scientific reports

OPEN

The mediating role of primary sclerosing cholangitis in the association between ulcerative colitis and hepatobiliary cancer investigated through Mendelian randomization

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This study explored the causal relationships among primary sclerosing cholangitis (PSC), ulcerative colitis (UC), and hepatobiliary cancer (HBC) by using bidirectional two-sample, two-step Mendelian randomization (MR) analysis. Genetic variants associated with PSC and UC from the FinnGen research database were used for instrumental variable-based analyses. Mediation analyses were conducted to examine the role of PSC and UC in HBC risk. The findings revealed a causal effect of genetic predisposition to UC on PSC risk (inverse-variance-weighted [IVW] analysis odds ratio [OR] 1.145, $p < 0.001$), whereas no reverse causality was observed. Although UC showed no direct causal effect on HBC risk, genetic susceptibility to PSC significantly increased the risk of HBC (IVW analysis OR = 1.855, $p < 0.001$). Mediation analysis further identified PSC as a significant mediator amplifying the causal effect of UC on HBC risk (effect size = 0.083). These results established a causal link between genetic susceptibility to UC and increased risk of PSC, and highlighted the critical role of PSC in mediating the impact of UC on HBC risk.

Keywords causal relationship, hepatobiliary cancer, Mendelian randomization, mediator, primary sclerosing cholangitis, ulcerative colitis

Hepatobiliary cancer (HBC) includes primary liver cancers, such as hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and gallbladder cancer (GBC). HBC is the sixth most common malignancy and the third leading cause of cancer-related mortality. Given the marked increase in susceptibility to HCC as compared to the general population, primary sclerosing cholangitis (PSC) has garnered attention as a potential predisposing factor for hepatobiliary cancer[1]. PSC is a rare, chronic, immune-mediated cholangiopathy characterized by inflammation and progressive fibrotic narrowing of the bile ducts[2,3]. Existing literature demonstrates a recent increase in its prevalence, with reported rates ranging from 0.78 to 31.7 per 100,000 individuals[4]. Although the prevailing consensus attributes PSC to a combination of environmental and genetic factors, the precise etiology of this condition remains elusive[3]. The natural progression of PSC culminates in the emergence of biliary cirrhosis and eventually in liver failure, with the potential for further advancement to HBC[5]. However, the underlying causes of PSC have not been definitively characterized[6].

Furthermore, the presence of inflammatory bowel disease (IBD), a persistent inflammatory condition affecting the gastrointestinal tract, particularly ulcerative colitis (UC), has been observed in approximately 70–80% of individuals diagnosed with PSC[7], and significantly increases the risk of colorectal cancer to greater than that associated with UC alone[8,9]. Despite the well-established and extensively researched link between PSC and UC, the precise nature of this relationship, in terms of chronological precedence and severity, remains unclear[10].

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Hence, establishing a definitive causal connection between UC and PSC is of paramount importance for disease management. Such determination serves as a pivotal foundation for developing efficacious treatment strategies. Furthermore, a pertinent question is whether UC contributes to the pathogenesis of HBC.

Specific genetic variants that exhibit clear associations with potential risk factors can function as unbiased proxies for these risk factors. This approach facilitates the establishment of causality and is known as Mendelian randomization (MR), a genetic methodology that bolsters the precision of inferences pertaining to the causal link between an exposure and its corresponding outcome. Notably, MR mitigates susceptibility to confounding factors and effectively eliminates concerns related to reverse causality, both of which are prevalent limitations of conventional observational investigations[11]. Compared with conventional multivariate regression methods, MR analysis offers enhanced robustness against measurement errors, confounding factors, and reverse causation[12].

In this study, we used summary statistics extracted from a genome-wide association study (GWAS) that encompassed UC, PSC, and HBC. We endeavored to investigate the genetic underpinnings of these traits. To this end, we comprehensively evaluated genome-wide genetic correlations. Furthermore, we conducted a two-sample, bidirectional MR analysis, which enabled exploration of the potential causal relationship between UC and PSC. Additionally, we investigated the intricate interplay among UC, PSC, and the risk of HBC using a meticulous two-step MR analysis.

Methods

Study design and data sources

To derive causal insights encompassing PSC, UC, and HBC, we employed a bidirectional two-sample, two-step MR design, following the guidelines outlined in the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) statement[13]. Figure 1 shows a schematic diagram summarizing the MR investigation.

The MR study was based on three fundamental assumptions. First, genetic variants meticulously chosen as instrumental variables (IVs) exhibited robust associations with their respective exposures. Second, the selected IVs were devoid of connections to potential confounding factors. Third, IVs exerted a direct influence on the outcomes solely through exposure pathways, rather than via alternative routes.

Our study obtained summary statistics from a GWAS conducted as part of the FinnGen Research Project. The sample sizes were as follows: PSC cases, 1,715; PSC controls, 330,903; UC cases, 5,034; UC controls, 371,530; HBC cases, 304; and HBC controls, 174,006. The FinnGen initiative facilitates investigation of the intricate interplay between disease patterns and genetic variations in isolated populations. Supplementary Table 1 provides comprehensive details of all GWAS included in our study.

Genetic instrument selection

Genome-wide significant single nucleotide polymorphisms (SNPs) (p-value $< 5.0E-08$) were extracted from the dataset[14]. Subsequently, SNP selection and filtering were performed to ensure data quality. SNPs exhibiting a low linkage disequilibrium ($r2 < 0.1$) within a region of 500 kb[15] were excluded, adhering to the principle

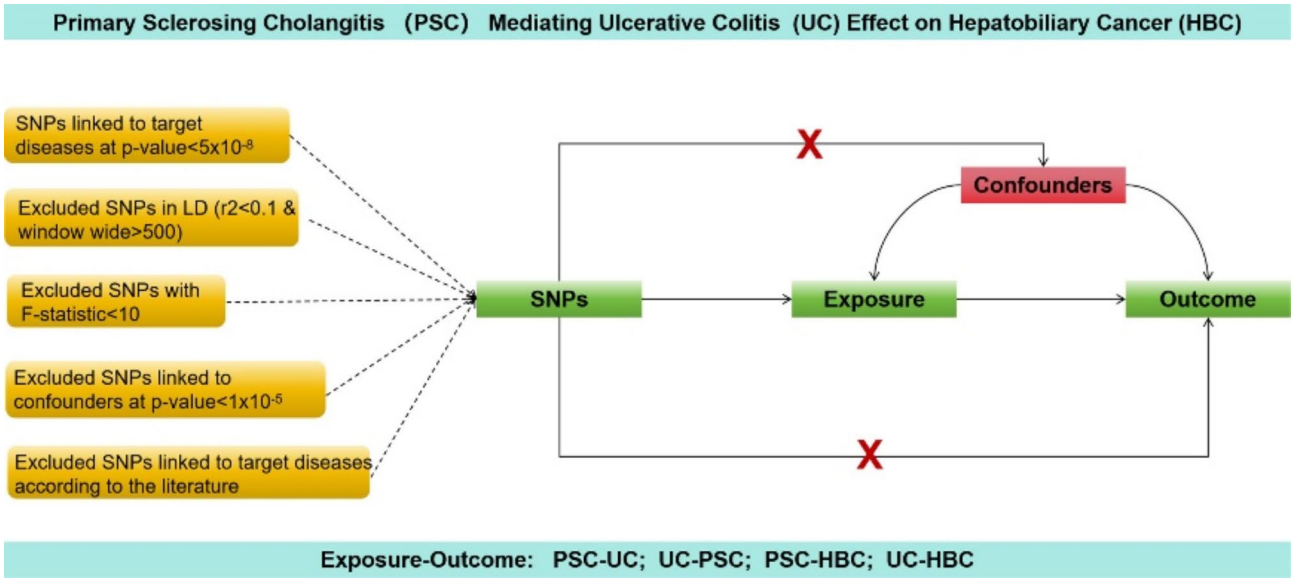

Fig. 1. This Figure illustrates a diagram of the MR study. The study's flowchart is structured upon three core assumptions. (I) Relevance to Exposure: The instrumental variables (IVs) must exhibit a statistically significant connection to the exposure under investigation (II) Independence from Confounding: The IVs should remain disconnected from any recognized confounding factors that could potentially modify the relationship between an exposure and its associated outcome. (III) Exclusion Restriction: The IVs should bear no direct influence on the outcomes; their impact is solely mediated by their effects on the exposure of interest.

of diminishing confounding due to genetic correlations. To assess the strength of each SNP, F-statistics were computed using the formula F = R2 [(*N* − 2) / (1 - R2)], where R2 represents the proportion of exposure factor variance elucidated by the IVs, and N signifies the overall sample size[16,17]. SNPs with weak instrumental characteristics were discarded using a threshold of F = 10 as the cut-off value.

Furthermore, SNPs linked to potential confounders (Supplementary Tables S2–S5) were removed from the analysis. SNPs demonstrating horizontal pleiotropic effects were pruned using the PhenoScanner database[18]. This meticulous curation process was designed to uphold two pivotal assumptions in Mendelian randomization: the independence of the IVs from potential confounding factors and the exclusion restriction.

MR analysis

A bidirectional two-sample MR analysis was performed to investigate the potential association between PSC and UC. Subsequently, two-step MR analysis was conducted to delve deeper into the intricate relationships among PSC, UC, and HBC. This comprehensive approach aimed to ascertain not only pairwise connections, but also meticulously assess conceivable mediating pathways within the causal framework.

In our primary analyses, we employed the inverse variance-weighted (IVW) method, assuming the validity of all genetic variants as IVs[19,20]. To investigate the third hypothesis of MR, addressing potential violations of the IV assumption stemming from directional-level pleiotropy, we employed the MR-Egger method[21]. Additionally, we employed the weighted median approach, which computes the median of weighted estimates and can provide reliable effect estimates, even when pleiotropy affects up to 50% of the IVs[22,23].

Three supplementary analytical methods were also used. These encompass weighted pattern estimation, simple pattern estimation, and IVW analysis for random-effects models. The inclusion of weighted and simple pattern estimates facilitates the relaxation of the assumptions intrinsic to IV analysis, consequently augmenting the dependability and consistency of causal effect detection. Notably, IVW analysis for the random-effects models exhibited robustness, even in the presence of heterogeneity.

The outcome results were presented as the odds ratio (OR) of the respective outcome variable, accompanied by its corresponding 95% confidence interval, signifying a unit standard deviation change in the OR exposure factor. This comprehensive process was predominantly executed using the TwoSample MR package[24].

The mediation proportions were computed utilizing the following formula: $\beta \times \beta 2 / [(\beta 1 \times \beta 2) + \beta]$, where β represents the total effect derived from the primary analysis, β1 represents the effect of exposure on the mediator, and β2 represents the effect of the mediator on HBC (Fig. S1)[25].

Sensitivity analysis

Heterogeneity testing was performed for both the IVW analysis and MR-Egger regression. Cochran's Q test was employed to evaluate IV heterogeneity, with a p-value of less than 0.05 indicating significant heterogeneity[26]. When a genetic variant utilized as an IV is associated with risk factors beyond the intended exposure of interest and exerts a causal effect on the outcome, a bias due to horizontal pleiotropy may occur, thus violating MR assumptions 2 and 3. To gauge and mitigate such bias stemming from horizontal pleiotropy, an MR-Egger regression was employed. The absence of horizontal pleiotropy was indicated by an intercept close to zero ($p > 0.05$).

In addition, a leave-one-out approach was integrated into the analyses. This entailed the sequential removal of each SNP and subsequent calculation of the pooled effect of the remaining SNPs. This step aimed to identify potential SNPs that might influence the causal effect.

Statistical analysis

All statistical analyses conducted in this study were executed utilizing the "Two-sample MR" package within R version 4.0.1 (https://cran.r-project.org/bin/windows/base/old/). Statistical significance was defined as a two-si ded p-value of less than 0.05.

Results

Quality control for genetic instruments

The MR results are reported in accordance with the STROBE-MR guidelines (Supplementary Material 2).

Supplementary Tables S2–S5 give the details of the included SNPs. All PSC SNPs had an F-statistic > 199, which was above the threshold of 10, suggesting sufficient instrumental strength[27,28]. Horizontal pleiotropy was not detected for individual SNPs associated with PSC in the pleiotropy test (Supplementary Table S6). Furthermore, no indications of inter-SNP heterogeneity were observed in either IVW or MR-Egger regression analyses of UC (Supplementary Table S6, Figs. S2, S3). In the context of PSC, although some heterogeneity in SNP selection may emerge when UC is the outcome, it did not exert any influence on IVW outcomes within the random-effects model, confirming the reliability of our conclusions (Supplementary Table S6, Fig. S2).

Causal estimates between PSC and UC

Our IVW analysis findings ($p = 0.059$, OR = 1.263) indicated that PSC did not exert a discernible influence on the risk of UC. These outcomes demonstrated remarkable consistency with the results derived from alternative MR techniques: MR-Egger (p-value = 0.440, OR = 0.230), simple mode (p-value = 0.317, OR = 1.187), weighted mode (p-value = 0.308, OR = 1.153), and weighted median (p-value = 0.017, OR = 1.202) (Fig. 2A).

In contrast, the outcomes of the reverse MR analyses consistently suggested a conspicuous causal connection between UC and the risk of PSC: IVW $p < 0.001$, OR = 1.145; weighted median $p = 0.010$, OR = 1.123; MR-Egger $p = 0.058$, OR = 1.165; simple model $p = 0.141$, OR = 1.150; and weighted model $p = 0.251$, OR = 1.097 (Fig. 2A).

Notwithstanding the varying significance of the different methods, the findings indicated a substantial level of concurrence in the direction of the estimated causal effect (Fig. 2B, C). The reported estimates signified

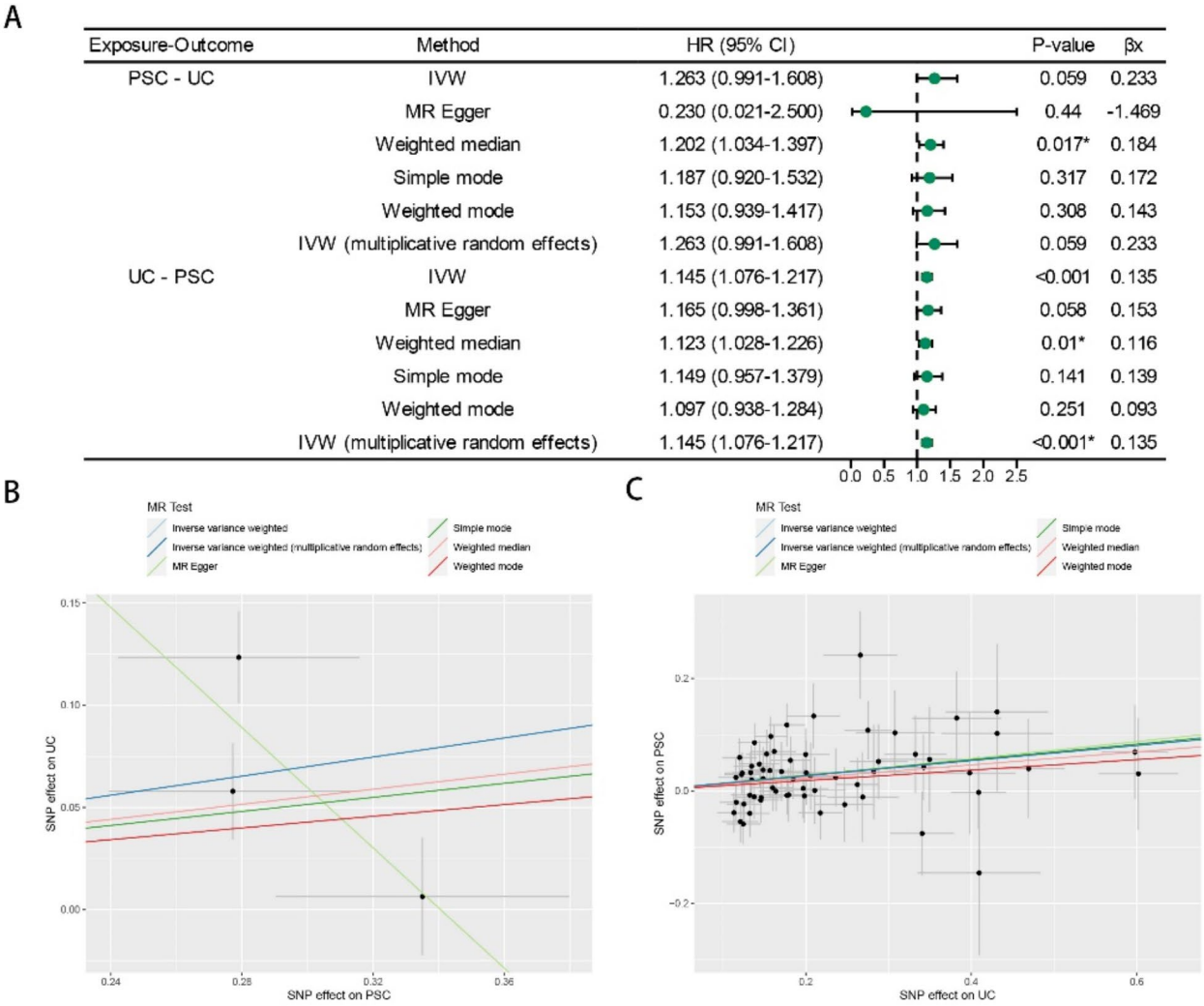

Fig. 2. MR estimates derived from the fixed-effect IVW method, MR-Egger regression, weighted median method, weighted-mode method, simple-mode and random-effect IVW method to assess the causal effect between PSC and UC. (**A**) Results of forward MR and reverse MR analysis between PSC and UC. (**B**) Scatter plots showing the MR effect of each exposure on PSC. (**C**) Scatter plots showing the MR effect of each exposure on UC. *PSC* primary sclerosing cholangitis, *UC* ulcerative colitis.

the effects of genetic susceptibility to PSC (or UC) on the risk of UC (or PSC). Furthermore, the outcomes of the leave-one-out sensitivity assessments confirmed that the non-causal associations were not influenced by individual genetic variants in either the MR or reverse MR analyses (Supplementary Fig. S4).

Causal estimates between PSC and HBC or UC and HBC

The IVW analysis yielded compelling evidence of a positive association between PSC and HBC ($p<0.001$, OR = 1.855), with consistent results found using the weighted median method ($p=0.001$, OR = 1.778) (Fig. 3A). Notably, the outcomes from the MR-Egger test ($p=0.215$, OR = 2.950), simple mode ($p=0.070$, OR = 1.787), and weighted mode ($p=0.067$, OR = 1.756) did not reveal any association between PSC and HBC. Nevertheless, these results did not influence our principal assessment based on IVW analysis.

Conversely, MR analyses investigating the association between UC and an increased risk of HBC showed no significant association: IVW $p=0.683$, OR = 1.029; weighted median $p=0.766$, OR = 1.030; MR-Egger $p=0.117$, OR = 1.343; simple mode $p=0.403$, OR = 1.214; or weighted mode $p=0.641$, OR = 0.915 (Fig. 3A).

Notwithstanding the varying significance of the different methods, the findings indicated a substantial level of concurrence in the direction of the estimated causal effect (Fig. 3B, C). This finding suggested a noteworthy causal relationship between genetic susceptibility to PSC and HBC risk, whereas UC has no direct causal relationship with HBC risk. Moreover, leave-one-out sensitivity tests indicated that non-causal associations were unaffected by individual genetic variants in both the MR and reverse MR analyses (Supplementary Fig. S5).

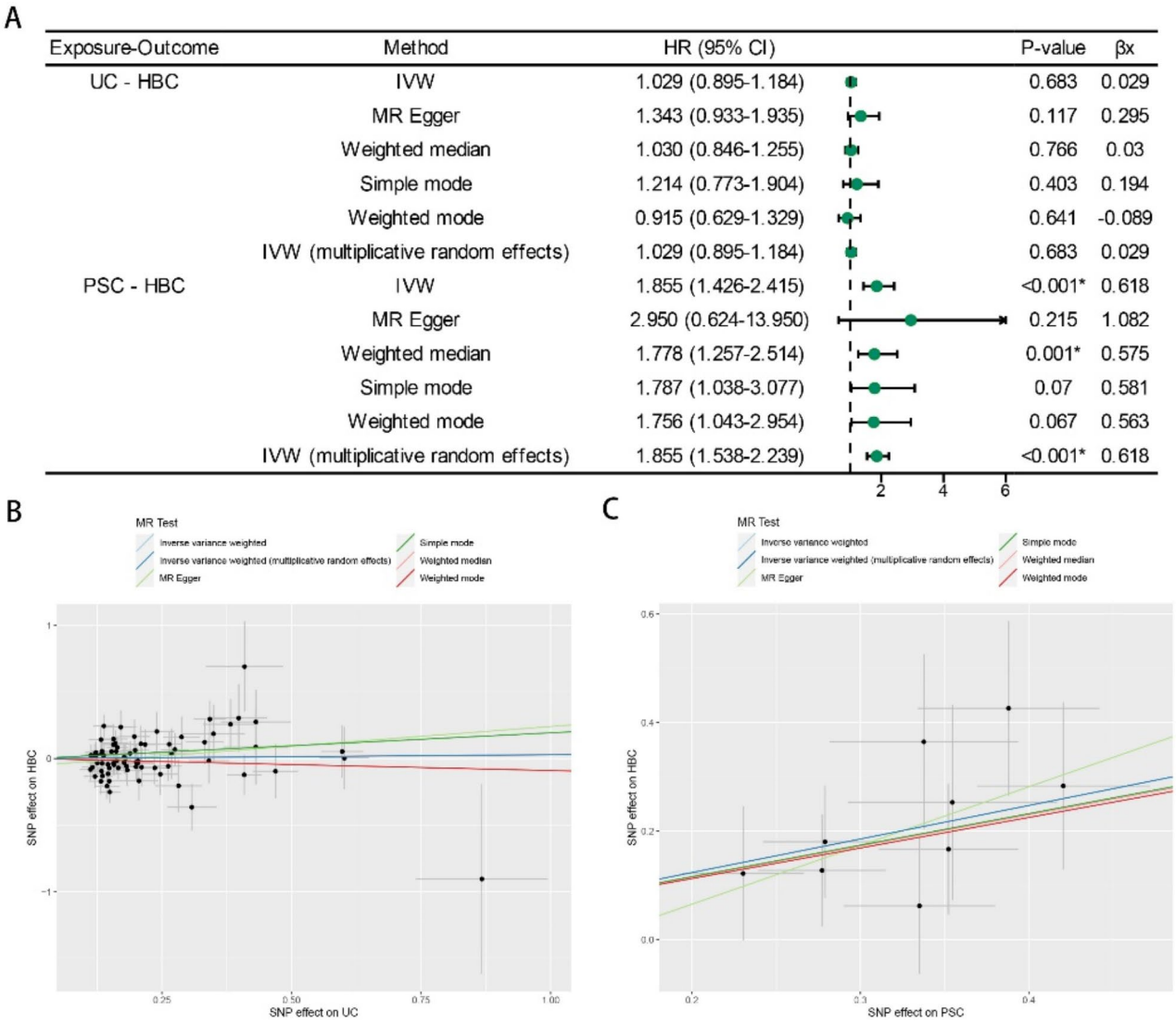

Fig. 3. MR estimates derived from the fixed-effect IVW method, MR-Egger regression, weighted median method, weighted-mode method, simple-mode and random-effect IVW method to assess the causal effect of UC on HBC and PSC on HBC. (**A**) Results of MR analysis between liability to UC or PSC and the HBC risk. (**B**) Scatter plots showing the MR effect of each exposure on PSC. (**C**) Scatter plots showing the MR effect of each exposure on UC. *UC* ulcerative colitis, *PSC* primary sclerosing cholangitis, *HBC* hepatobiliary cancer.

PSC as mediator in UC progression to HBC

These findings indicate a causal link between the predisposition to UC and an elevated risk of PSC (IVW: $p < 0.001$). Similarly, a causal relationship between PSC susceptibility and an increased risk of HBC was evident (IVW: $p < 0.001$). Importantly, the results did not support a direct causal relationship between UC and HBC. These results imply that PSC potentially functions as a comprehensive mediator, wherein susceptibility to UC precipitates an augmented risk of HBC, largely via PSC.

Regarding the causal impact of UC on HBC, according to the IVW method, the mediating effect of PSC was 0.083, representing 73.8% of the total mediating effect. Moreover, the direct and overall effects of UC on HBC were calculated as 0.029 and 0.113, respectively (Figs. 2 and 3).

Discussion

To the best of our knowledge, this study represents a pioneering endeavor to scrutinize the causal associations among UC, PSC, and HBC by applying a bidirectional two-sample, two-step MR analysis. Our comprehensive findings underscore the lack of substantial evidence supporting a causal relationship between genetic susceptibility to PSC and the risk of UC. However, a distinct causal association emerged between the genetic predisposition to UC and the heightened risk of PSC, suggesting a potential convergence of shared genetic mechanisms between these two conditions. This conclusion was supported by a recent study on the relationship between IBD and PSC[29].

UC, as a long-term exposure factor, evidently increases the risk of HBC. A study by Huang et al. provides further support for our findings[30]. Although direct causality between PSC and HBC has not been established, our results unmistakably confirmed the causal role of PSC in an increased risk of HBC. This inference lends credence to the notion that PSC operates as an essential mediator of the induction of HBC development from UC. The insights gleaned from our study provide a compelling rationale for the early implementation of preventive measures and therapeutic interventions targeting both PSC and HBC.

Cancers affecting the hepatobiliary tract represent a particularly pernicious group of malignancies, and previous studies have also investigated the relationship between PSC and the risk of HBC[31]. Following the diagnosis of PSC, the annual incidence of CC ranges from 1 to 2%, with a cumulative risk of 5–10% over a patient's lifetime[32]. Furthermore, individuals with PSC display heightened vulnerability to GBC. This is evidenced by the fact that 14% of patients with PSC undergoing cholecystectomy present with a gallbladder mass, of which 57% involves malignancy[33,34]. While HCC is relatively less prevalent in the context of PSC, a concern remains that the risk of HCC could be similar to that observed for other forms of cirrhosis once cirrhosis manifests[5]. Despite the perceived association between UC and HBC, existing evidence remains limited and scholarly discourse on this topic is scarce[35,36]. Considering the frequent coexistence of UC and PSC[37,38], we hypothesized that UC might exacerbate HBC risk by inducing PSC. Our findings lend credence to this hypothesis, indicating that PSC may act as a comprehensive mediator of HBC induced by UC. The collective impact of UC on HBC was estimated at 0.1125, with PSC accounting for 73.8% of this effect through indirect mediation. Consequently, prevention and management of PSC are of paramount significance in the broader context of disease control and intervention.

PSC is a relatively uncommon yet increasingly more prevalent cholestatic liver disorder. Despite its increasing incidence, the underlying etiological factors remain unclear. Moreover, established medical interventions currently do not confer a verified benefit in curtailing disease progression[39]. Previous epidemiological studies have consistently underscored the high prevalence of IBD in approximately 70–80% of patients with PSC, with the majority of these cases (approximately 80%) having UC[7]. In contrast, only a small subset of patients with IBD, ranging from 2 to 8%, ultimately develop PSC during the course of their ailment[40,41]. The nuanced clinical presentation of PSC, which often manifests with few symptoms, and its frequent incidental diagnosis may inadvertently lead to underdiagnosis of PSC in individuals with UC[42]. However, asymptomatic individuals do not equal the absence of risk. Research has shown that approximately 22% of patients diagnosed with asymptomatic PSC exhibit clinical symptoms within 5 years of diagnosis[43]. The median survival period for symptomatic patients prior to succumbing or undergoing liver transplantation is estimated to be approximately 9 years. Additionally, the coexistence of PSC and UC engenders a parallel risk, leading to more extensive colitis and a four-fold increased susceptibility to colorectal tumors compared to isolated UC[42,44]. The outcomes of our study unequivocally pointed to a causal link between susceptibility to UC and PSC risk, although insufficient evidence exists to establish a causal effect of PSC on UC risk. This underscores the importance of diligently monitoring liver function in individuals with UC and actively participating in regular PSC screenings. Notably, the effective management and treatment of UC are pivotal for facilitating the early prevention and diagnosis of PSC.

At present, only liver transplant has demonstrated the ability to alter the natural trajectory of PSC. Nevertheless, a substantial proportion of patients (approximately 20–25%) experience PSC relapse within a decade of undergoing liver transplant[43]. Research has highlighted that total colectomy performed either before or during liver transplant can mitigate the risk of PSC recurrence by up to 50%[45]. Certain studies have investigated the plausible association between UC and PSC from the point of view of the "leaky gut" hypothesis[46–48]. The intricate interplay between the intestines and liver is upheld via the biliary tract, portal vein, and systemic circulation. According to this hypothesis, the breach of the intestinal epithelial barrier could result in the migration of intestinal flora into the portal vein circulation, facilitated by the "enterohepatic axis"[48]. This sequence of events can induce an immune response and foster chronic inflammation within the hepatic biliary system[46,48,49]. Concomitantly, interventions targeting the intestinal microbiota may offer potential efficacy in managing UC–PSC interfaces. These findings align with our evidence for a causal relationship between UC and PSC.

Our study had several strengths. First, we harnessed the potency of bidirectional, two-sample MR analysis, a strategy adept at uncovering causal relationships between UC and PSC, while attenuating the impacts of reverse causality and confounding variables. Second, our employment of MR revealed a fresh vantage point, highlighting the pivotal role of PSC in driving the emergence of HBC triggered by UC. Furthermore, our stringent selection criteria for SNPs ensured their significant association with the exposure variable, without direct links to the outcome variable. The F-statistics for each SNP surpassed the customary threshold of 10 ($F > 199$), reaffirming the robustness and trustworthiness of the statistical findings.

Despite these strengths, some limitations of the study should also be acknowledged. Given that all GWAS summary statistics incorporated in our analysis originated from samples of European descent, the generalizability of the observed estimates across other racial backgrounds remains uncertain. Subgroup analyses based on variables, such as sex, age, and comorbidities were constrained, prompting a cautious interpretation of the individual conclusions. Therefore, validation of our outcomes in future studies encompassing larger, diverse ethnic cohorts is imperative. In addition, MR studies have inherent limitations. Exposure factors may influence genetic risk through multiple pathways, which we attempted to address using the MR-Egger regression and weighted median methods. However, these approaches may not fully account for all the complexities. Genetic pleiotropy, in which a genetic variant affects multiple traits, remains a challenge despite our adjustments. In addition, our analysis did not consider epigenetic factors that could have affected our results. Future research should incorporate these elements to enhance the robustness of the causal inferences.

In summary, our study underscores the causal role of susceptibility to UC in increasing the risk of PSC, although no evidence for bidirectionality was found. Moreover, our findings indicate that heightened

susceptibility to PSC substantially amplifies the likelihood of developing HBC while concurrently operating as a pivotal mediator in augmenting UC's influence of UC on HBC. The implications of our findings underscore the importance of vigilant early surveillance and ongoing management of both UC and PSC, which are pivotal for preventing PSC recurrence and for reducing the risk of HBC.

Data availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the corresponding author.

Received: 28 August 2023; Accepted: 11 December 2024
Published online: 28 December 2024

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Acknowledgements
We would like to thank Editage (www.editage.cn) for English language editing.

Author contributions
Fangming W: Conceptualization, Formal analysis, Methodology, Writing-original draft, Writing-review & editing; Junhui X: Investigation, Validation, Visualization, Writing-original draft.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-83085-0.

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