International Journal of Chronic Obstructive Pulmonary Disease

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Nicotine Metabolism and Chronic Obstructive Pulmonary Disease: Mendelian Randomization and Phenotypic Characterization Analysis

Ting-Fen Ji[1–7,*], Shi-Wen Song[2–7,*], Ying Xie[2–8], Zhao Liu[2–7], Ying-Man Gao[2–8], An-Qi Cheng[2–7], Xin-Mei Zhou[2–7], Min Wang[2–7], Hua-Ping Dai[3–6,9], Liang Zhao[2–7], Dan Xiao[2–7], Chen Wang[2–7]

[1]Department of Tobacco Control and Prevention of Respiratory Diseases, China-Japan Friendship Hospital, Capital Medical University, Beijing, People's Republic of China; [2]Department of Tobacco Control and Prevention of Respiratory Diseases, Center of Respiratory Medicine, China-Japan Friendship Hospital, Beijing, People's Republic of China; [3]National Center for Respiratory Medicine, Beijing, People's Republic of China; [4]State Key Laboratory of Respiratory Health and Multimorbidity, Beijing, People's Republic of China; [5]National Clinical Research Center for Respiratory Diseases, Beijing, People's Republic of China; [6]Institute of Respiratory Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China; [7]WHO Collaborating Center for Tobacco Cessation and Respiratory Diseases Prevention, Beijing, People's Republic of China; [8]School of Health Policy and Management, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China; [9]Department of Pulmonary and Critical Care Medicine, Center of Respiratory Medicine, China-Japan Friendship Hospital, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Dan Xiao, Email danxiao@263.net

Background: Smokers with similar smoking exposure show markedly different risks of chronic obstructive pulmonary disease (COPD), and it remains unclear whether genetically determined nicotine metabolism, measured by the nicotine metabolite ratio (NMR), is independently associated with COPD beyond smoking exposure.

Methods: This study integrated genetic evidence and population-based phenotypic analyses. First, two-sample Mendelian randomization (MR) analyses were conducted using genome-wide association study summary statistics to evaluate the causal effect of the NMR on COPD, with colocalization analyses to assess shared causal genetic variants. Second, phenotypic analyses in the China National Tobacco Cessation Cohort Study were conducted in a cross-sectional setting to compare COPD prevalence across metabolic phenotypes under comparable smoking exposure, with mediation analyses evaluating the contribution of smoking behavior.

Results: MR analysis showed a causal association between a higher NMR and increased risk of COPD (OR = 1.06, 95% CI: 1.05–1.08). Colocalization analyses identified shared causal variants. Population-based phenotypic analyses showed that COPD prevalence was consistently higher in normal metabolizers (NMR $\geq$ 0.31) than slow metabolizers (NMR < 0.31), regardless of smoking exposure strata, and more importantly, remained higher within comparable levels of cumulative smoking. Mediation analysis showed that smoking behavior partially mediated this association (indirect effect $\beta = 0.0049$, $P < 0.001$), accounting for 14.5% of the total effect.

Conclusion: Nicotine metabolism, as captured by NMR, is causally associated with COPD susceptibility. These findings suggest that incorporating nicotine metabolism into smoking exposure assessment may have potential value in improving COPD risk stratification.

Keywords: COPD, nicotine metabolism, smoking behavior

Introduction

Chronic obstructive pulmonary disease (COPD) is highly prevalent in China, with estimates from the China Pulmonary Health study indicating that more than 8.6% of individuals are affected.[1] Cigarette smoking is the most important and modifiable environmental factor for COPD, contributing to more than 70% of the overall disease burden.[1,2]

Among smokers who develop COPD, studies have demonstrated dose-response associations with smoking exposure.[3,4] However, traditional self-reported smoking indicators, including cigarettes per day (CPD), smoking

https://doi.org/10.2147/COPD.S594021
Received: 7 January 2026
Accepted: 14 April 2026
Published: 23 April 2026

duration, and pack-years, are susceptible to influences from social desirability, interview context, and changes in health awareness, which may lead to recall bias.[5,6]

To overcome these limitations, researchers have increasingly focused on physiological indicators of exposure derived from nicotine metabolites, such as cotinine (COT) and its further metabolite trans-3'-hydroxycotinine (3-HC).[7] In particular, the nicotine metabolite ratio (NMR), defined as the 3-HC/COT ratio, is strongly genetically influenced, with 60%–80% of its variation explained by genetic factors, resulting in high temporal stability.[8,9]

Smokers with faster nicotine metabolism typically need to inhale more nicotine to maintain comparable steady-state concentrations, which may lead to greater smoking intensity and increased toxicant exposure.[10] In addition, the primary enzymes responsible for nicotine metabolism also participate in the biotransformation of multiple other toxic constituents of cigarette smoke.[11] Thus, interindividual variation in metabolic rate may influence both smoking behavior and toxicant metabolism among smokers, ultimately resulting in differences in systemic exposure levels. Animal studies further indicate that Cyp2a5, the mouse homolog of human CYP2A6, is upregulated and accompanied by increased cotinine-induced ROS production, which may promote oxidative stress–related lung injury.[12]

A retrospective study has suggested that a higher NMR may be associated with the presence of COPD, raising the possibility that nicotine metabolism could be clinically relevant to disease susceptibility. However, the available evidence remains limited based on relatively small samples, restricting their generalizability.[13] To date, such evidence in Chinese populations is lacking.

To address these gaps, we integrated genetic causal inference with population-based phenotypic analyses to examine whether interindividual differences in nicotine metabolism are associated with COPD under comparable levels of smoking exposure, and to assess the contribution of smoking behavior to this association.

Methods

Study Design

This study integrated genetic epidemiology with population-based phenotypic analyses to systematically evaluate the relationship between NMR and COPD. First, using GWAS summary statistics, we performed MR analyses to assess the causal effect of NMR on COPD and conducted colocalization analyses to evaluate shared causal variants. Second, we carried out a cross-sectional analysis within the CNTCCS to compare COPD prevalence across metabolic phenotypes under equivalent levels of smoking exposure, to evaluate the association between NMR and COPD, and to further examine the mediating role of smoking behavior (Figure 1).

The study was approved by the Ethics Committee of China-Japan Friendship Hospital (2024-KY-336), and all procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Mendelian Randomization

GWAS Summary Data Sources

GWAS summary statistics for NMR were derived from a large-scale study conducted by Buchwald et al, including 5,185 individuals of European ancestry.[14] Summary data for COPD were initially sourced from the FinnGen Consortium (J10_COPD, Early-onset COPD, Later-onset COPD), as detailed in the FinnGen study's data repository (https://www.finngen.fi/en), featuring 18,266 cases and 311,286 controls.[15] COPD summary statistics were also obtained from the U.S. Veterans Affairs Million Veteran Program (MVP_COPD), including 103,054 cases and 315,405 controls of European ancestry.[16]

Instrument Selection and Functional Pathway Enrichment Analysis

Single-nucleotide polymorphisms (SNPs) associated with NMR at genome-wide significance ($P < 5\times10^{-8}$) were selected as instrumental variables and pruned for linkage disequilibrium ($r^2 < 0.2$, 500 kb window) using the 1000 Genomes reference panel. Palindromic SNPs with high minor allele frequency (>0.40) and variants unavailable in outcome datasets were excluded. Instrument strength was assessed using F-statistics ($F > 10$). 68 independent SNPs were included for analyses in the FinnGen dataset, of which 59 were also available in the MVP dataset (Supplementary Tables 1 and 2). Potential outliers and horizontal pleiotropy were evaluated using MR-PRESSO and RadialMR.

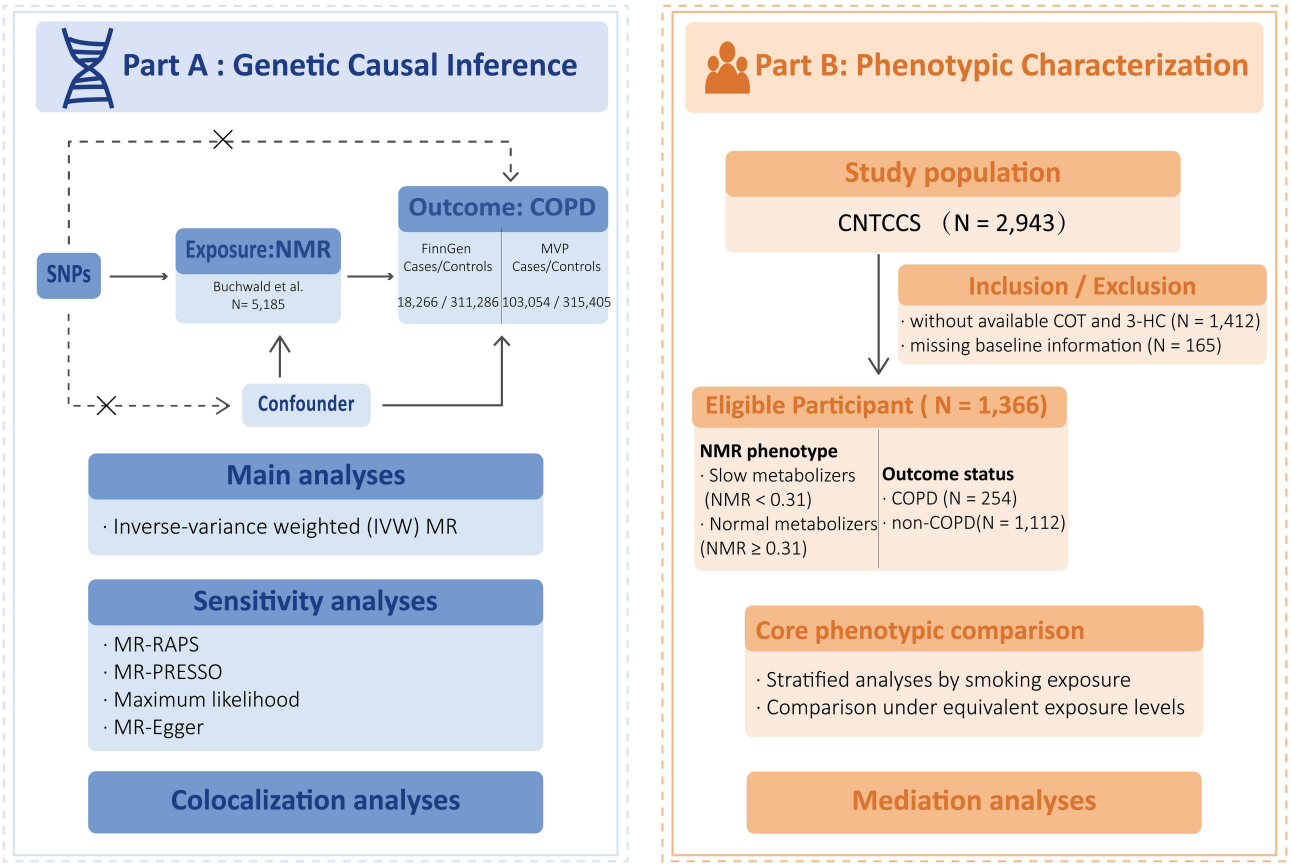

Figure 1 Flowchart of the study. Note: Lines with cross symbols indicate assumed absence of direct causal pathways in MR.

Two-Sample MR

Univariable two-sample MR was conducted to estimate the causal effect of NMR on COPD using the inverse-variance weighted (IVW) as the primary method.[16] Sensitivity analyses included maximum likelihood,[16] MR-RAPS,[17] and MR-PRESSO.[18] Reverse MR and leave-one-out analyses were performed to assess potential reverse causation and the influence of individual SNPs. All analyses were conducted using the "TwoSampleMR" R package.

Stratified two-sample MR analyses were conducted for Early-onset COPD (defined as first COPD diagnosis before age 65) and Later-onset COPD (defined as first COPD diagnosis at or after age 65).

Colocalization Analyses

Colocalization analyses were conducted to evaluate whether NMR and COPD shared causal variants within ±500 kb of each instrumental SNP. Posterior probabilities for five hypotheses (H0–H4) were estimated using the "coloc R" package, with a posterior probability for shared causality (PPH4) >75% considered evidence of colocalization.[19]

Cross-Sectional Analysis

Study Population

The study population was drawn from the CNTCCS, a large-scale national cohort described previously.[20,21] Our participants were eligible for inclusion if they met the following criteria: (1) aged 20 years or older; (2) had complete baseline questionnaire data; (3) provided blood samples for laboratory testing; and (4) provided written informed consent. Individuals with a self-reported history of other pulmonary diseases, including asthma, pulmonary tuberculosis, lung cancer, or pulmonary infections, as well as those with missing key variables, were excluded from the analysis.

Measurement

COPD was defined according to a post-bronchodilator ratio of forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) of less than 0.7.[22]

NMR was calculated as the 3-HC/COT ratio. Plasma levels of COT and 3-HC were quantified using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with a lower limit of quantification of 0.5 ng/mL.[23]

Other covariates, including demographic characteristics and smoking behaviors, were obtained from baseline questionnaires.

Statistical Analysis

NMR was dichotomized using a predefined cutoff value of 0.31 (according to our previous research),[20] with NMR < 0.31 defined as slow metabolism and NMR ≥ 0.31 defined as normal metabolism. Prevalence was calculated as the proportion of COPD participants within each metabolic phenotype and smoking exposure stratum. Prevalence comparisons between metabolic phenotypes were conducted across strata of cigarettes per day, smoking duration, and pack-years.

Mediation analysis was conducted using the "mediation" R package based on the Baron and Kenny framework.[24] To improve comparability with the genetic analyses, mediation analyses were conducted among participants aged <65 years. Regression models were specified to estimate the total effect of NMR on COPD, the association between NMR and smoking behavior (pack-years), and the association between smoking behavior and COPD conditional on NMR, with adjustment for relevant covariates. Indirect effects were estimated using a bootstrap approach with 5,000 resamples. All regression and mediation models were adjusted for age, sex, nationality, marital status, and education level. Participants with missing key variables were excluded from the analysis.

A two-sided P value < 0.05 was considered statistically significant. All analyses were performed using R software (version 4.3.2) and SPSS software (version 26.0).

Results

Genetic Evidence for a Causal Association Between NMR and COPD

NMR Was Associated with Increased COPD Susceptibility

The IVW method showed that genetically predicted NMR was positively associated with COPD (FinnGen: OR = 1.065, 95% CI: 1.052–1.078, P < 0.001; MVP: OR = 1.058, 95% CI: 1.050–1.065, P < 0.001). These associations were robust across multiple sensitivity analyses, including maximum likelihood (FinnGen: OR = 1.063, 95% CI: 1.050–1.077; MVP: OR = 1.056, 95% CI: 1.048–1.064), MR-RAPS, and MR-PRESSO (Figure 2).

Scatter, forest, and leave-one-out plots consistently supported the robustness of the MR findings (Supplementary Figures 1–5). No evidence of horizontal pleiotropy was detected based on MR-Egger and MR-PRESSO tests (all P > 0.05; Supplementary Table 3), and funnel plot symmetry further indicated the absence of directional bias (Supplementary Figure 6).

In reverse MR analyses treating COPD as the exposure, no evidence was found to support a causal effect of COPD on NMR across all applied methods (Supplementary Table 4).

Stratified Mendelian randomization analyses based on age-of-onset categories defined in the FinnGen study showed that the OR_{ivw} for Early-onset COPD was 1.118 (95% CI: 1.099–1.136, P < 0.001), and the estimate for Later-onset COPD was 1.026 (95% CI: 1.009–1.042, P = 0.001). MR-Egger and MR-PRESSO analyses showed no evidence of horizontal pleiotropy (P > 0.05; see Supplementary Table 5 and Supplementary Table 6).

Colocalization Analyses Identified Shared Genetic Signals

Colocalization analyses identified shared genetic signals between NMR and COPD within the CYP2A6–EGLN2 locus on chromosome 19. In the FinnGen dataset, the lead variant rs8102683 demonstrated strong evidence of colocalization, with a posterior probability for a shared causal variant (PPH4) of 0.935. Consistent colocalization signals were observed in the MVP dataset for rs56113850, rs12610432, and rs7937 (PPH4 range: 0.864–0.996) (Supplementary Table 7 and Supplementary Figure S7).

Dataset	Method	IVs	OR(95%CI)	P-value
FINN_COPD	Inverse variance weighted	68	1.065(1.052,1.078)	<0.001
	MR-RAPS	68	1.068(1.056,1.081)	<0.001
	Maximum likelihood	68	1.065(1.053,1.077)	<0.001
	MR-PRESSO	68	1.064(1.052,1.078)	<0.001
	MR-Egger	68	1.072(1.043,1.110)	<0.001
MVP_COPD	Inverse variance weighted	59	1.058(1.050,1.065)	<0.001
	MR-RAPS	59	1.058(1.049,1.066)	<0.001
	Maximum likelihood	59	1.058(1.050,1.066)	<0.001
	MR-PRESSO	59	1.058(1.050,1.065)	<0.001
	MR-Egger	59	1.070(1.053,1.088)	<0.001

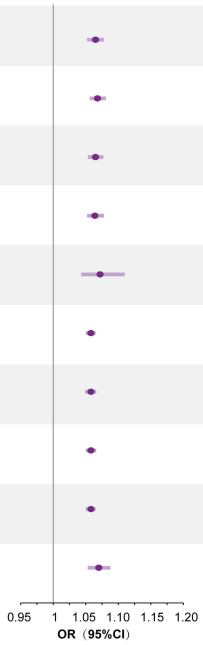

Figure 2 Two-sample Mendelian randomization reveals causal evidence for NMR on COPD.
Note: The forest plots illustrate the standardized beta (95% CI) for each two-sample MR method.

Phenotypic Association of NMR with COPD in the CNTCCS

Baseline Characteristics

Among 2,943 participants in the CNTCCS, 1,531 had available COT and 3-HC data. After excluding individuals with missing baseline information, 1,366 participants were included in the final analysis, of whom 254 (18.6%) had COPD.

Participants with COPD had longer smoking duration (37.78 ± 9.99 vs. 24.74 ± 11.48 years) and higher cumulative smoking exposure measured by pack-years (37.24 ± 20.01 vs. 24.06 ± 16.30), compared with those without COPD (both $P < 0.001$). Participants with COPD were more likely to have normal nicotine metabolism, and their mean NMR was higher than individuals without COPD (0.31 vs. 0.26, $P < 0.001$). Table 1 summarizes the baseline characteristics.

Table 1 Characteristics of Participants in the CNTCCS

	COPD (n=254)	Non-COPD (n=1112)	P-value
Age			
Mean, SD	57.61 ± 9.80	44.25 ± 11.59	<0.001
Sex			
Male	246(96.9)	1053(94.7)	0.203
Female	8(3.1)	59 (5.3)	
Nationality			
Han	215(84.6)	1032(92.8)	<0.001
Minorities	39(15.4)	80(7.2)	
Marital status			
Married	240(94.5)	874(78.6)	<0.001
Unmarried/Divorced/Widowed/Separated	14(5.5)	238(21.4)	
Education level			
Primary school and below	34(13.4)	33(3.0)	<0.001
Middle school	140(55.1)	344(30.9)	
College and above	80(31.5)	735(66.1)	

(*Continued*)

https://doi.org/10.2147/COPD.S594021

Table 1 (Continued).

	COPD (n=254)	Non-COPD (n=1112)	P-value
CPD			
Mean, SD	19.7 ± 9.22	19.06 ± 8.07	0.624
Smoking Years			
Mean, SD	37.78 ± 9.99	24.74 ± 11.48	<0.001
Pack-years			
Mean, SD	37.24 ± 20.01	24.06 ± 16.3	<0.001
NMR			
Mean, SD	0.31 ± 0.18	0.26 ± 0.16	<0.001

NMR Phenotype is Associated with COPD Prevalence Across Smoking Exposure Strata

Across all smoking exposure strata, COPD prevalence was consistently higher in the normal metabolizer group than in the slow metabolizer group, with statistically significant differences observed primarily among participants with heavier smoking exposure. Specifically, among participants with <20 and ≥20 pack-years of smoking, COPD prevalence was 10.5% and 33.0% in the normal metabolizer group, compared with 7.9% and 20.7% in the slow metabolizer group, respectively. Similar patterns were observed in analyses stratified by cigarettes per day and smoking duration, with statistically significant differences in strata with ≥20 cigarettes per day and ≥20 years of smoking (Figure 3).

NMR Phenotype Stratifies COPD Prevalence at Comparable Levels of Smoking Exposure

To further control for cumulative smoking exposure, COPD prevalence increased progressively in both slow and normal metabolizers; however, within each pack-year stratum, normal nicotine metabolizers consistently exhibited a higher prevalence of COPD than slow metabolizers (Supplementary Figure 8).

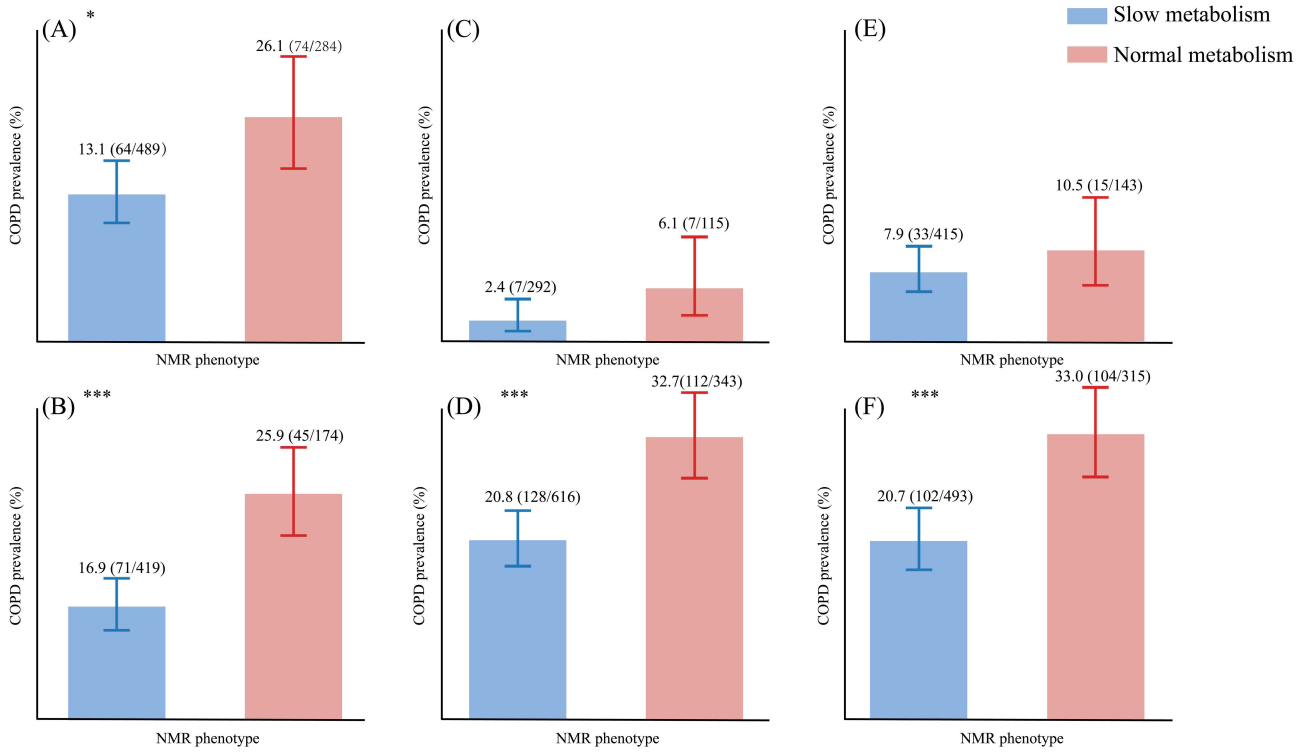

Figure 3 COPD prevalence across NMR metabolic phenotypes under different levels of smoking exposure. Note: (**A** and **B**) Cigarettes per day (<20 and ≥20 cigarettes/day); (**C** and **D**) Smoking duration (<20 and ≥20 years); (**E** and **F**) Pack-years (<20 and ≥20 pack-years). Error bars represent 95% confidence intervals. * P < 0.05, *** P < 0.001.

https://doi.org/10.2147/COPD.S594021

Table 2 Mediating Effect of Smoking Behavior[a] (Mediating Variable) on the Association Between NMR[b] (Independent Variable) and COPD[c] (Dependent Variable) in All Participants Aged Under 65 Years[d]

Path	β (SE)	P value
[e]NMR on COPD	0.0337 (0.0101)	<0.001
[f]NMR on Smoking behavior	0.1705 (0.0434)	<0.001
[g]Smoking behavior on COPD given NMR	0.2353 (0.0479)	<0.001
[h]Indirect: NMR on COPD	0.0049 (0.0017)	<0.001
[i]Direct: NMR on COPD given smoking behavior	0.0288 (0.0101)	0.004
Proportion mediated	14.5%	<0.001

Notes: [a]Smoking behavior: Pack-years; [b]NMR: Nicotine metabolite ratio; [c]COPD: chronic obstructive pulmonary disease; [d]All analyses adjusted for age, sex, nationality, marital status, and education level; [e]Total effect of independent variables on dependent variables. [f]Coefficients of independent variables on mediating variables after adjustment for covariates; [g]Coefficients of mediating variables on dependent variables after adjusted for covariates and IVs. [h]Indirect effect of independent variables on dependent variables. [i]Direct effect of independent variables on dependent variables.

Smoking Behavior Partially Mediates the Association Between NMR and COPD

Mediation analysis indicated that smoking behavior partially mediated the association between NMR and COPD, with a significant indirect effect (β = 0.0049, P < 0.001), accounting for approximately 14.5% of the total effect. After adjustment for smoking behavior, the direct effect of NMR on COPD remained significant (β = 0.0288, P = 0.004) (Table 2).

Discussion

To our knowledge, this is the first study to systematically evaluate the association between the NMR and COPD by integrating genetic causal inference with population-based phenotypic analyses.

Our study reveals several meaningful findings. First, our results demonstrate that NMR, as a highly stable metabolic phenotype, is associated with COPD. Second, normal metabolizers showed a higher prevalence of COPD than slow metabolizers across strata of smoking behavior, including within comparable pack-year categories, indicating that smoking exposure alone does not fully account for the observed differences. Finally, mediation analyses indicated that smoking behavior explained only a modest proportion of the association, suggesting that interindividual differences in nicotine metabolism may contribute to heterogeneity in COPD susceptibility.

Our findings are consistent with previous studies suggesting a role of nicotine metabolism and CYP2A6 in respiratory disease. An observational study conducted in a smoking cessation clinic involving 78 participants reported that a higher NMR was independently associated with the presence of COPD, even after accounting for conventional smoking exposure measures.[13] Genetic studies further support this relationship. Variants in CYP2A6 that reduce nicotine metabolism have been associated with a lower risk of emphysema, whereas higher CYP2A6 enzymatic activity has been linked to increased susceptibility to respiratory diseases.[25,26] In addition, several prospective cohort studies have shown that lung injury in younger smokers is more sensitive to differences in inhalation patterns and toxicant processing, highlighting the potential importance of metabolic and behavioral heterogeneity in early disease stages.[26–28] It should also be noted that COPD diagnosed in younger individuals may represent a heterogeneous group. Conditions such as alpha-1 antitrypsin deficiency or early-life airway abnormalities may contribute to airflow limitation in this population, which differ from typical smoking-related COPD and may partially influence the observed associations.[29]

Moreover, our study further demonstrates that nicotine metabolism represents a distinct phenotypic characteristic associated with COPD. Smoking duration reflects the cumulative exposure to tobacco over time, whereas CPD and short-term biomarkers such as cotinine primarily capture recent exposure intensity.[30] CPD is particularly susceptible to recall bias and short-term behavioral modification, especially among individuals who have already developed respiratory symptoms and may intentionally reduce their daily cigarette consumption.[31] In addition, CPD does not capture qualitative aspects of smoking behavior, such as inhalation depth or puff frequency, which substantially influence the actual intake of harmful smoke constituents.

In contrast, NMR is a stable biological trait and is less influenced by short-term changes in smoking behavior. Smokers with faster nicotine metabolism may compensate by inhaling more deeply or more frequently to maintain desired nicotine levels, thereby increasing the intake of harmful tobacco constituents over a shorter period and potentially elevating the risk of COPD. Colocalization analyses suggest that NMR and COPD may share potential causal variants within the CYP2A6–EGLN2 locus. EGLN2 is involved in hypoxia signaling and inflammatory regulation, processes that are closely linked to early airway remodeling and small airway injury.[32–34]

Our findings have important public health implications. Current COPD risk assessment among smokers still relies heavily on self-reported smoking history, potentially leading to underestimation or misclassification of true tobacco exposure. Indicators of individual nicotine metabolism may provide a more comprehensive characterization of tobacco exposure and help identify individuals with higher COPD susceptibility despite similar smoking levels. Incorporating metabolic phenotypes into smoking exposure or COPD susceptibility assessment frameworks may facilitate more refined and individualized strategies for COPD prevention.

The strengths of our study include the identification of nicotine metabolism as a stable metabolic phenotype associated with COPD susceptibility, supported by convergent genetic and population-based evidence. However, this study has several limitations. First, the MR analyses were based on publicly available GWAS summary statistics and therefore lacked individual-level data, precluding stratified genetic analyses by smoking behavior or disease progression. Second, the genetic analyses were based on European ancestry GWAS datasets, whereas the phenotypic analysis was conducted in a Chinese population, which may affect generalizability. Third, the population-based analysis was cross-sectional in nature and did not include year-long follow-up, limiting the assessment of temporal relationships and disease incidence. BMI was not included as a covariate due to a high proportion of missing data, which may result in residual confounding. In addition, the moderate sample size of the phenotypic cohort may have limited the statistical power for certain subgroup analyses, and the corresponding results should therefore be interpreted with caution. Furthermore, the shared variants suggested by colocalization in the CYP2A6–EGLN2 region require further validation through functional studies. Further longitudinal and mechanistic studies are needed.

Conclusion

In conclusion, our study suggests that interindividual variation in nicotine metabolism, as captured by NMR, is associated with increased susceptibility to COPD and is only partially explained by smoking behavior. These findings suggest that incorporating nicotine metabolism into smoking exposure assessment may have potential value in improving COPD risk stratification.

Data Sharing Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical Statement

The study was approved by the Ethics Committee of China-Japan Friendship Hospital (2024-KY-336), and all procedures were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Acknowledgments

We sincerely thank the U.S. Veterans Affairs Million Veteran Program and FinnGen consortia for providing open-access GWAS and transcriptomic summary statistics. Their contributions to public data sharing have made this research possible.

Funding

National Natural Science Foundation of China (82570030); Science and Technology Project of Heilongjiang Province of China (2022ZXJ03C02); Noncommunicable Chronic Diseases-National Science and Technology Major Project (2023ZD0506400).

Disclosure

Authors report no conflicts of interest in this work.

References

1. Wang C, Xu J, Yang L, et al. Prevalence and risk factors of chronic obstructive pulmonary disease in China (the China Pulmonary Health [CPH] study): a national cross-sectional study. *Lancet*. 2018;391(10131):1706–1717. doi:10.1016/S0140-6736(18)30841-9
2. Soriano JB, Kendrick PJ, Paulson KR. GBD Chronic Respiratory Disease Collaborators. Prevalence and attributable health burden of chronic respiratory diseases, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Respir Med*. 2020;8(6):585–596. doi:10.1016/S2213-2600(20)30105-3
3. Li J, Xu J, Yang L. China Pulmonary Health Study Group. et al. mediating effect of tobacco dependence on the association between maternal smoking during pregnancy and chronic obstructive pulmonary disease: case-control study. *JMIR Public Health Surveill*. 10;2024:e53170. doi:10.2196/53170
4. Chen L, Xiong H, Wen Q, et al. The role of active and passive smoking in chronic obstructive pulmonary disease and systemic inflammation: a 12-year prospective study in China. *J Epidemiol Glob Health*. 2024;14(3):1332–1340. doi:10.1007/s44197-024-00290-w
5. Chang CM, Edwards SH, Arab A, Del Valle-Pinero AY, Yang L, Hatsukami DK. Biomarkers of tobacco exposure: summary of an fda-sponsored public workshop. *Cancer Epidemiol Biomarkers Prev*. 2017;26(3):291–302. doi:10.1158/1055-9965.EPI-16-0675
6. Turner RM, DePietro M, Ding B. Overlap of asthma and chronic obstructive pulmonary disease in patients in the united states: analysis of prevalence, features, and subtypes. *JMIR Public Health Surveill*. 2018;4(3):e60. doi:10.2196/publichealth.9930
7. Mahabee-Gittens EM, Merianos AL, Lopez-Galvez N, et al. Thirdhand smoke exposes children living with smokers and nonsmokers to tobacco smoke toxicants: racial, income, and housing disparities in hand nicotine and saliva cotinine levels. *Environ Health Perspect*. 2025. doi:10.1289/EHP16332
8. Medaglio D, Bilker WB, Han X, et al. Nicotine metabolite ratio decreases after switching off efavirenz-based therapy in people with hiv who smoke. *Clin Pharmacol Ther*. 2024;115(1):80–85. doi:10.1002/cpt.3068
9. Pouget JG, Giratallah H, Langlois AWR, et al. Fine-mapping the CYP2A6 regional association with nicotine metabolism among African American smokers. *Mol Psychiatry*. 2025;30(3):943–953. doi:10.1038/s41380-024-02703-5
10. Chen A, Krebs NM, Zhu J, Muscat JE. Nicotine metabolite ratio predicts smoking topography: the pennsylvania adult smoking study. *Drug Alcohol Depend*. 2018;190:89–93. doi:10.1016/j.drugalcdep.2018.06.003
11. Winters BR, Clapp PW, Simmons SO, Kochar TK, Jaspers I, Madden MC. E-cigarette liquids and aldehyde flavoring agents inhibit cyp2a6 activity in lung epithelial cells. *ACS Omega*. 2023;8(12):11261–11266. doi:10.1021/acsomega.2c08258
12. Kanamori K, Ahmad SM, Hamid A, Lutfy K. Chronic exposure to e-cigarettes elevates cyp2a5 activity, protein expression, and cotinine-induced production of reactive oxygen species in mice. *Drug Metab Dispos*. 2024;52(3):171–179. doi:10.1124/dmd.123.001348
13. Pezzuto A, Lionetto L, Ricci A, Simmaco M, Borro M. Inter-individual variation in CYP2A6 activity and chronic obstructive pulmonary disease in smokers: perspectives for an early predictive marker. *Biochim Biophys Acta Mol Basis Dis*. 2021;1867(1):165990. doi:10.1016/j.bbadis.2020.165990
14. Buchwald J, Chenoweth MJ, Palviainen T, et al. Genome-wide association meta-analysis of nicotine metabolism and cigarette consumption measures in smokers of European descent. *Mol Psychiatry*. 2021;26(6):2212–2223. doi:10.1038/s41380-020-0702-z
15. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023;613(7944):508–518. doi:10.1038/s41586-022-05473-8
16. Verma A, Huffman JE, Rodriguez A, et al. Diversity and scale: genetic architecture of 2068 traits in the VA Million Veteran Program. *Science*. 2024;385(6706):eadj1182. doi:10.1126/science.adj1182
17. Zhao Q, Wang J, Hemani G, Bowden J, Small DS. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. *Ann Stat*. 2019;47(3):1742–1769.
18. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50(5):693–698. doi:10.1038/s41588-018-0099-7
19. Cao X, Sun H, Feng R, et al. Integrative multi-omics QTL colocalization maps regulatory architecture in aging human brain. *medRxiv*. 2025;25326042.
20. Qin R, Liu Z, Cheng AQ, et al. Efficacy of varenicline or bupropion and its association with nicotine metabolite ratio among smokers with COPD. *Respirology*. 2024;29(6):479–488. doi:10.1111/resp.14702
21. Liu Z, Qin R, Hu XJ, et al. Real-world tobacco cessation practice in China: findings from the prospective, nationwide multicenter China National Tobacco Cessation Cohort Study (CNTCCS). *Lancet Reg Health West Pac*. 2023;39:10082. doi:10.1016/j.lanwpc.2023.100826
22. Huang K, Han X, Pan Z, et al. Impact of using pre- and postbronchodilator spirometry reference values in a Chinese Population. *Am J Respir Crit Care Med*. 2024;210(7):881–889. doi:10.1164/rccm.202308-1488OC
23. Mooney ME, Li ZZ, Murphy SE, Pentel PR, Le C, Hatsukami DK. Stability of the nicotine metabolite ratio in ad libitum and reducing smokers. *Cancer Epidemiol Biomarkers Prev*. 2008;17(6):1396–1400. doi:10.1158/1055-9965.EPI-08-0242
24. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol*. 1986;51(6):1173–1182. doi:10.1037/0022-3514.51.6.1173

25. Minematsu N, Nakamura H, Iwata M, et al. Association of CYP2A6 deletion polymorphism with smoking habit and development of pulmonary emphysema. *Thorax*. 2003;58(7):623–628. doi:10.1136/thorax.58.7.623
26. Giratallah H, Chenoweth MJ, Pouget JG, et al. CYP2A6 associates with respiratory disease risk and younger age of diagnosis: a phenome-wide association Mendelian Randomization study. *Hum Mol Genet*. 2024;33(2):198–210. doi:10.1093/hmg/ddad172
27. Cho MH, Castaldi PJ, Wan ES, et al. A genome-wide association study of COPD identifies a susceptibility locus on chromosome 19q13. *Hum Mol Genet*. 2012;21(4):947–957. doi:10.1093/hmg/ddr524
28. Sakornsakolpat P, McCormack M, Bakke P, et al. Genome-wide association analysis of single-breath dl_{co}. *Am J Respir Cell Mol Biol*. 2019;60(5):523–531. doi:10.1165/rcmb.2018-0384OC
29. Cazzola M, Stolz D, Rogliani P, Matera MG. α1-Antitrypsin deficiency and chronic respiratory disorders. *Eur Respir Rev*. 2020;29(155):190073. doi:10.1183/16000617.0073-2019
30. Zhang P, Chen PL, Li ZH, et al. Association of smoking and polygenic risk with the incidence of lung cancer: a prospective cohort study. *Br J Cancer*. 2022;126(11):1637–1646. doi:10.1038/s41416-022-01736-3
31. Bandiera FC, Ross KC, Taghavi S, et al. Nicotine dependence, nicotine metabolism, and the extent of compensation in response to reduced nicotine content cigarettes. *Nicotine Tob Res*. 2015;17(9):1167–1172. doi:10.1093/ntr/ntu337
32. Bloom AJ, Baker TB, Chen LS, et al. Variants in two adjacent genes, EGLN2 and CYP2A6, influence smoking behavior related to disease risk via different mechanisms. *Hum Mol Genet*. 2014;23(2):555–561. doi:10.1093/hmg/ddt432
33. Li J, Wu X, Wu Y, et al. Physical, mental, and health empowerment disparities across chronic obstructive pulmonary disease, asthma, and combined groups and the moderating role of ehealth literacy: cross-sectional study. *J Med Internet Res*. 2025;27:e70822. doi:10.2196/70822
34. Bray MJ, Chen LS, Fox L, et al. Dissecting the genetic overlap of smoking behaviors, lung cancer, and chronic obstructive pulmonary disease: a focus on nicotinic receptors and nicotine metabolizing enzyme. *Genet Epidemiol*. 2020;44(7):748–758. doi:10.1002/gepi.22331