Causal Associations Between Inflammatory Bowel Disease and Primary Biliary Cholangitis: A Two-Sample Bidirectional Mendelian Randomization Study

Jiaxi Zhao (zhaojia_xi@126.com)
General Practice Medical Center, West China Hospital, Sichuan University

Kaixin Li
Huadong Hospital Affiliated to Fudan University

Xiaoyang Liao
General Practice Medical Center, West China Hospital, Sichuan University

Keywords: inflammatory bowel disease, ulcerative colitis, Crohn's disease, primary biliary cholangitis, Mendelian randomization

Posted Date: January 9th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2435433/v1

License: ☑️ This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background

Inflammatory bowel disease (IBD) was reported to be associated with hepatobiliary disease. Previous observational and Mendelian randomization (MR) studies suggested a causal association between IBD and primary sclerosing cholangitis (PSC). However, it is unclear whether IBD has causal association with primary biliary cholangitis (PBC): another autoimmune liver disease.

Methods

We obtained genome-wide association study (GWAS) statistics from published GWASs for PBC, UC and CD. We screened qualified instrumental variables (IVs) based on the three major assumptions of MR. To determine the causal relationship between UC or CD and PBC, two-sample MR analyses were performed using inverse variance weighted (IVW), MR-Egger, and weighted median (WM) methods, and sensitivity analyses were conducted to validate the robustness of the results. We also conducted reverse MR analysis to reveal the causal association between PBC and UC or CD.

Results

UC were associated with a higher risk of PBC (OR = 1.35, 95% CI: 1.05–1.73, P = 0.02) in IVW method. And CD was associated with an increased risk of PBC (OR = 1.18, 95% CI: 1.03–1.36, P = 0.02) in IVW method. The weighted median and MR-Egger regression of both diseases showed a consistent direction but not statistically significant. Results of reverse MR analysis did not suggest genetic susceptibility to psoriasis was associated with increased risk of UC (OR = 1.05, 95% CI: 0.95–1.17, P = 0.34) or CD (OR = 1.1, 95% CI: 0.99–1.20, P = 0.06).

Conclusion

The present study revealed that IBD subtypes could increase the incidence of PBC, but in turn PBC did not increase the incidence of IBD subtypes. Understanding that IBD and PBC constitute mutual risk factors can help with clinical management of both diseases.

Introduction

Primary biliary cholangitis (PBC), formerly known as primary biliary cirrhosis, is an autoimmune liver disease characterized clinically by chronic cholestasis and histologically by non-suppurative destructive cholangitis. Along with primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH), it is an autoimmune liver disease.[1] γ-glutamyl-transferase (GGT) and alkaline phosphate (ALP) levels, which
represent hepatic bile acid excretion, are crucial for clinical management of PBC\cite{2,3}. Ursodeoxycholic acid (UDCA) is currently used as first-line therapy, but up to 40% of PBC patients have an inadequate clinical response\cite{4}. Previous studies showed that PBC was linked to extrahepatic immune-mediated diseases, such as Hashimoto's thyroiditis and rheumatoid arthritis\cite{5}.

Inflammatory bowel disease (IBD) is a chronic, idiopathic inflammatory diseases of the gastrointestinal tract that mainly includes two categories: Crohn's disease (CD) and ulcerative colitis (UC). The etiology of IBD is related to genetic, autonomic immune, endocrine, and other factors \cite{6-8}. With a prevalence of more than 0.3% in Western countries, IBD has become a global disease, emphasizing the importance of IBD prevention and management\cite{9}. As one of the autoimmune liver disease, PSC is strongly linked to IBD\cite{10}. However, the connection between IBD and PBC has yet to be determined. In recent years, there have been some case reports of concomitant PBC and IBD\cite{11,12}. However, there are currently very limited observational studies and intervention trials on IBD in conjunction with PBC, so a reliable method is required to confirm the causal relationship between IBD and PBC.

Traditional observational studies' ability to infer causality is hampered by potential confounding and reverse causality. Mendelian randomization (MR) analysis detects and quantifies causality by using genetic variations as instrumental variables (IVs). There are three key hypotheses that must be met: the IV: 1. has a strong correlation with exposure, 2. independent of confounding factors between exposure and outcome, 3. influences outcome directly through exposure\cite{13,14}. This study conduct a two-sample bidirectional MR analysis using up-to-date data from genome-wide association studies (GWASs) to to assess the causal relationship between IBD subtypes and PBC.

**Materials And Methods**

**Study Design**

The bidirectional causal relationships between IBD subtypes and PBC were evaluated using MR analysis. Three key hypotheses were shown in Fig. 1. We first looked into causality by using IBD subtypes as exposures and PBC as an outcome. Then, using PBC as an exposure and IBD subtypes as outcomes, reverse causality was investigated.

**Data Source**

We used the International Inflammatory Bowel Disease Genetics Consortium (IIBDGC) GWAS data, which included 42,950 cases with IBD and a control group of 53,536 people without IBD. Genetic association data included 72,647 UC participants (n\text{case} = 20,417, n\text{control} = 52,230) and 69,268 CD participants (n\text{case} = 22,575, n\text{control} = 46,693), respectively\cite{15}. IBD and its subtypes are diagnosed using endoscopic, radiological, and histopathological criteria. The GWAS data of PBC were obtained from a meta-analyses
of European subjects (n case = 2,764 cases, n control = 10,475). PBC is diagnosed using the International Classification of Diseases (ICD-10) criteria.

GWAS data are obtained by the IEU OpenGWAS online database, which could be used in extensive analysis. The ethical approval were not necessary for this study, since participant consent and ethical approval were obtained for the IBD and PBC preliminary study.

**Snp Selection**

We found single-nucleotide polymorphisms (SNPs) that were strongly linked to UC, CD, and PBC, with genome-wide significance of $P < 5 \times 10^{-8}$. We clumped all SNPs in linkage disequilibrium (LD) ($r^2 < 0.001$). Then, to ensure the strength of the IVs and to mitigate the effects of weak instrument bias, we filtered SNPs with F-statistics greater than 10. By harmonizing, we also eliminated ambiguous and palindromic SNPs. Finally, 44, 59, and 16 SNPs were used as IVs for UC, CD, and PBC for subsequent MR analysis, respectively. Details of these IVs are provided in Supplementary Tables 1–3.

**Mendelian Randomization Analyses**

We combined several statistical methods in the MR analysis. The primary method was the inverse variance weighted (IVW), which is with balanced pleiotropy that is expected to be stable. In addition, MR-Egger regression, weighted median, and MR pleiotropy residual sum and outlier (MR-PRESSO) test were used as supplements to the IVW method to estimate the causal relationship under different conditions $[^{16-18}]$. The findings were presented in the form of odds ratios (ORs) and 95% confidence intervals (CIs). Statistical significance was considered when the two-sided p-value was less than 0.05. All statistical analyses have been performed using TwoSampleMR package (version 0.5.6) in R (2022.02.3) $[^{19}]$.

**Sensitivity Analysis**

Horizontal pleiotropy occurs when IVs associated with the exposure influence the outcome through multiple factors other than the exposure. We used the MR-Egger intercept test to detect pleiotropy, pleiotropy exists when $P < 0.05$, and the results are not reliable which should be abandoned, the results of MR-Egger intercept test were visualized by scatter plots. We used Cochran's Q statistics to examine heterogeneity, heterogeneity exists when $P < 0.05$, and the results were visualized by funnel plots. MR-PRESSO test was used to correct for heterogeneity: detecting outliers and excluding them, then testing for heterogeneity again. In the leave-one-out analysis, results were re-analyzed after removing SNPs once at a time, and forest plots were drawn to intuitively judge the robustness of the results.

**Results**
Mendelian randomization analyses

Effect of UC or CD on PBC

According to the IVW method, UC were associated with a higher risk of PBC (OR = 1.35, 95% CI: 1.05–1.73, \( P = 0.02 \)). Both MR-Egger regression and weighted median showed a consistent direction but unsignificant results (OR = 1.12, 95% CI: 0.43–1.95, \( P = 0.824 \) and OR = 1.17, 95% CI: 0.99–1.38, \( P = 0.057 \), respectively).

In the IVW method, CD was associated with an increased risk of PBC (OR = 1.18, 95% CI: 1.03–1.36, \( P = 0.02 \)). The weighted median (OR = 1.13, 95% CI: 0.99–1.28, \( P = 0.056 \)) and MR-Egger regression (OR = 1.17, 95% CI: 0.81–1.69, \( P = 0.413 \)) showed a consistent direction but not statistically significant results. (Supplementary Table 4)

Effect Of Pbc On Uc Or Cd

We also conducted a reverse MR analysis between PBC and IBD subtypes, but the IVW method did not reveal any reverse causal relationships between them. Supplemental Table 5 provides the results of the reverse MR analysis.

Sensitivity Analysis

We conducted the Cochran's Q statistics and its funnel plots, MR-Egger intercept test and its scatter plots, leave-one-out analysis and its forest plots in the sensitivity analysis. And MR-PRESSO test was used to correct for heterogeneity. The analyses of UC on PBC revealed significant heterogeneity (Q = 270.452, \( P = 9.660 \times 10^{-35} \)). There was no evidence of directional pleiotropy (intercept = 0.0422, \( P = 0.295 \)). (Supplementary Table 6) After outliers were removed, the association was significant (outlier-corrected: OR = 0.14, 95% CI: 0.08–1.74, \( P = 0.031 \)). The leave-one-out analyses demonstrated the robustness of the findings.

There was significant heterogeneity in the Cochran's Q statistics between CD and PBC (Q = 192.294, \( P = 1.445 \times 10^{-16} \)). But there was no evidence of pleiotropy between CD and PBC (intercept = 0.0013, \( P = 0.956 \)). (Supplementary Table 5) To correct the heterogeneity, we removed the outliers and conducted the MR-PRESSO test, the results shown that the association was significant (outlier-corrected: OR = 0.14, 95% CI: 0.06–2.52, \( P = 0.015 \)).

The funnel plots of the MR analysis' Cochran's Q statistics was displayed in Fig. 2. Figure 3 and Fig. 4 depicted the scatter plots of the causal relationships between IBD subtypes and risk of PBC, as well as the forest plots of the leave-one-out analyses, respectively.
The IVW method in the MR analysis revealed no reverse causality between PBC and UC or CD. As a result, additional heterogeneity and pleiotropy testing was not required.

**Discussion**

The mechanism of occurrence of PBC, an autoimmune liver disease, is currently not clear, and the treatment response is unreliable. Therefore, it is fairly important to explore diseases that may increase the susceptibility to PBC. Starting from the already-known causal relationship between IBD and PSC, this study innovatively explored the causal relationship between IBD and PBC, providing new ideas for the prevention and treatment of PBC.

The GWAS data from the International Inflammatory Bowel Disease Genetics Consortium (IIBDGC) large-scale and a PBC meta-analysis were used in this study. In addition, we used multiple MR methods to investigate the possible causal relationship between IBD and PBC susceptibility. We also used reverse MR to investigate the relationship between PBC and IBD susceptibility. The primary MR analysis indicated that both UC and CD were causally associated with an increased risk of PBC (OR = 1.35, 95% CI: 1.05–1.73, P = 0.02; OR = 1.18, 95% CI: 1.03–1.36, P = 0.02, respectively). However, reverse MR analysis did not find a causal relationship between PBC and IBD.

Both UC and CD are linked to a variety of hepatobiliary symptoms\[^{20}\]. PSC is the best known, and nonalcoholic fatty liver disease (NAFLD) is the most common. A study published in 1999 by Koulentaki M found that the prevalence of PBC in IBD patients was higher than in the general population\[^{21}\]. In a clinical study of six patients with IBD and PBC, PBC was diagnosed after IBD in six patients (the mean time between IBD and PBC diagnosis was 7.1 years, ranging from 1.1 to 22.2 years)\[^{12}\].

Genetics plays an important role in the association between IBD and PBC. The association of UC and PBC with genes on the short arm of chromosome 6 may imply that the genes of inflammation play a pathogenic role in both diseases. Some drugs commonly used in IBD can also cause damage to the liver\[^{22}\]. A mild increase in liver function tests can occur following treatment with 5-ASA in the first 6 months to several years\[^{23}\]. TNF-α blocking agents, on the other hand, were linked to the onset of autoimmune diseases, including autoimmune liver disease\[^{12}\]. Furthermore, cholestasis occurred after infliximab infusion in one case and resolved after the drug was discontinued\[^{24}\].

Alleles of specific genes linked to PBC predispose people to specific infections. And the characterization of the genetic components of PBC may indirectly reveal associations with specific infectious agents. Surprisingly, recent epidemiological studies have repeatedly shown a link between PBC and infectious agents\[^{25–27}\]. More than 200 risk gene loci are known to be associated with IBD, and genes are associated with susceptibility to infection\[^{15,28}\]. As a result, an infection may activate the body’s cross-immune response, which is linked to the pathogenesis of PBC and IBD. Similarly, for both PBC and IBD, strong genetic associations within the major histocompatibility complex (MHC) have been consistently reported\[^{29,30}\]. (Fig. 5)
However, current clinical studies on the relationship between IBD and PBC were limited, consisting mostly of case reports, including one in which a patient developed PBC after having a colectomy, implying that surgery may be a factor in promoting disease progression[31]. In addition, gut permeability disruption in IBD may result in bacterial translocation, subsequent activation of cholangiocytes via the portal system, and activation of inflammatory responses and fibrosis in the liver, ultimately leading to the development of PBC[32].

We’d like to highlight some of our study’s strengths while also acknowledging some of its limitations. On one hand, this was the first study to use the 2-sample MR method to assess bidirectional causality between IBD and PBC. This approach had the advantage of being less susceptible to confounding factors, and reverse causality, when compared to observational studies and intervention experiments. On other hand, we studied the rare clinical disease of IBD combined with PBC in a novel way, going beyond the limitations of previous case reports. The study also had limitations. First, due to data availability, we limited the population to people of European ancestry. As a result, applying the findings of this study to other populations should be careful. Second, while methods such as removing linkage disequilibrium and detecting pleiotropy were used in the selection and processing of IVs to minimize and monitor their effects on the results, it was difficult to completely avoid them.

**Conclusions**

This is the first MR study to explore the bidirectional causal relationship between IBD and PBC. Our MR analysis suggested that IBD may increase the incidence of PBC, but in turn, PBC did not increase the incidence of IBD. The underlying mechanism of IBD on the occurrence of PBC needs to be investigated further.

**Declarations**

**Funding statement**

There was no funding to report.

**Conflict of interest disclosure**

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

**Ethics approval statement and patient consent statement**

The ethical approval were not necessary for this study, since participant consent and ethical approval were obtained for the IBD and PBC preliminary study.

**Permission to reproduce material from other sources**
The use and citation of the two sets of GWAS data included in the article have been approved by the authors.

**Authors’ Contributions:**

Jiaxi Zhao wrote the main manuscript text. Jiaxi Zhao prepared figure1-4, Kaixin Li and Jiaxi Zhao prepared figure 5. Xiaoyang Liao provided methodological assistance. Jiaxi Zhao prepared the supplementary tables. All authors reviewed the manuscript.

**Data Availability**

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

**References**


**Figures**

![Diagram A](image)

![Diagram B](image)

**Figure 1**
The flow diagram of the MR analysis.

(A) Using IBD SNPs as IVs to study the causal impact of IBD subtypes on PBC. (B) Using PBC SNPs as IVs to study the causal impact of PBC on IBD subtypes. Solid lines indicate IVs (SNPs) are associated with exposure and can only influence outcome through exposure. Dashed lines indicate IVs (SNPs) are independent of any confounding variables between exposure and outcome.

Figure 2
Causal relationships between UC or CD and PBC in funnel plots

UC: ulcerative colitis; CD: Crohn's disease; PBC: primary biliary cholangitis; MR: mendelian randomization

Figure 3

Causal relationships between UC or CD and PBC in scatter plots
UC: ulcerative colitis; CD: Crohn's disease; PBC: primary biliary cholangitis; SNPs: single-nucleotide polymorphisms; MR: mendelian randomization

Figure 4

Forest plots of SNPs associated with UC or CD and risk of PBC
UC: ulcerative colitis; CD: Crohn's disease; PBC: primary biliary cholangitis; SNPs: single-nucleotide polymorphisms; MR: mendelian randomization

Figure 5

The relationships between infection, genetic code, IBD and PBC

Infection can directly affect the occurrence of IBD and PBC, and can also lead to susceptibility to IBD and PBC by affecting genetic changes; Genes also cause susceptibility to certain infections; IBD may increase the incidence of PBC, but PBC did not increase the incidence of IBD. IBD: Inflammatory bowel disease; PBC: primary biliary cholangitis

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable.xlsx