additionally, the shared embryonic origin and structural resemblance between the gastrointestinal and respiratory systems may underlie the observed changes in intestinal microbiota in response to the inflammatory milieu in COPD patients. Nevertheless, the precise mechanistic underpinnings of these alterations remain elusive.[46]

The genus Butyricicoccus can degrade polysaccharides through the autocrine secretion of multienzyme complexes, producing butyrate and other SCFAs.[47] These SCFAs act as effective anti-inflammatory mediators, maintaining the intestinal epithelial barrier, balancing the gut microbiota, inhibiting the expression of destructive cytokines, and regulating immunity and inflammation, thereby providing protective effects against ulcerative colitis.[48] Our study reports a negative impact on their abundance following the onset of COPD. In a cohort study, it was also observed that patients with lung cancer combined with COPD had a general reduction in SCFA-producing bacteria, including genus Butyricicoccus, compared to patients with lung cancer alone.[49] Additionally, patients with lung cancer combined with COPD often had lower SCFA concentrations compared to those with lung cancer alone or healthy controls. This may be positively correlated with a reduction in SCFA-producing bacteria in the gut following the onset of COPD, which could further affect the integrity of the intestinal barrier. In other words, the gut microbiota changes caused by COPD, leading to reduced SCFA production, may promote the development of gastrointestinal diseases.[47] This could be a potential mechanism of the "lung-gut axis", warranting further research.

Among the metabolite profiles examined, notable findings emerged, with 3 amino acid metabolites showing a nominally significant contribution to the onset of COPD. The compound 5-Oxoproline, a derivative of proline, assumes a pivotal role in glutathione metabolism, undergoing hydrolysis to generate glutamate for the regeneration of glutathione through 5-Oxoprolinase activity. Its accumulation suggests potential oxidative stress occurrences.[50] However, the validation of a definitive causal link between 5-oxoproline and COPD awaits substantiation through clinical and experimental investigations. The findings from our study may offer preliminary insights for guiding future research endeavors. Conversely, there is no relevant research to prove that the accumulation of creatine promotes the pathogenesis of COPD, and it may even be beneficial for COPD patients.[51] Further studies may be needed to elucidate its related effects. In addition, phenyllactate was found has wide antimicrobial activity, it is lactic acid bacteria to produce more, for its potential is still related literature reports to the causal effect of COPD, but some studies have found that the production of phenyllactic acid may interfere with the oxidation state, lead to the occurrence of mitochondrial damage, and even induce apoptosis.[52]

Our study found that different changes in lipid metabolite concentration have different causal effects on the pathogenesis of COPD, shedding light on the integral role of lipid metabolism in COPD pathogenesis.[42] Sphingolipids, specifically sphingomyelin, were notably implicated in exacerbating oxidative stress and damage induced by tobacco smoke. This exacerbation occurs through the inhibition of antioxidant enzymes, particularly superoxide dismutase, resulting in the accumulation of superoxide radicals.[53] Noteworthy is the association of Palmitoyl sphingomyelin with an elevated risk of cardiovascular disease in individuals with type 2 diabetes.[54] Additionally, glycerophospholipid metabolism exhibited a correlation with increased airflow obstruction and heightened COPD exacerbations.[55] The role of cholesterol in COPD remains a subject of controversy, underscored by recent research findings. Toru et al's[56] study revealed a negative correlation between HDL cholesterol levels and muscle mass, as well as trunk muscle density in COPD patients. Conversely, LDL levels exhibited a positive correlation with physical activity levels in the same cohort.[56] Another study reported that hyperlipidemia was linked to a reduced risk of pneumonia and improved all-cause mortality in COPD patients.[57] Our investigation aligns with the nuanced nature of these findings, shedding light on potential protective effects associated with cholesterol in the context of COPD. However, the intricacies of this relationship necessitate further exploration, particularly in delineating the specific roles of HDL and LDL cholesterol. Smoking can also influence lipid and cholesterol metabolism and interfere with the cytochrome enzyme system responsible for their transport, leading to an increase in triglycerides and cholesterol lipoproteins.[58] This underscores the imperative for ongoing research to elucidate the underlying mechanisms and contribute to a comprehensive understanding of the interplay between cholesterol and COPD. While lipid metabolism serves diverse roles in the body, the intricate balance among various lipid types adds complexity to our understanding of their specific relationship to COPD pathogenesis.[59,60] In our investigation, we identified two metabolites, synstachydrine and N-acetylornithine, demonstrating a protective effect against COPD. However, the precise underlying mechanisms governing this association remain elusive. These findings suggest potential therapeutic targets for future interventions, subject to validation through rigorous prospective studies. Further research is imperative to substantiate these observations and elucidate the pathways through which synstachydrine and N-acetylornithine may contribute to COPD protection.

Numerous empirical investigations have underscored a discernible correlation between the metabolic characteristics inherent in COPD and the severity of accompanying lung function impairment. Pertinent studies have delineated specific metabolites demonstrating either positive or negative associations with diverse lung function variables, as documented in the scientific literature.[51,61,62] This nexus serves to enrich our understanding of the nuanced spectrum of disease severity within the COPD paradigm. However, it is imperative to acknowledge the inherent limitations stemming from the observational nature of these inquiries, which preclude the unequivocal establishment of a causal relationship between metabolites and COPD. The employment of Reverse MR analysis has brought to light direct repercussions of COPD on metabolic profiles, encompassing noteworthy alterations in fatty acid, protein, lipoprotein, amino acid, and nucleotide metabolism. The specific mechanisms precipitating these metabolic shifts remain elusive, yet plausible connections to heightened inflammation and oxidative stress in vivo, alongside chronic hypoxia-induced metabolic perturbations in COPD patients, merit consideration. Despite these insights, the imperative for further clinical validation remains, serving as a necessary step to authenticate and substantiate these intricate associations, thereby advancing our comprehension of the dynamic interplay between metabolic dynamics and the pathophysiology of COPD. Alpha-tocopherol is an exogenous antioxidant that exerts potent antioxidant effects through chain-breaking activity, membrane repair, and free radical

scavenging.[63] In COPD patients, systemic oxidative stress is elevated, which correspondingly leads to an increase in systemic antioxidant defenses, peaking within 48 hours during acute exacerbations.[64] However, the consumption of non-enzymatic antioxidants is substantial, making it necessary to supplement them to prevent disease exacerbation caused by oxidative stress.

Yi et al and Hanyu et al have also utilized MR analysis to validate the causal relationship between gut microbiota and COPD.[13,14] Nevertheless, our investigation diverges markedly from these antecedent studies on several fronts. Primarily, our research adopts a more exhaustive approach in elucidating the etiological landscape of COPD, encompassing a comprehensive examination of the causal connections between metabolites and COPD. Secondly, our study adeptly establishes bidirectional causality, unraveling the intricate associations between gut microbiota, metabolites, and COPD. Notably, our methodology distinguishes itself by leveraging a more expansive GWAS dataset inclusive of COPD, coupled with a more rigorous selection process for IVs in comparison to the study conducted by Yi et al. These refinements bolster the precision and reliability of our findings, thereby contributing to an enhanced understanding of the nuanced interplay between gut microbiota, metabolites, and the underlying pathogenic mechanisms in COPD.

However, our study is not without its limitations. Firstly, the GWAS summary data employed for COPD, blood metabolites, and the majority of gut microbiota predominantly originate from European populations. A minor fraction of the gut microbiome data is sourced from other ethnic backgrounds, potentially introducing bias into our results. Secondly, to ensure an adequate number of SNPs as IVs, we adopted a more lenient threshold ($1*10^{-5}$) instead of the commonly used threshold in other MR studies ($5*10^{-8}$). This choice may impact the accuracy of our findings. Additionally, the gut microbiome summary dataset we selected classifies at the genus level rather than the species or strain level. Moreover, within the GWAS summary data for metabolites, 177 metabolites were designated as unknown. Although our study successfully identified causal relationships involving some of these unknown metabolites and COPD, conducting further analysis through a literature review proved challenging. Finally, our study did not establish specific causal relationships between gut microbiota and metabolites, nor could it elucidate the specific mechanisms through which they influence COPD. These limitations underscore the importance of careful consideration when interpreting our findings. Therefore, conducting prospective studies for additional validation, given the limitations of GWAS data and MR analysis, remains imperative.

Conclusion

Utilizing MR analysis, our research has effectively disentangled the reciprocal causal relationships between gut microbiota richness, metabolite concentrations, and the onset of COPD. This nuanced analytical framework has not only facilitated the identification of causative and protective factors pertinent to COPD but has also shed light on the repercussions of COPD development on gut microbiota richness and metabolite concentrations. The implications of our findings are far-reaching, potentially yielding indispensable biomarkers for early diagnostic interventions and the dynamic monitoring of COPD progression. Furthermore, our study introduces novel dimensions to the preventive and therapeutic landscapes of COPD. It is imperative, however, to underscore the need for a deeper understanding of the intricate mechanistic underpinnings governing these associations, which necessitates further exploration through prospective research initiatives.

Data Sharing Statement

Data on gut microbiota can be accessed at www.mibiogen.org, blood metabolite data are available from the IEU Open GWAS project (https://gwas.mrcieu.ac.uk/), and the COPD dataset can be obtained through the Finngen website (https://www.finngen.fi/).

Ethical Approval

GWAS summary statistics for the studies used for analysis were composed and obtained from published studies. All studies had prior approval from their respective institutional review boards (IRBs). The Institutional Review Board of the China-Japan Union Hospital of Jilin University approved the protocol for this study, and in accordance with their guidelines, this study only used publicly available data and did not use any individual-level data. Therefore, no additional IRB approval was required.

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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.

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Disclosure

The authors declare that this study was conducted in the absence of any commercial or financial relationship that could be interpreted as a potential conflict of interest.

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