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ORIGINAL RESEARCH

The Causal Relationship Between Gastroesophageal Reflux Disease and Chronic Obstructive Pulmonary Disease: A Bidirectional Two-Sample Mendelian Randomization Study

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Background: Gastroesophageal reflux disease (GERD) and Chronic Obstructive Pulmonary Disease (COPD) often coexist and have been associated in observational studies. However, the real potential causal relationship between GERD and COPD is unknown and not well established.

Methods: In this study, we conducted a bidirectional two-sample Mendelian randomization(MR) to estimate whether GERD and COPD are causal. The GERD genetic data is from summary level data of a genome-wide association (GWAS) meta-analysis (Ncases = 71,522, Ncontrol=26,079). The COPD GWAS are available from the FinnGen (Ncases=16,410, Ncontrol=283,589). MR-Egger regression, Weighted Median, and Inverse-variance weighted (IVW) were used for MR analysis from the R package "TwoSampleMR", and IVW was the dominant estimation method. Additionally, the MR pleiotropy residual sum and outlier (MR-PRESSO), Cochran Q statistic, and leave-one-out analysis were used to detect and correct for the effect of heterogeneity and horizontal pleiotropy.

Results: MR analysis indicated that GERD was causally associated with an increased risk of COPD (IVW odds ratio (OR): 1.3760, 95% confidence interval (CI): 1.1565–1.6371, P=0.0003), and vice versa (IVW OR: 1.1728, 95% CI:1.0613–1.2961, P=0.0018). The analyses did not reveal any pleiotropy or heterogeneity.

Conclusion: Our study revealed possible evidence for a bidirectional causal relationship between GERD and COPD. Implementing screening and preventive strategies for GERD in individuals with COPD, and vice versa, will be crucial in future healthcare management. Further studies are needed to elucidate the mechanisms underlying the causal relationship between GERD and COPD.

Keywords: human genetics, Mendelian randomization, gastroesophageal reflux disease, causal relationship, chronic obstructive pulmonary disease

Introduction

Chronic obstructive pulmonary disease (COPD) and Gastroesophageal reflux disease (GERD) are two common and debilitating diseases that significantly impact global health. COPD is a progressive chronic respiratory disease (CRD) characterized by persistent airflow limitation, chronic systemic inflammation, respiratory symptoms such as coughing, wheezing, and shortness of breath, and pathological changes in the airways.[1,2] COPD has become the third leading cause of death worldwide, with high morbidity, mortality, and disability rates, and exacerbating the burden on healthcare systems and economies.[3,4] The incidence of COPD increases with age, with smoking being the major risk factor.[2,5] In 2019, the global prevalence of COPD was estimated to be approximately 212.3 million cases, with an additional 16.2 million new cases reported. The disease claimed the lives of 3.3 million people, making it the deadliest of all CRD.[5] Gastroesophageal reflux disease (GERD) is a common gastrointestinal

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disorder that affects approximately 13% of the world's population,[6,7] and 20% of adults in Western countries.[8] In recent years, an increasing number of observational studies have shown bidirectional associations between COPD and GERD.[9–11] It has been reported that the incidence of GERD in patients with COPD ranges from 17% to 78%.[12] In addition, a systematic review showed that the incidence of mixed reflux in patients with GERD was much higher in individuals with COPD than in those without COPD,[13] and exacerbations of COPD have also been associated with GERD.[14] While GERD and COPD are distinct clinical entities, several studies suggested a potential association between these two conditions. However, the results are inconclusive and susceptible because bias due to confounding and reverse causation cannot be excluded in observational studies. Therefore, the direction of this association and the underlying mechanisms remain to be elucidated.

This is the first study to evaluate the causal relationship between COPD and GERD. We conducted a bidirectional two-sample Mendelian randomization (MR) study using summary-level genome-wide association study (GWAS) data to evaluate the causal relationship of COPD on the risk of GERD and vice versa. MR is a genetic epidemiological approach that uses genetic variants as instrumental variables (IVs) to evaluate the causal effect of an exposure on an outcome and overcomes many of the limitations of traditional observational studies, including confounding and reverse causation.[15,16] The bidirectional MR will help to clarify whether GERD is a risk factor for the development of COPD or whether COPD contributes to the development of GERD. This study can advance our understanding of both diseases' pathophysiology by detecting the causal relationship between COPD and GERD. In addition, understanding the nature of this relationship is critical as it may have important clinical implications for disease management and treatment strategies.

Materials and Methods

We conducted a bidirectional two-sample MR to explore the causal relationship between COPD and GERD. This study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University. Because of this study relied on the publicly available summary data from the GWAS study, the requirement for informed consent was waived.

Data Sources of GERD

The GWAS summary statistics from a large meta-analysis of GERD in UK Biobank and QSkin Sun and Health Study cohorts by An et al,[17] including 332,601 participants of European-descent (71,522 GERD and 261,079 controls) and can be downloaded from URL (10.6084/m9.figshare.8986589). The data of patients diagnosed with GERD were collated by data fields code in the UKBB (68,535 GERD and 250,910 controls): self-report (field ID: 20002—Noncancer illness code, self-reported Medical conditions), ICD-10 (41202—main diagnoses, 41204—secondary diagnosis), ICD-9 (41203—main diagnoses, 41205—secondary diagnosis), the Office of Population Censuses and Surveys (41200—main operative procedures; 41210—secondary operative procedures) and treatment/medicine. And GRED cases were defined by self-reported heartburn and medical records of reflux medications taken in QSkin Sun and Health Study (2987 cases and 10,169 controls)[17] (Table 1).

Data Sources of COPD

The COPD GWAS summary data were obtained from FinnGen (https://www.finngen.fi/en), a large-scale genomic research project developed in Finland.[18] The COPD GWAS data from FinnGen included 16,410 COPD cases and 283,589 control cases. In the FinnGen project, the diagnosis of COPD was based on the International Classification of Diseases, 10th edition (ICD-10), 9th edition (ICD-9), and 8th edition (ICD-8) codes. The definition of endpoints events in the study is determined by the presence of COPD ICD codes (ICD-10:J43/44; ICD-9:491.2/492; ICD-8:491.04/492). All of the participants were of European ancestry (Table 1).

Table 1 Details of the GWASs Data

Traits	Sample Size (Cases/Controls)	Population	Consortium	Web Source
GERD	71,522/261,079	European descent	A meta-analysis of GWAS	https://doi.org/10.1038/s41467-019-11968-2
COPD	16,410/283,589	European	FinnGen	https://r8.finngen.fi/pheno/J10_COPD

Abbreviations: GERD, Gastroesophageal Reflux Disease; COPD, Chronic Obstructive Pulmonary Disease.

Selection of IVs

Three basic principles of MR must be met: (a) the IVs are strongly linked with exposure; (b) the IVs are not strongly linked with potential confounders, and (c) the IVs do not directly affect the outcome.[16]

For IVs, We chose single nuclear polymorphisms (SNPs) for GERD/COPD with a genome-wide significant threshold of $P < 5\times10^{-8}$. Then, we clumped the selected SNPs to avoid linkage disequilibrium (LD) with each other.[19] The clumping window with stringent threshold of $r^2 < 0.001$, window size = 10,000 kb. We searched the PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) to filter out the SNPs associated with known confounders to ensure that the SNPs are independent of the confounders. In the next step, we harmonized the exposure and outcome variation effect estimates and removed any SNPs with incompatible alleles or palindromic SNPs. We did not use proxies to replace missing outcome data.[20] In addition, we estimated the F-statistic ($F = beta^2/se^2$) for each SNP to assess its power, and SNPs were removed if the F<10.[21] We only used SNPs available for all traits analyzed as IVs to maintain consistency. Ultimately, 5 SNPs were available for COPD as exposures to analyses of the causal effect on GERD, and in the reverse direction, 18 SNPs were available for GERD (Table 2).

Statistical Analysis

In this study, we conducted a bidirectional MR study to estimate the potential causal associations between GERD and COPD, and the random-effects IVW method was used as the top-choice statistical method. Then, to ensure the robustness of the main analysis, we performed sensitivity analyses. Cochran's Q test was used to assess the heterogeneity among each IV. The horizontal pleiotropy of IVs was evaluated by the MR-Egger intercept test.[22] To identify potential horizontal pleiotropic outliers and to address any potential bias caused by these outliers, we used the MR-PRESSO test.[23] This test was used as a corrective measure in our analysis. In addition, we performed a leave-one-SNP-out analysis to evaluate the effect of excluding single SNP of exposure on the results of the MR study. All statistical analyses mentioned above were

Table 2 The Detailed Information of the Instrumental Variables in Each Trait

Traits	SNP	Effect Allele	Other Allele	P-value	Beta	SE	F
COPD	rs143031266	G	A	2.74E-08	0.164683	0.0296348	30.88115523
	rs2273500	C	T	2.19E-11	0.0951338	0.0142143	44.79388539
	rs2904259	C	T	1.97E-08	0.0664499	0.0118358	31.52052393
	rs66694560	A	G	5.19E-10	0.0763852	0.0122937	38.60585883
	rs9270664	A	G	1.25E-08	0.067614	0.0118783	32.40146741
GERD	rs111472920	T	G	4.47E-08	0.0987	0.018	30.06694444
	rs12706746	A	G	4.24E-09	0.0369	0.0063	34.30612245
	rs12939066	T	C	3.13E-11	0.0415	0.0062	44.80359001
	rs13167137	T	G	2.82E-08	−0.0328	0.0059	30.90606148
	rs1473115	T	C	3.95E-10	0.0396	0.0063	39.51020408
	rs15071	T	C	1.01E-08	−0.0428	0.0075	32.56604444
	rs1858828	T	G	3.23E-08	0.0327	0.0059	30.71789716
	rs3072	T	C	1.86E-08	−0.0345	0.0061	31.98736899
	rs62046253	T	C	1.30E-08	−0.0352	0.0062	32.23309053
	rs62442944	T	G	3.49E-08	0.0418	0.0076	30.25
	rs6683411	A	G	4.82E-08	0.0324	0.0059	30.15685148
	rs6710685	T	C	1.96E-09	0.0391	0.0065	36.18485207
	rs6762606	T	C	4.18E-08	−0.0359	0.0065	30.5043787
	rs6809836	A	G	3.70E-09	0.0383	0.0065	34.71928994
	rs6991878	T	C	2.71E-08	−0.0338	0.0061	30.70249933
	rs72704785	A	G	6.81E-09	0.0459	0.0079	33.7575709
	rs7552188	T	C	1.07E-08	0.0413	0.0072	32.90297068
	rs769671	T	C	9.28E-10	−0.0385	0.0063	37.34567901

Abbreviations: SNP, single-nucleotide polymorphism; Beta, the per-allele effect on each trait; SE, standard error; F, F-statistic.

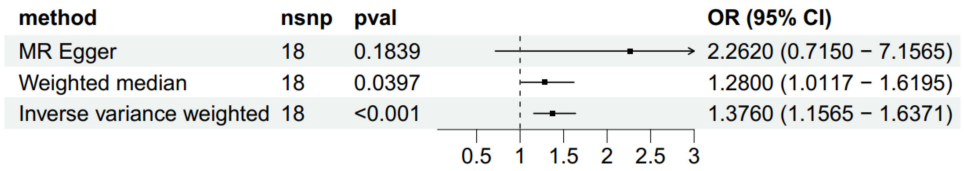

Figure 1 Estimated causal effect of GERD on COPD using different MR methods.
Abbreviations: SNP, single-nucleotide polymorphism; pval, p value; OR, Odds Ratio; CI, Confidence Interval.

two-sided and conducted using the TwoSampleMR packages in R 4.2.3. $P < 0.05$ were considered statistically significant.[24]

Results

The Causal Effect of GERD on COPD

For the forward MR analysis, 1 SNP rs11171710 associated with emphysema was excluded. 4 SNPs (rs7590354, rs2271856, rs2927089, and rs1050458) were not found in FinnGen of COPD GWAS data. 2 SNPs (rs10228350; rs2108959) for being palindromic were excluded after the harmonization process. Finally, 18 eligible SNPs were selected as IVs of GERD as the exposure and COPD as the outcome, and the F statistic of all SNP was greater than 10 (Table 2). The study of the main result showed IVW (OR: 1.3760, 95% CI: 1.1565–1.6371, *P*=0.0003) and indicated that genetic predictions for GERD showed a significant association with the incidence of COPD, increasing the risk by 37.6%. MR-egger (OR: 2.2620, 95% CI: 0.7150–7.1565, *P*=0.1839), weight median (OR: 1.2800, 95% CI: 1.0117–1.6195, *P*=0.0397).(Figures 1 and S1A) There was no significant bias in the causal effect of GERD on COPD in the sensitivity analysis (all *P* values >0.05 for Cochran's Q test; MR-egger intercept test: Intercept =−0.0191, *SE*=0.0223, *P* =0.4047; MR-PRESSO global test *P*=0.3400) (Table 3). The leave-one-SNP-out analysis showed that the effect estimates were not affected by the presence of a single SNP (Figure S1B).

The Causal Effect of COPD on GERD

For the reverse-direction MR analysis, 1 SNP rs708461 for being palindromic was excluded after the harmonization process. The trait of SNP rs8040868 and SNP rs113623975 were associated with smoking and were eliminated, which was the potential confounder of GERD.[8] 1 SNP rs28929474 was the outlier MR-PRESSO detected and was excluded. Finally, 5 eligible SNPs remained as IVs of COPD as the exposure and GERD as the outcome. The F statistic of all remained SNP is greater than 10(Table 2). In the IVW mode analysis, we found that COPD had a significant causal risk effect on GERD (OR: 1.1728, 95% CI:1.0613–1.2961, P=0.0018). MR-egger (OR: 1.4337, 95% CI: 0.8934–2.3009, *P*=0.2323), weight median (OR: 1.1754, 95% CI: 1.0451–1.3219, *P*=0.0070) (Figures 2 and S2A). There was no significant bias in the causal effect of COPD on GERD in the sensitivity analysis (all *P* values >0.05 for Cochran's Q test; MR-egger intercept test: Intercept =−0.0160, *SE*=0.0188, *P* =0.4564; MR-PRESSO global test *P*=0.2673) (Table 3). The leave-one-SNP-out analysis did not find any high-impact points (Figure S2B).

Table 3 Sensitivity Analysis of MR

Exposure	Outcome	nSNPs	Heterogeneity Test		MR-Egger Pleiotropy Test		MR-PRESSO Global Outlier Test		
			Q	P-value	Intercept	P-value	RSSobs	P-value	Outlier
GERD	COPD	18	19.275370	0.3129	−0.0191	0.4047	21.77467	0.3400	None
COPD	GERD	6	14.371421	0.0134	0.0141	0.2562	24.56575	0.0367	rs28929474
After remove the outlier									
COPD	GERD	5	5.964387	0.2018	−0.0160	0.4564	9.919114	0.2673	None

Abbreviations: GERD, Gastroesophageal Reflux Disease; COPD, Chronic Obstructive Pulmonary Disease; SNP, single-nucleotide polymorphism; MR-PRESSO, MR pleiotropy residual sum and outlier.

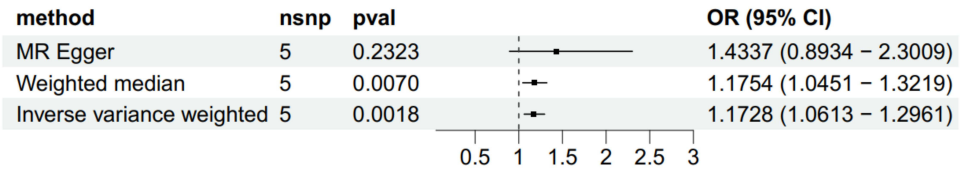

Figure 2 Estimated causal effect of COPD on GERD using different MR methods.
Abbreviations: SNP, single-nucleotide polymorphism; pval, p value; OR, Odds Ratio; CI, Confidence Interval.

Discussion

To our knowledge, this is the first bidirectional MR study to evaluate the causal association between COPD and GERD. The results of our MR study may provide evidence supporting that genetically predicted COPD was positively associated with the risk of GERD and vice versa. The sensitivity analyses conducted in this study consistently and robustly supported the causal association between COPD and GERD. Although MR-egger results do not support a causal association between GERD and COPD, the results are in the same direction as IVW. Some researchers believe that if the IVW method produces a significant result, even if the results of the other methods are not significant and there is no evidence of pleiotropy and heterogeneity, this result can be considered positive as long as the beta values of the other methods point in the same direction.[25–27]

For the forward MR analysis, our results showed a significant positive causal association of GERD with COPD risk, consistent with previous study findings. In this case, the presence of GERD-associated genetic variants may causally associated with an elevated risk of developing COPD. This finding implies that the genetic factors influencing GERD contribute, at least in part, to the development of COPD. García Rodríguez LA et al[10] used the UK General Practice Research Database to perform a study that found that patients diagnosed with GERD had a 17% increased risk of developing COPD. Faulty anti-reflux barriers cause GERD, one of the primary mechanisms.[28] Current perspectives and some studies show that GERD may contribute to the progression of COPD by inhaling gastric contents, acid reflux, leading to broncho-constriction and airway inflammation and potentially causing or exacerbating COPD.[29–32] These findings could have significant implications for the clinical management of individuals with GERD, highlighting the importance of early diagnosis and treatment of GERD to potentially reduce the risk of subsequent development of COPD.

Conversely, in the reversal MR analysis, we also discovered that COPD has a potential causal association with GERD risk. Current extensive research indicates a relatively high prevalence of GERD in COPD patients.[12,33,34] Furthermore, the coexistence of GERD in patients with COPD appears to be associated with worsened pulmonary symptoms, decreased quality of life, and increased frequency of COPD exacerbations.[35–38] A comprehensive systematic review and meta-analysis showed that GERD was a significant risk factor for exacerbations of COPD (OR: 5.37; 95% CI 2.71–10.64).[39] According to Rascon-Aguilar et al,[40] patients with GERD-positive COPD were twice as likely to experience exacerbations as patients without GERD. Patients with COPD experience significant lung function impairment and reduced lung compliance, which can lead to dyspnea, breathing way changes, and respiratory muscle fatigue, which increases intrathoracic pressure.[26] Elevated intrathoracic pressure can increase intra-abdominal pressure, resulting in decreased pressure on the lower esophageal sphincter (LES), causing reflux of gastric acid and digestive fluid into the esophagus.[29,41] In addition, patients with COPD require long-term use of respiratory medications such as inhaled bronchodilators, anticholinergics, and corticosteroids, which may increase the risk of developing GERD.[31,42,43] Obviously, the mechanisms described in the previous paragraph are closely related to this section, often resulting in a vicious cycle. However, because GERD is often silent and imperceptible in patients with COPD, it is may always overlooked as a potential cause of respiratory illness and symptoms.[44] Therefore, screening for GERD in patients with COPD is necessary.

As mentioned above, the underlying mechanisms of the interaction between GERD and COPD remain unclear. Based on our research, we speculate on possible mechanisms underlying the association between these two diseases. rs15071 is located on chromosome 5, in the 3' UTR of the VCAN gene. The VCAN gene encodes a proteoglycan called versican in the extracellular matrix (ECM). A study conducted by Liao et al[45] found a significant association between the VCAN gene and lung function through dmGWAS network analysis and validation. It has been reported that versican expression

negatively correlates with FEV1 in human alveolar walls and rims.[46] Versican has been implicated in the tissue remodeling process in the lungs of COPD patients. A study conducted by Wu et al[47] demonstrated that inhibition of versican synthesis may be a potential therapeutic strategy to increase the deposition of insoluble elastic proteins and stimulate elastic fiber repair in the lungs of COPD patients. In addition, COPD patients are known to have persistent chronic inflammation. Versican plays a critical role in the regulation of immune and inflammatory responses.[48,49] Versican can interact with inflammatory cells indirectly or directly; these interactions activate signaling pathways that promote the synthesis and secretion of inflammatory cytokines such as TNF-α, IL-6, and NF-kB.[49,50]

In addition, we identified rs6683411 located on chromosome 1 in the intergenic region of the hnRNPA1P46 gene. Heterogeneous nuclear ribonucleoprotein A/B (hnRNPA/B) is a core member of the hnRNP family of RNA-binding proteins (RBPs), which includes four major subtypes, A0, A1, A2/B1, and A3.[51] Previous studies suggest that hnRNPs were involved in and induce epithelial-mesenchymal transition (EMT), a process closely associated with the development of COPD and a potential mechanism leading to small airway fibrosis.[52–55] rs6762606 is located on chromosome 3, in the intronic region of the EPHB1 gene. As mentioned above, the study by Liao et al[45] also clearly shows a significant association between the EPHB1 gene and lung function. rs72704785 on chromosome 5 is located in the upstream promoter region of the miR-4456 gene. Existing research suggests that miR-4456 may potentially inhibit the tight junction(TJ) damage caused by tobacco exposure in COPD through its interaction with chemokine ligands.[56]

Our study has some strengths. First, the MR design allowed us to assess causality by using genetic variants as IVs, thereby reducing the effects of confounding and reverse causation. Second, we used large-scale GWAS to obtain robust genetic instruments and summary statistics for COPD and GERD. Third, bidirectional MR analysis provided insight into the directionality of the causal relationship between COPD and GERD. Of course, there are some potential limitations. First, the prior observation studies have explored this relationship, so our research findings may have a limited impact on advancing the development of this field. Second, as with all MR studies, our results depend on certain assumptions, including that the genetic variants used as IVs are valid instruments and that there is no pleiotropy. Although we conducted sensitivity analyses to assess the robustness of our results, the possibility of unmeasured pleiotropy or violation of these assumptions cannot be completely ruled out. Third, the generalizability of our findings may be limited to populations of European ancestry, as the genetic tools were primarily derived from studies conducted in these populations. Finally, additional subgroup analyses were not possible due to the use of summary statistics rather than raw data in the study.

In conclusion, our bidirectional two-sample Mendelian randomization study may provide potential evidence for a causal association between COPD and an increased risk of GERD. These findings have important clinical implications, emphasizing the need for clinicians to consider both diseases simultaneously in their management strategies and the importance of early intervention and management. Further research is warranted to elucidate the underlying mechanisms linking GERD and COPD, explore potential therapeutic interventions, and investigate this relationship's bidirectional nature in different populations.

Conclusion

This study represents the first MR study to investigation of the causal relationship between GERD and COPD. Our comprehensive MR analysis revealed possible evidence for supporting a bidirectional causal relationship between GERD and COPD. By using genetic variants as IVs, we overcame confounding factors and gained a more robust understanding of the causal nature of this relationship. Further research is warranted to delve deeper into the mechanisms underlying this bidirectional causality and explore potential therapeutic interventions that could improve outcomes for individuals affected by GERD and COPD.

Abbreviations

GERD, Gastroesophageal reflux disease; COPD, Chronic Obstructive Pulmonary Disease; GWAS, genome-wide association study; MR, Mendelian randomization; IVW, Inverse-variance weighted; MR-PRESSO, MR pleiotropy residual sum and outlier; OR, odds ratio; CI, confidence interval; CRD, chronic respiratory disease; IVs, instrumental variables; SNPs, single nuclear polymorphisms; LD, linkage disequilibrium; SE, standard error; LES, lower esophageal sphincter; ECM, extracellular matrix; RBPs, RNA-binding proteins; EMT, epithelial-mesenchymal transition; TJ, tight junction.

Data Sharing Statement

All data generated or analysed during this study are included in this published article and its Supplementary Information Files.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University. Because of this study relied on the publicly available summary data from the GWAS study, the requirement for informed consent was waived.

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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 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 they have no competing interests.

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