ORIGINAL RESEARCH

A Two-Step Mendelian Randomization Involving Chronic Obstructive Pulmonary Disease, COVID-19 and Risk Factors

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ABSTRACT

Introduction and Objectives • Smoking is a risk factor for Covid-19 due to the destruction of heart and lungs from tobacco products. Increased smoking increases complications related to COVID-19, however, the association between chronic obstructive pulmonary disorder (COPD), environmental factors, and how the lung function mediates the association remains unclear. Therefore, our primary objective is to conduct a Mendelian randomization to investigate whether COPD, environmental factors and lung function has a mediating effect between smoking and the severity of COVID-19.

Methods • A two-step Mendelian randomization design was employed using genetic data from genome-wide association studies (GWAS). The instrumental variable was the genetic variants (Z) associated with smoking, COPD, lung function (forced expiratory volume per second (FEV_1), and COVID-19 phenotypes (hospitalized, severe and overall covid-19) were selected. The first step involved estimating the associations between instruments and their respective phenotypes, while the second step examined the relationships between instruments and outcomes, as well as instruments and mediators. Various sensitivity analyses were conducted to assess the robustness of the findings.

Participants • A sample size ranging between 195773 to 289887

Measurements • Lung function was measured per second [forced expiratory volume per second (FEV_1)], genetic determinants of lifetime smoking index, and varying severities of COVID-19 and COPD.

Results • COVID-19 Severe (OR =1.48, 95% CI = 1.10 to 1.98) and COVID-19 Hospitalized (OR = 1.67, 95% CI = 1.42 to 1.97), alongside additional sensitivity analyses showed consistent directional effects. Smoking exacerbated COVID-19's risk in the experimental group more than in control populations: Odds Rations (OR) of 1.19 per standard deviation (SD), based on the lifetime smoking index, and a 95% Confidence Interval (CI) of 1.11 to 1.27. COPD and lung function did not mediate the associations.

Conclusions • There exists strong genetic evidence linking environmental factors, smoking and lung function, and COVID-19's severity. Mild COVID-19 is also captured, but to a lesser extent, through minimal evidence. Low lung function exacerbates COPD but does not mediate the implications of smoking on the risk of COVID-19. Our study has implications in the public health policy and messaging for smokers and risks of COVID -19. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

The World Health Organization (WHO) has identified COPD as the third leading cause of global mortality, with 1.23 million reported deaths in 2019.[1] WHO attributes major risk factors to environmental smoke, occupational dust, and indoor air pollutants such as chemicals and fumes. Singh et al.,[2] indicated negative associations in the relationship between COVID-19 and COPD, where worse clinical outcomes were observed in COPD patients with COVID-19. Also, Gerayeli et al.[3] noted that COPD patients more vulnerable to COVID-19 and likely to experience severe outcomes compared to individuals without COVID-19.

Zhao et al.[4] suggested that the risk of severe covid-19 in patients with pre-existing COPD was 4 times greater than those without. These studies displayed inconsistent outcomes regarding the association between smoking and COVID-19-associated mortalities.[5] In contrast, other studies emphasized the possibility of symptomatic COVID-19 as a result of smoking and issues that unmask these discrepancies have been analysed in observation studies, for example, challenges facing the interpretation of mutually-adjusted models of statistical analysis confounding and selection bias.[6-9] COVID-19 and environmental factors affect the health of lungs and consequently promotes the development of COPD.

Mendelian studies offer many advantages over observational studies as they allocate genetic variants at conception and create a solid evidence of confounding variables than observational studies. Fadista et al.[10] and Relton et al.[11] have reported and emphasized that obesity and smoking are risk factors for COVID-19 as they make persons more susceptible to severe illness. Even though smoking has been found to increase the severity of Covid-19, the underlying mechanism is yet to be elaborated.

The updated Genome-Wide Association Study (GWAS) data obtained from the COVID-19 Host Genetic Initiatives play a crucial role in advancing the understanding of the genetic factors influencing COVID-19 susceptibility and severity. For instance, identifying specific genetic variants linked with the severity of covid-19 for accuracy in pinpointing genomes. Moreover, it allows assessment of polygenic risk factors associated with genetic variants and an individual's susceptibility to COVID-19.

In light of the previous studies, with COPD as the outcome variable, our study seeks to investigate COVID-19, lung function and environmental factors and establish their relationships with COPD using Mendelian Randomization. COVID-19, smoking and environmental factors will be studied to establish the basis of the relationship and ascertain whether and how they induce or worsen COPD development by taking into account the resulting effects of COVID-19 on the development of COPD through forced expiratory volume per second of the lung function.

We hypothesized a strong genetic link to the severity of COVID-19 with the presence of genetic factors that amplified the effects of smoking.

METHODS

We used the two-step Mendelian randomization of studies to investigate mediation in a Mendelian framework.[12,13] With the aid of the univariable Mendelian randomization, we performed the association between COPD and the following: exposure with mediators, exposure and outcomes, and outcomes and mediators. The two-step Mendelian randomization was based on the possibility of an unpredictable direction of bias or a weak sense of instrument bias.[14] Like the Mendelian randomization technique, the instrumental variable analysis used in the present randomization encompassed the three assumptions:

- **Relevance:** It was assumed that there is a strong relationship between the exposure of interest (X) and the genetic variant (Z).
- **Independence:** It was assumed that there is no relationship between founding factors (*U*) and the genetic variant (Z) that could affect the outcome of (Y).
- **Exclusion Restriction:** The outcome (Y) is affected by the genetic variant (Z), but only via the exposure (X);[13] there is no direct effect on the outcome, apart from the impact mediated by the exposure.

Table 1. Strength of the instruments

	Lifetime smoking	FEV1	FVC	FEV1/FVC	Liability to COPD
Number of SNPs	118	254	309	94	69
Variance explained (R2)	0.012	0.034	0.047	0.035	NA
F statistics	49.225	58.472	66.654	152.275	56.9[a]

[a]Mean of F statistics

The genetic variant (Z) was the instrumental variable and was not linked with any of the confounders that may shift the course of the results or outcomes. In the first step, we estimated the association between genetic variations or instruments with their respective phenotypes regardless of whether there are exposures or mediators. We selected instruments associated with confounding variables that could shape the outcome. After this, we will examine the relationships between instruments and the resulting outcomes alongside the instruments and the mediators. F-statistics are used to assess the overall significance of a regression model. In the context of genetic association studies, F-statistics help determine whether the genetic variants included in the model collectively have a significant effect on the trait being studied. The larger the F-statistic, the more evidence there is against the null hypothesis that none of the genetic variants have an effect (see Table 1).

We analyzed the correlations between the genetic variants or the instruments and their respective phenotypes or measurable traits. In our approach, the phenotypes were the "mediators" that could potentially affect the association between the health outcomes and the genetic variants, or "exposures," which are the health risk factors.

Exposure Populations

The genetic information determinants of COPD in general were obtained from GWAS in Biobank (n = 900 000 participants of European descent) by Ahlberg et al.[15] In light of this finding, Chen et al.[16] performed a study and provided insight into the susceptibility, severity and environmental risk factors of COVID-19. The study provided eight genetic loci of COVID-19 infection indexing, with a genome-wide significance ($P = 5 \times 10^{-8}$) rather than linkage equilibrium (LD): r^2<0.001. The statistics of the above-mentioned risk factors in standard deviation (SD) were obtained. Adjustments for genotype and sex arrays were made in GWAS for the stratification of the population and relatedness.

Mediators of COPD Development

We obtained genetic determinants of COVID-19 and environmental factors from the United Kingdom Biobank GWAS statistical summaries by the Medical Research Council Integrative Epidemiology Unit (MRC IEU), the University of Bristol,[17,18] and in accordance with our previous study where we identified 42 986 participants of European descent.[19] Health care staff performed tests for the risk factors and mediators for COPD development using the Vitalograph Pneumotrac 260 equipment and the spirometer (Maids Moreton, United Kingdom). The GWAS produced 320 and 260 instruments for the forced vital capacity (FVC) and forced expiratory volume per second (FEV_1),

respectively, that attained genome-wide significance but low LD. Some of the measurement equipment predicted FVC and FEV_1 as COPD risk factors. As mentioned above, adjustments for genotype and sex arrays were made in GWAS for the stratification of the population and relatedness used by MRC IEU researchers: BOLT-LMM. Also, the GWAS was used as an outcome for the exposure-mediator association assessment.

We obtained the genetic determinants of FVC and FEV_1 from the GWAS of lung function. The data consisted of 500 000 volunteer British participants.[20] The methodologies used for SpiroMeta measurements varied across the studies, whereas the previously described measurement techniques in UK Biobank were used to measure FVC and FEV_1. GWAS' primary researchers reported that investigations reported in UK Biobank adjusted sex, height, genotyping array, age, and environmouchental factors predisposing individuals to COPD and age^2 and accounted for relatedness via BOLT-LMM and stratification of the population. Sex, age^2, age, principle components via linear regression, and height adjustments were made for studies in SpiroMeta, whereas the association between lung health and COPD was controlled through stratification. Studies with related specimens were analyzed using different techniques. The study produced 99 instruments for FEV_1/ FVC attaining genome-wide significance rather than LD. Additionally, exposure-mediator associations were assessed using a similar GWAS (n = 355 971).

Genetic Liability to COPD

Genes determining the liability to COPD were acquired from a GWAS consisting of the International COPD Genetics Consortium and the UK Biobank, where 15 256 study cases and 47 936 controls of European ancestry were examined.[21] Unlike the International COPD Genetics Consortium, GWAS were adjusted: sex, age, genotyping array (only in the UK Biobank), duration of exposure to environmental risk factors, the current health status of the lungs and principal components, with the aid of logistic regression, reported by the initial investigators. The study yielded 77 instruments attaining the genome-wide significance concerning the liability for COPD rather than low LD.

Outcomes: Covid-19 Phenotypes

COVID-19's phenotypes for the genetic association were acquired from the COVID-19 Host Genetics Initiative (https://www.covid19hg.org/), and the release date is June 6 2021. Most of the studies reporting the genetic data included study participants of European descent. The phenotypes included all COVID-19 cases reported (152 764 cases, with a total of 3 988, 902 case controls. 89% of the populations were Europeans), hospitalized patients (27 874 cases, with a total of 3 101 765 controls. 88% were Europeans), alongside patients experiencing severe respiratory effects of COVID-19 (10 873 cases, with a total of 1 234 756 controls. 95% of the population were Europeans). COVID-19 status was differently and independently assessed in each study. This includes serology or RNA tests for SARS-CoV-2 infections and self-reports for hospital and physician-confirmed COVID-19 infections. The latter was denoted as "hospitalized laboratory-conformed" SARS-CoV-2 infections, where corona-associated symptoms informed hospitalization. COVID-19's severity was defined based on the need for respiratory support services like intubation or continuous airway pressure and death. The initial investigators deliberated GWAS adjustments, which included variables like sex, age^2, age, and covariates specific to the study, alongside principal components. SAIGE was deliberated as the analytical approach.

Exposure

The primary exposure was predictable environmental factors: the standard deviation of a lifetime smoking index.

Mediators

The included predictable variables: FVC, FEV_1, FEV_1/ FVC, alongside genetic liability towards COPD.

Outcomes

Overall, COVID-19 was the primary outcome, whereas its severity and hospitalization were the secondary outcomes.

Confounders

We assessed the interplay between possible confounders (by use of relevant GWAS like alcohol consumption [n = 537 349 obtained from GWAS and Sequencing Consortium of Alcohol and Nicotine use (GSCAN), which was measured in terms of drinks per week],[22] education and literacy levels [n = 328 917, data obtained from the Social Science Genetic Association Consortium (SSAGC), in terms of educational years] [23], body-mass index [n = 681 275 obtained from the Genetic Investigation of anthropometric Trials (GIANT) consortium]).[24] We used the standard approach to meta-analysis, where we calculated the random effects of the individual studies,[19] after which we deliberated bi-directional Mendelian randomization to establish whether pleiotropy was horizontal or vertical: smoking causes and the resulting biased pathways and the side effects of smoking and the associated unbiased pathways, respectively.

Statistical Analyses

In this study, The term "mediators" refers to variables like FVC, FEV1, FEV1/FVC, and genetic liability toward COPD, which are factors that could potentially influence the relationship between health outcomes and genetic variants (exposures). "Exposures" in this context are the health risk factors, specifically the standard deviation of a lifetime smoking index, that are being studied for their impact on health outcomes, particularly their association with COVID-19. Phenotypes refer to COVID-19 cases, hospitalized patients, and patients with severe respiratory effects as different outcomes or manifestations of COVID-19. Confounding factors refer to alcohol consumption, education, literacy levels, and body mass index. These are variables that can influence the relationship between exposures and outcomes and are taken into account in the analysis. The

term "instruments" refer to genetic variants (Z) used as instrumental variables in Mendelian randomization analyses. These genetic variants are assumed to meet the three assumptions of relevance, independence, and exclusion restriction in the instrumental variable analysis.

In accordance with our previous investigations, we verified the instruments' strength and fitness for a valid analysis: the derived overall statistical summaries (using F-statistic) and calculating total variance explained by the instruments (R^2).[19,25] Secondly, *F*-statistics for every instrument for COPD's genetic liability was estimated by the instrument association with exposure, alongside the respective standard error. Also, all genetic associations were aligned with the alleles, producing a similar effect. The frequencies of the effect alleles for palindromic instruments were used in the latter process.

In the analysis of the exposure-outcome of smoking effects on the risk of COVID-19, we used the SD alongside the inverse variance weighted (IVW) and multiplicative random effects. The Bonferroni correction was used to correct multiple comparisons (0.05/3 = 0.016). This approach regressed the association between instruments and outcomes based on instrument-outcome associations (for the assessed instruments), weighted by the use of variance of instrument-outcome associations. The intercept was constrained to 0. Likewise, the IVW approach was used to investigate the exposure to mediators: association between COPD, lung function, and smoking index (SD), alongside the logarithm of COPD's association with lung function (the SD of FEV_1, FVC, FEV_1/FVC) with liability with COVID-19's risk (mediators to outcomes), with the assumption that the method balanced pleiotropy.[26]

Cochran's *Q*-statistics was used to assess the instrument's heterogeneity: high heterogeneity indicated invalid instruments, whereas the Mendelian randomization Egger's intercept (MR-Egger) and the I^2_{GX} was used to assess the evidence of overall horizontal pleiotropy and the possibility of MR-Egger's dilution of regression, respectively. Additionally, different assumptions (plurality valid, majority valid, and balanced pleiotropy) were used as the foundation of other forms of sensitivity analysis like valid inferences. In contrast, MR-Egger, weighted mode, weighted mean, and MR-robust adjusted profile scores were used to provide detailed supportive data.[27-30] The certainty of the observations on the associations was strengthened by the consistency of the estimate directions of the analyses.[31]

Because height could have generated bias in the Mendelian randomization of FVC and FEV_1 estimates, we deliberated an additional analysis approach: height adjustment using the multivariate (MVMR) for analyses involving FEV_1/FVC, FVC, and FEV_1 as exposures. The use of this approach was based on its advantage over the traditional techniques where an instrument associated with height could have been removed. The latter's implication is the reduction of statistical power. We measured the robustness of the study outcomes using GWAS heights [Genetic Investigation of Anthropometric Traits (GIANT) and the UK Biobank].[32] The conditional *F*-statistic was calculated as the indicator of instrument bias, whereas the MVMR-Egger was used in the repeated analysis. This approach was considered more rational than the MVMR.[19,33,34] We performed all statistical analyses using the R program (version 4.2.2, Core Team; 2022). We used the environment and the language for statistical computing based on the fundamentals and the packages "TwoSampleMR" and the "MVMR."

Ethical approval

The present Mendelian randomization involved publicly available information. Thus, there was no need for ethical considerations. However, the original publications used in the GWAS contain ethical considerations and approval details.

RESULTS

Table 1 reports the instruments used to measure lifetime smoking, where we found an R^2 of 1.2% and an overall *F*-statistic of 49.2, suggesting low evidence for the weak instrument bias. Likewise, there was no solid evidence of weak instruments regarding traits related to lung functions or COPD. There was neither evidence for traits relating COPD to lung functions nor weak evidence for weak instruments regarding traits related to COPD or lung functions (Tables 1-5). A positive relationship was established between smoking-enhancing alleles and Body Mass Index (BMI), whereas an inverse relationship was established between alcohol consumption and education levels (Table 2). The two associations were bi-directional, indicating a mixture of vertical and horizontal pleiotropy. Table 3 summarizes the instruments used in the final analysis.

Table 2. Assessment of (A) lifestime smoking index instruments' relation with confounders and (B) bi-directional Mendelian randomization analyses

A.

Outcome	#SNP	Beta per risk allele	95% CI	*P* value
BMI (SD)	84	0.0043	0.0024 to 0.0061	<.0001
Education years (SD)	118	-0.006	-0.007 to -0.005	<.0001
Alcoohl use (SD of log drinks/ week)	118	0.0026	0.0018 to 0.0033	<.0001

B.

Direction of association	#SNPs[a]	Beta per exposure unit	95%CI
Lifetime smoking			
BMI	84	0.27	0.14 to 0.39
Education	118	-0.38	-0.44 to -0.31
BMI	118	0.17	0.12 to 0.22
Smoking			
BMI	492	0.11	0.09 to 0.13
Education	56	-0.2	-0.25 to -0.15
Alcohol	36	0.07	0.00 to 0.15

[a]BMI instruments extracted from MR-Base directly ("ieu-b-40"). Educaton and alcohol instruments extracted directly from the summary statistics ($P < 5E\text{-}8$ and $R^2 < 0.001$).

Table 3. The number of instruments included in the final analysis

Instrument	Number included in the final analysis
Lifetime smoking index	118
FEV1/FVC	94
FEV1	254
FVC	309
Genetic liability COPD	69

Table 4. IVW estimates for the three phenotypes

Phenotype	OR per SD	*P* value	95% CI	
			CI+	CI-
COVID-19	1.19	3.5×10^{-7}	1.11	1.27
Hospitalized COVID-19	1.67	9.7×10^{-10}	1.42	1.97
Severe COVID-19	1.48	.009	1.10	1.98

Table 5. I^2_{GX} of the analyses in this study

Analysis	I^2_{GX}
Smoking	
COVID-19	0.98
Hospitalized COVID-19	0.98
Severe COVID-19	0.98
FEV1	0.98
FVC	0.98
FEV1FVC	0.98
COPD	0.98
FEV1	
COVID-19	0.98
Hospitalized COVID-19	0.98
Severe COVID-19	0.98
FVC	
COVID-19	0.98
Hospitalized COVID-19	0.98
Severe COVID-19	0.98
FEV1FVC	
COVID-19	0.99
Hospitalized COVID-19	0.99
Severe COVID-19	0.99
COPD	
COVID-19	0.83
Hospitalized COVID-19	0.83
Severe COVID-19	0.83

Table 6. The assocaition of FEV1 and FVC in COVID-19 risk using Mendelian randoimization, adjusted for height

Using height estimates from GIANT (ieu-a-89)			IVW			MR-Egger		
Exposure	Outcome	# SNPs	OR	95%CI	Conditional F statistic	OR	95%CI	Egger intecept *P* value
Forced expiratory volume in 1-second (FEV1) || id:ukb-b-19657	Overall COVID-19	99	0.98	0.90 to 1.07	9.5	1.02	0.92 to 1.13	0.22
Forced expiratory volume in 1-second (FEV1) || id:ukb-b-19657	Hospitalized COVID-19	99	1.07	0.86 to 1.33	9.5	1.16	0.90 to 1.50	0.24
Forced expiratory volume in 1-second (FEV1) || id:ukb-b-19657	Severe COVID-19	98	0.98	0.67 to 1.42	9.5	1.05	0.67 to 1.65	0.54
Forced vital capacity (FVC) || id:ukb-b-7953	Overall COVID-19	145	1.04	0.96 to 1.12	11.4	1.05	0.96 to 1.16	0.48
Forced vital capacity (FVC) || id:ukb-b-7953	Hospitalized COVID-19	145	1.17	0.95 to 1.45	11.4	1.22	0.95 to 1.57	0.50
Forced vital capacity (FVC) || id:ukb-b-7953	Severe COVID-19	144	0.93	0.66 to 1.31	11.5	0.98	0.66 to 1.47	0.60
Lung function (FEV1/FVC) || id:ebi-a-GCST007431	Overall COVID-19	35	1.02	0.97 to 1.07	23.3	1.06	1.00 to 1.12	0.02
Lung function (FEV1/FVC) || id:ebi-a-GCST007431	Hospitalized COVID-19	35	1.06	0.94 to 1.20	23.3	1.09	0.94 to 1.27	0.52
Lung function (FEV1/FVC) || id:ebi-a-GCST007431	Severe COVID-19	35	1.10	0.90 to 1.38	23.3	1.17	0.90 to 1.51	0.50
Using height estimates from UK Biobank (ukb-b-10787)			**IVW**			**MR-Egger**		
Exposure	**Outcome**	**# SNPs**	**OR**	**95%CI**	**Conditional F statistic**	**OR**	**95%CI**	**Egger intecept *P* value**
Forced expiratory volume in 1-second (FEV1) || id:ukb-b-19657	Overall COVID-19	118	1.01	0.93 to 1.09	5.6	1.00	0.91 to 1.09	0.64
Forced expiratory volume in 1-second (FEV1) || id:ukb-b-19657	Hospitalized COVID-19	118	1.20	0.97 to 1.48	5.6	1.23	0.97 to 1.57	0.63
Forced expiratory volume in 1-second (FEV1) || id:ukb-b-19657	Severe COVID-19	116	1.28	0.90 to 1.84	5.7	1.10	0.73 to 1.67	0.13
Forced vital capacity (FVC) || id:ukb-b-7953	Overall COVID-19	160	1.02	0.94 to 1.11	5.9	1.02	0.94 to 1.11	0.91
Forced vital capacity (FVC) || id:ukb-b-7953	Hospitalized COVID-19	160	1.22	0.99 to 1.52	5.9	1.27	1.01 to 1.60	0.40
Forced vital capacity (FVC) || id:ukb-b-7953	Severe COVID-19	159	1.27	0.88 to 1.83	6.0	1.27	0.86 to 1.88	0.97
Lung function (FEV1/FVC) || id:ebi-a-GCST007431	Overall COVID-19	41	1.02	0.98 to 1.06	14.0	1.04	0.99 to 1.09	0.13
Lung function (FEV1/FVC) || id:ebi-a-GCST007431	Hospitalized COVID-19	41	1.13	1.02 to 1.25	14.0	1.14	1.00 to 1.29	0.83
Lung function (FEV1/FVC) || id:ebi-a-GCST007431	Severe COVID-19	41	1.27	1.07 to 1.52	14.3	1.15	0.93 to 1.44	0.12

Figure 1. The association between FVC, FEV_1, and lung function when height estimates were used for GIANT

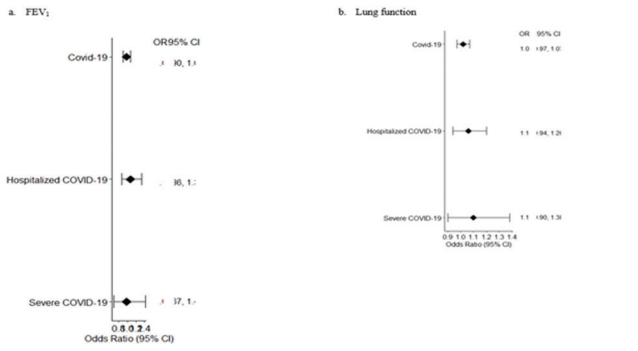
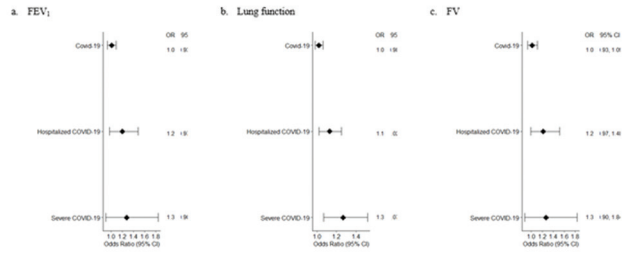


Figure 2. The association between FVC, FEV_1, and lung function when height estimates obtained from the UK Biobank.



Figures 1 and 2 show the association of FVC and FEV_1 in COVID-19 risk using Mendelian randomization, adjusted for height. The effect estimates indicate the association between COVID-19, environmental factors, and lung functions, which are all related to the development of COPD. Figure. 1 shows a high index of lifetime smoking is linked with low lung function. All the analyses produced consistent evidence of the same. Likewise, a high index of lifetime smoking is linked with high COVID-19 risk, despite horizontal pleiotropy (Figure 1b). The two analytic results, more so, low lung function, are associated with COPD development.

COVID-19 was found to be associated with FVC and FEV_1 (Figure 2). The Cochran's *Q*-test established heterogeneity ranging from 1.4×10^{-10} to 0.007, whereas the MR-Egger intercept did not produce overall horizontal pleiotropy. Even after adjusting height by the multivariate Mendelian randomization, the findings did not change. Only

F-statistics were low (Table 6). Severe and high hospitalization of COVID-19 was associated with a high ratio of FEV1/FVC (Figure 2a). We found strong evidence of heterogeneity among IVW instruments: Cochran's Q *P* value; 3.74×10^{-17} to .00598, whereas the MR-Egger intercept could not support directional horizontal pleiotropy. The associations were absent during sensitivity analyses, together with the analysis of multivariate Mendelian randomization (Table 6). We found a similar trend with the liability for COVID-19, where the association with severe and hospitalized COVID-19 was evident (Figure 2b). However, these associations were not present in the rest of the sensitivity analyses. I^2_{GX} was 98%, which is approximately 100%, in most cases because there was minimal evidence supporting regression dilution. Nonetheless, the I^2_{GX} of 83% supported possible regression in the analysis of COPD's liability for COVID-19.

We considered multiple comparisons and calculated IVW estimates for the three phenotypes. Table 4 summarizes the IVW estimates for the three comparisons, where severe COVID-19 reported consistent outcomes with a positive association. We measured heterogeneity using Cochran's *Q*-test and found *P* = .0019, whereas the MR-Egger intercept test reported negative horizontal pleiotropy.

DISCUSSION

We found that COVID-19, environmental risk factors, and lung health could exacerbate COPD, with reference to hospitalization and severity. True to our expectations, COVID-19 and environmental factors are at the core of the association between lung function and the development of COPD. Addressing the environmental risk factors and smoke cessation can significantly reduce the burden of COVID-19 and COPD equally.[9] We based our investigation on the mediating role of traits associated with lung function and the consequential development of COPD. Our study findings are inconsistent with previous investigations that reported that COPD is an agent for poor prognosis in COVID-19.[36] By adjusting COPD GWAS for smoking, we acknowledge potential genetic variants in our study, and this could result from the influence of collider bias. Notably, collider bias is a kind of selection bias that can affect observable associations, especially when investigating or looking for a common outcome of two different variables.[37] Inconsistencies could have been generated by many environmental risk factors reported by the previous studies, lung function based on other health issues like COVID-19 and obesity, alongside selection bias as the Mendelian randomization process could have scrambled for COPD survivors and the risk of COVID-19. We could not establish the effect of lung function on COVID-19-related risks courtesy of the robust effects of pleitropic implications of height. Nonetheless, banking on multivariate Mendelian randomization accounted for this shortcoming.

As environmental factors and COVID-19 are linked to severe health outcomes, there are unexamined pathways that play a mediating role. Specifically, similar to COVID-19, environmental factors such as smoking have been found to be associated with inflammation. Smoking has garnered significant attention because of its potential impact on the immune system, which could explain the poorer prognosis seen in some COVID-19 patients. However, it's important to note that there is insufficient genetic evidence to support this argument [38,39] fully. In simpler terms, smoking is connected to weakened immunity, which, in turn, may lead to a worse outlook for COVID-19 patients. This concept is vital in the study of COPD, as a weakened immune system is closely linked to a higher incidence of the disease.

The present investigation found that COVID-19 and environmental factors are associated with lung functions and the subsequent development of COPD. The risk and severity of COVID-19 are key to these pathways and the eventual development of COPD. The outcomes of the present investigation put smoking at the center of lung health when discussing COPD's development. Tobacco control and smoking cessation were found to be health risks and concerns for COVID-19 patients. Susceptibility to COVID-19 emerged as a health hazard of tobacco smoking, whose cessation was deemed as an effective mechanism for improving lung health.[40-42] While emphasizing the benefits of smoking cessation, it is crucial to recognize the challenges individuals with nicotine addiction face. Healthcare providers should offer comprehensive support and resources for smokers attempting to quit. This includes behavioral counseling, pharmacotherapy, and access to support groups. Recognizing the difficulty of quitting smoking underscores the importance of tailored interventions and ongoing assistance to increase the likelihood of successful smoking cessation among individuals at risk for COVID-19 and COPD.

Smoking's association with worse outcomes in COVID-19 patients can be attributed to several biological mechanisms. Smoking significantly impacts the respiratory system by impairing mucociliary clearance and damaging cilia, hindering the removal of mucus and pathogens from the respiratory tract.[43] This compromised defense mechanism creates an environment conducive to viral replication and infection. Additionally, smoking induces chronic inflammation in the lungs, releasing pro-inflammatory cytokines that contribute to lung damage and exacerbate the inflammatory response triggered by viral infections, potentially leading to more severe outcomes in COVID-19. Furthermore, smoking has immunosuppressive effects, weakening both innate and adaptive immune responses. This compromised immune system struggles to mount an effective defense against viral infections, allowing for more extensive viral replication and severe disease. Smoking is also associated with an upregulation of ACE2 receptors, the entry point for SARS-CoV-2. Increased expression of ACE2 receptors may enhance the virus's ability to infect respiratory cells, contributing to a higher viral load and more severe disease.

Moreover, smoking exacerbates existing comorbidities such as COPD, cardiovascular diseases, and diabetes, which are already linked to severe outcomes in COVID-19 patients. The oxidative stress induced by smoking damages cells and tissues,

impairing lung function and increasing susceptibility to respiratory infections.[44] Finally, smoking alters the function of immune cells, including macrophages and neutrophils, essential components of the immune response. Impaired function of these cells compromises the ability to efficiently clear the virus.

Clinical Implications

The findings of this study have significant implications for healthcare and public health strategies. Smoking cessation programs should be prioritized and tailored to specifically address the increased risk of COVID-19 severity associated with smoking. Public health campaigns should highlight the dual impact of smoking on respiratory health and COVID-19 outcomes, emphasizing the potential benefits of quitting. Healthcare providers should incorporate smoking cessation counseling into routine clinical care, and interventions should consider the specific needs of individuals with a history of smoking to enhance effectiveness.

Study limitations and Future Directions

The present study has several limitations. Firstly, the study relies on three key assumptions, including the selection of strong instruments derived from a large Genome-Wide Association Study (GWAS) to minimize the risk of bias associated with weak instruments. However, the potential for a false-negative outcome exists if the instruments have a low R2 despite the known detrimental effects of smoking on both the severity and risk of COVID-19. Moreover, biases resulting from assumptions on instrumental variables, particularly the association between confounders and instruments, where alleles increasing smoking are linked to confounders, pose a potential risk, thereby enhancing the overall risk of COVID-19. Education levels and the impact of a high Body Mass Index (BMI) are identified as crucial factors that could exaggerate the overall effect of smoking. While sensitivity analyses and the main analysis consistently show adverse effects of smoking on COVID-19, the MR-Egger analysis deviates, characterized by low statistical power and sensitivity to outliers. The design of the study, employing a two-step Mendelian randomization to explore possible mediation, did not yield substantial evidence regarding the effects of lung function or COPD on the risk of COVID-19. The study did not investigate mediation through alternative techniques, such as multivariate Mendelian randomization. F-statistics suggest susceptibility to weak instrument bias in alternative techniques assessing mediation, emphasizing the need for caution in the interpretation of these results. Additionally, the study's limitation to European participants raises concerns about the generalizability of outcomes to other ethnicities, highlighting the necessity for further research involving diverse populations. Furthermore, the inclusion of cases with possible asymptomatic participants or those with mild symptoms introduces potential bias, particularly toward non-differential misclassification. Exclusion of individuals who succumbed to COVID-19 without hospitalization may introduce bias, and the study acknowledges the need to explore the effects of smoking on the overall COVID-19 mortality rate. Lastly, the immeasurability of environmental factors and smoking poses challenges to the accuracy and precision of measurements, limiting the study's ability to comprehensively capture the effects of these factors on lung function and COVID-19.

Future research should focus on refining instrumental variable selection, considering the association between confounders and instruments. Alternative mediation analysis techniques, such as multivariate Mendelian randomization, could be explored to address the limitations of the two-step Mendelian randomization design. Additionally, investigating other potential mediators and examining diverse populations would strengthen the generalizability of the findings. While the study was limited to European participants, it provides valuable insights that can be considered in the context of other populations. However, it is crucial to acknowledge the need for further research involving diverse ethnicities to ensure the generalizability of the findings. Future studies should include participants from various ethnic backgrounds to capture the genetic and environmental diversity that may influence the observed associations.

CONCLUSION

The present study established the genetic evidence of smoking and environmental factors and demonstrated that they enhance COVID-19's severity and risks. We provided credible evidence pointing out that environmental factors, smoking, and COVID-19 are associated with lung function. The evidence suggests that smoking cessation and addressing the environmental factors could reduce the burden associated with the risk of COVID-19. However, we recommend the examination of mechanistic pathways of many dimensions, like social, biological, and behavioral, concerning their effects on the burden resulting from COVID-19 among smokers and those exposed to other environmental factors. The multi-dimensional approach in examining mechanistic pathways encompasses exploring genetic, environmental, and lifestyle factors. Future research should delve into the specific genetic variants associated with COVID-19 severity, considering the interplay with environmental factors. Understanding how smoking interacts with genetic predispositions and environmental influences in a multi-dimensional context will provide a more comprehensive understanding of the mechanisms involved.

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