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Primary biliary cholangitis and Sjogren's syndrome: bi-directional Mendelian randomization analysis

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ABSTRACT. – OBJECTIVE: Observational studies have shown a higher prevalence of Sjogren's syndrome (SjS) in patients with primary biliary cholangitis (PBC) than in the healthy population, but whether this correlation is causal needs further confirmation. This study aimed to investigate the bidirectional causal relationship between PBC and SjS using Mendelian randomization (MR) analysis.

MATERIALS AND METHODS: We used pooled data from a large-scale genome-wide association study (GWAS) to select mutually independent genetic loci associated with PBC and SjS in people of European ancestry as instrumental variables (IVs). The causal association between PBC and SjS was analyzed by MR analysis using inverse variance weighting (IVW) and weighted median methods, and the ratio of ratios (OR) was used as an evaluation index. In addition, sensitivity analyses, including Cochran's Q test, MR-PRESSO, MR-Egger intercept test, and leave-one-out test, were performed to ensure the stability of the results.

RESULTS: A total of 20 validated IVs were selected for PBC, and the number of IVs for SjS was seven. Positive MR analysis showed that genetically predicted PBC was significantly associated with the risk of SjS (IVW OR=1.174, 95% CI: 1.107-1.246, p<0.001). The weighted median method further confirmed this result (OR=1.146, 95% CI: 1.053-1.247, p=0.016). Inverse MR analysis showed that genetic susceptibility to SjS also increased the risk of PBC (IVW OR=1.737, 95% CI: 1.280-2.357, p<0.001), and this result was also confirmed by the weighted median method (OR=1.398, 95% CI: 1.120-1.746, p=0.003).

CONCLUSIONS: Our study found that genetically predicted SjS increased the risk of PBC and vice versa in a European population. This may shed light on the etiology of PBC and the management of patients with SjS.

Key Words:
Mendelian randomization, Primary biliary cholangitis, Sjogren's syndrome.

Introduction

Autoimmune diseases are common conditions in which an individual's immune system reacts against its healthy cells. This condition is a common cause of morbidity and mortality, with an estimated prevalence ranging from 5 per 100,000 to more than 500 per 100,000[1]. The etiology of autoimmune diseases includes genetic and environmental factors. Recent genomic-wide association studies (GWAS) have allowed the identification of various genetic loci associated with disease susceptibility and have revealed candidate genes that can be used in targeted therapeutics[1].

Primary biliary cholangitis (PBC) is an autoimmune disease characterized by elevated serum mitochondrial antibodies (AMA) and bile duct-specific injury, leading to chronic cholestatic liver disease and ultimately to cirrhosis and liver failure[2]. The PBC incidence and prevalence varied widely across regions, with North America being the highest, followed by Europe, and the lowest in the Asia-Pacific region. Both the incidence and prevalence showed an increasing tendency worldwide, especially in North America[3]. The pathogenesis of PBC remains poorly understood. The etiology of PBC appears to be a complex multistep process involving genetic and environmental factors in interactions[4,5]. Yu et al[6] found that PBC activates the Toll-like receptor 4 (TLR4)/Myeloid differentiation factor-88 (MyD88)/nuclear fac-

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tor-kappaB (NF-κB) signaling pathway, induces the release of inflammatory factors and produces a large number of apoptotic proteins, which results in liver damage and cell apoptosis. Ursodeoxycholic acid (UDCA) is considered the first-line treatment for PBC, but only 60% of UDCA treatments result in satisfactory outcomes[7].

Sjögren's syndrome (SjS) is a chronic multisystem autoimmune disease characterized by the inflammation of the exocrine glands, with subsequent hypofunction[8]. SjS is classified into primary SjS (pSS), which occurs in isolation, and secondary SjS, which is usually accompanied by a variety of other autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and PBC[9]. An observational study[10] has shown that the prevalence of PBC is higher in patients with SjS than in normal subjects. The development of PBC and SjS is the result of a combination of factors, which intersect in terms of genetic background and pathogenic mechanisms, leading to immune overlap. For example, in both PBC and SjS, B lymphocytes are abnormally activated and secrete associated cytokines and autoantibodies[11,12]. The iguratimod combined with methylprednisolone effectively attenuates autoimmune responses, reduces clinical symptoms and disease activity, and improves the functional status of the exocrine glands in patients with Sjögren's syndrome[13].

Understanding the relationship between SjS and PBC will help to explore the pathogenesis of both diseases and improve treatment and management. In epidemiologic studies, the presence of confounders greatly interferes with the inference of causality between exposure and outcome, as the inference of causality in observational studies is often challenged by potential confounding bias and reverse causality. In addition, randomized controlled trials (RCTs) have limitations in terms of ethical issues, observation time, and resources and costs. Mendelian randomization (MR) analysis is an epidemiological method that uses genetic variation as an instrumental variable (IV) to determine whether there is a causal relationship between exposure and outcome[14]. In MR analysis, alleles are randomly assigned, similar to RCTs, and are less subject to confounding than in traditional observational studies; furthermore, locus mutations precede the phenotype, avoiding reverse causation. This study will use data from a large-scale GWAS with a bidirectional MR design to examine causality between PBC and SjS, providing important guidance for further research and clinical practice.

Materials and Methods

Research Design

The study was reported according to the STROBE-MR guidelines[15]. Data were collected from public databases (GWAS Catalog and FinnGen databases). This bi-directional MR analysis explored the causal relationship between PBC and SjS. Single nucleotide polymorphisms (SNPs) were used as the IV in MR analysis to determine the causal effect of exposure variables[16]. Notably, MR analyses are subject to three assumptions[17]: (I) the variant is associated with exposure; (II) the variant is not associated with confounders that may bias the exposure-outcome association; and (III) there is no other way that the variant can influence the outcome other than through its association with exposure. The framework is shown in Figure 1.

Data Sources for PBC and SjS

In the forward MR analysis, data on PBC were obtained from a meta-analysis[3] of 2,764 cases and 10,475 controls of European ancestry. SNPs significantly associated with PBC were screened for preliminary IV from GWAS data ($p<5\times10^{-8}$ at genome-wide threshold). Also, independence between SNPs was ensured by linkage disequilibrium (LD) analysis[14]. Given that the main assumption of MR analysis is that IV can only influence outcomes through exposure, we manually eliminated SNPs associated with confounders using PhenoScanner[17]. Meanwhile, SjS outcome data were available from FinnGen (available at: https://www.finngen.fi/en/) with table code "M13_SJOGREN", including 416,757 samples (2,495 cases and 414,262 controls) with a total of 16, the 383,308 SNPs were genotyped.

In the reverse MR analysis, because the data in the FinnGen database was independently set and too few IVs were obtained after removing the chain imbalance, we combined the data published by Khatri et al[18] and Jia et al[19] as SNPs for SjS. We supplemented the SNPs that were omitted in the endpoints by searching for proxy SNPs. If no suitable proxy SNP was available, the SNP was excluded from our analysis.

Testing the Strength of the Tool's Variables and Statistical Power

To minimize the possible weak IV bias, we used the F statistic to assess the strength of IV[20]. We calculated the F value using the following formula: $F = (N-2)*R^2/(1-R^2)$ (n: sample size of GWAS)[21]. The larger the F statistic, the smaller

the bias[20]. If F>10, it indicates that the study is of sufficient strength. Meanwhile, R^2 was calculated as follows: $R^2 = 2\times\beta2\times(1\text{-eaf})\times eaf$ (β: allele effect value; eaf: effect allele frequency)[22].

MR Analysis

After reconciling the effect alleles in the GWAS for exposure and outcome data, inverse variance weighted (IVW) was used as the primary MR analysis, characterized by regression that did not take into account the presence of an intercept term and was fitted with the inverse of the outcome variance as weights. Among them, the IVW fixed-effects model was mainly used in the absence of any potential heterogeneity in the level of multivariate effects. Second, the above findings were further supplemented using the weighted median method. The weighted median method, defined as the weighted median of the ratio estimates, allows causality to be evaluated if at least 50% of the information in the analysis comes from valid instruments[23].

Sensitivity Analysis

First, IV heterogeneity was determined according to Cochran's Q statistic using either a random effects model ($p<0.05$) or a fixed effects model ($p>0.05$)[23]. Next, horizontal pleiotropy was checked by the MR-Egger intercept test. Also, based on the SNP level and global heterogeneity estimation, horizontal polytropy can be detected using the MR-Polytropic Residuals and Outliers method (MR-PRESSO)[23]. To identify outlier variants, the outlier test compares the expected and observed distributions for each variant. If any outlier variants are detected, they will be discarded to obtain unbiased causal estimates from the outlier-corrected MR analysis[23,24]. Third, the leave-one-out method excludes the included SNPs one by one and calculates the effect of the remaining IVs to assess whether the MR estimates are driven or biased by individual SNPs, which is performed in sensitivity analyses[23,25].

Statistical Analysis

The MR analyses were performed using R version 4.3.0 and "TwoSampleMR" (version 0.5.6) and the "MR-PRESSO" (version 1.0) R packages (Vienna, Austria). It was considered significant if the two-sided *p*-value was lower than 0.05.

Results

Causal Effect of PBC on SjS Risk

A total of 20 SNPs associated with PBC were included as IVs after removing SNPs with cascade

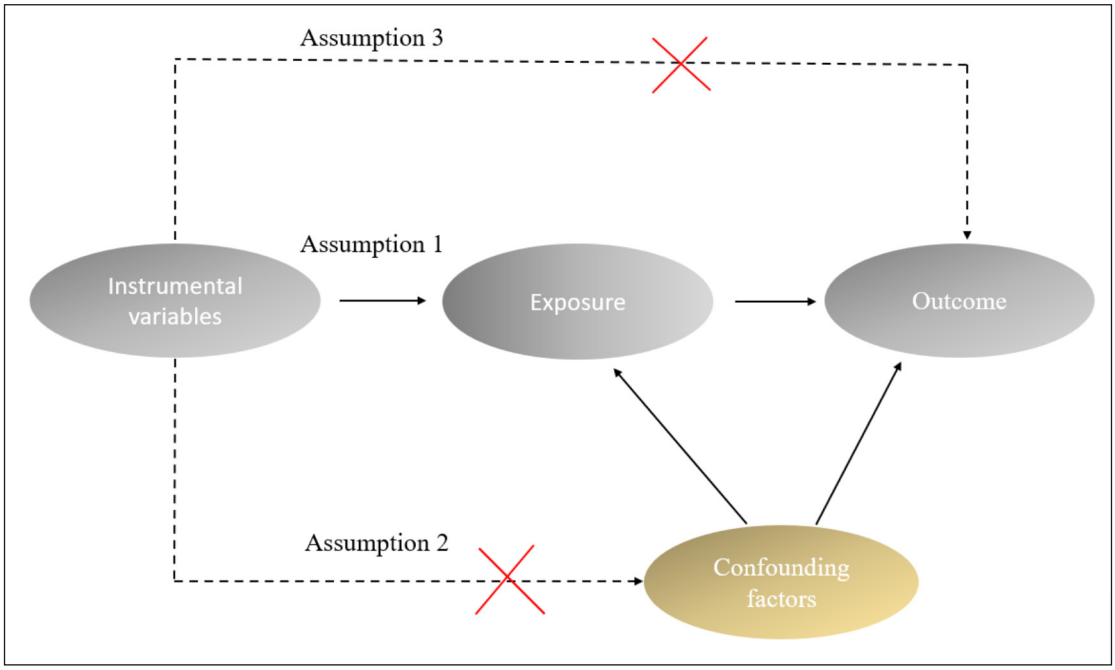

Figure 1. Overview of the MR study design. Assumption I, the variant is associated with exposure; assumption II, the variant is independent of confounders that might bias the exposure-outcome association; assumption III, there is no other way that the variant can affect outcome except through its association with exposure.

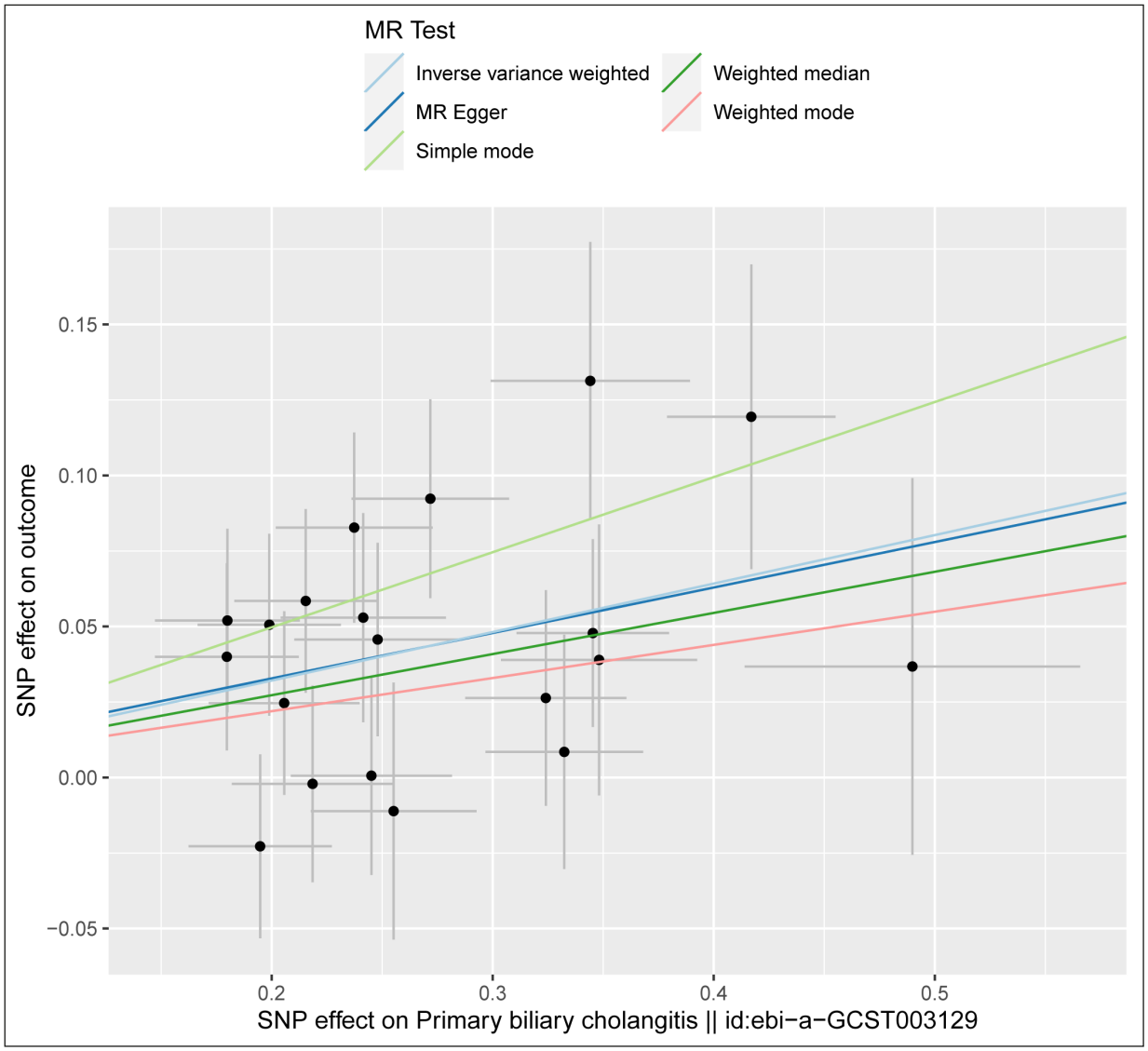

Figure 2. Scatter plot of the causal effect of PBC on SjS. The weighted median method similarly showed that PBC increased the risk of SjS (OR=1.146, 95% CI: 1.053-1.247, p=0.016).

imbalance in PBC. The results of IVW showed that PBC could increase the risk of SjS (OR=1.174, 95% CI: 1.107 to 1.246, p<0.001) (Table I). The weighted median method similarly showed that PBC increased the risk of SjS (OR=1.146, 95% CI: 1.053-1.247, p=0.016) (Table I and Figure 2).

Sensitivity analysis of MR analysis in PBC and SjS showed no heterogeneity between SNPs (Cochran's Q test, Q=19.837, p=0.342); MR-PRESSO results showed no abnormal SNPs were detected (p=0.430). Scatterplot results showed the stability of SNPs closely associated with PBC and SjS; the MR-Egger intercept test did not show horizontal pleiotropy in MR analysis (p=0.933). The leave-one-out sensitivity analysis showed that no single SNP had a large effect on the overall results (Figure 3).

Causal Effect of SjS on PBC

After removing SNPs with linkage disequilibrium with SjS, a total of seven SNPs associated with SjS were included as IVs. IVW showed that SjS increased the risk of PBC (OR=1.737, 95% CI: 1.280-2.357, p<0.001) (Table II). The weighted median method similarly showed that SjS increased the risk of PBC (OR=1.398, 95% CI: 1.120 to 1.746, p=0.003) (Table II and Figure 4).

Sensitivity analyses of MR analyses in SjS and PBC showed heterogeneity between SNPs (Cochran's Q-test, Q=39.241, p<0.001). The

Table I. MR results of PBC on SjS.

Method	β	SE	OR (95% CI)	*p*-value
IVW	0.161	0.030	1.174 (1.107-1.246)	$p<0.001$
WME	0.136	0.043	1.146 (1.053-1.247)	0.016
MR-Egger	0.151	0.122	1.162 (0.916-1.475)	0.232

MR, Mendelian randomization analysis; PBC, primary biliary cholangitis; SjS, Sjogren's syndrome; IVW, inverse variance weighting; WME, weighted median.

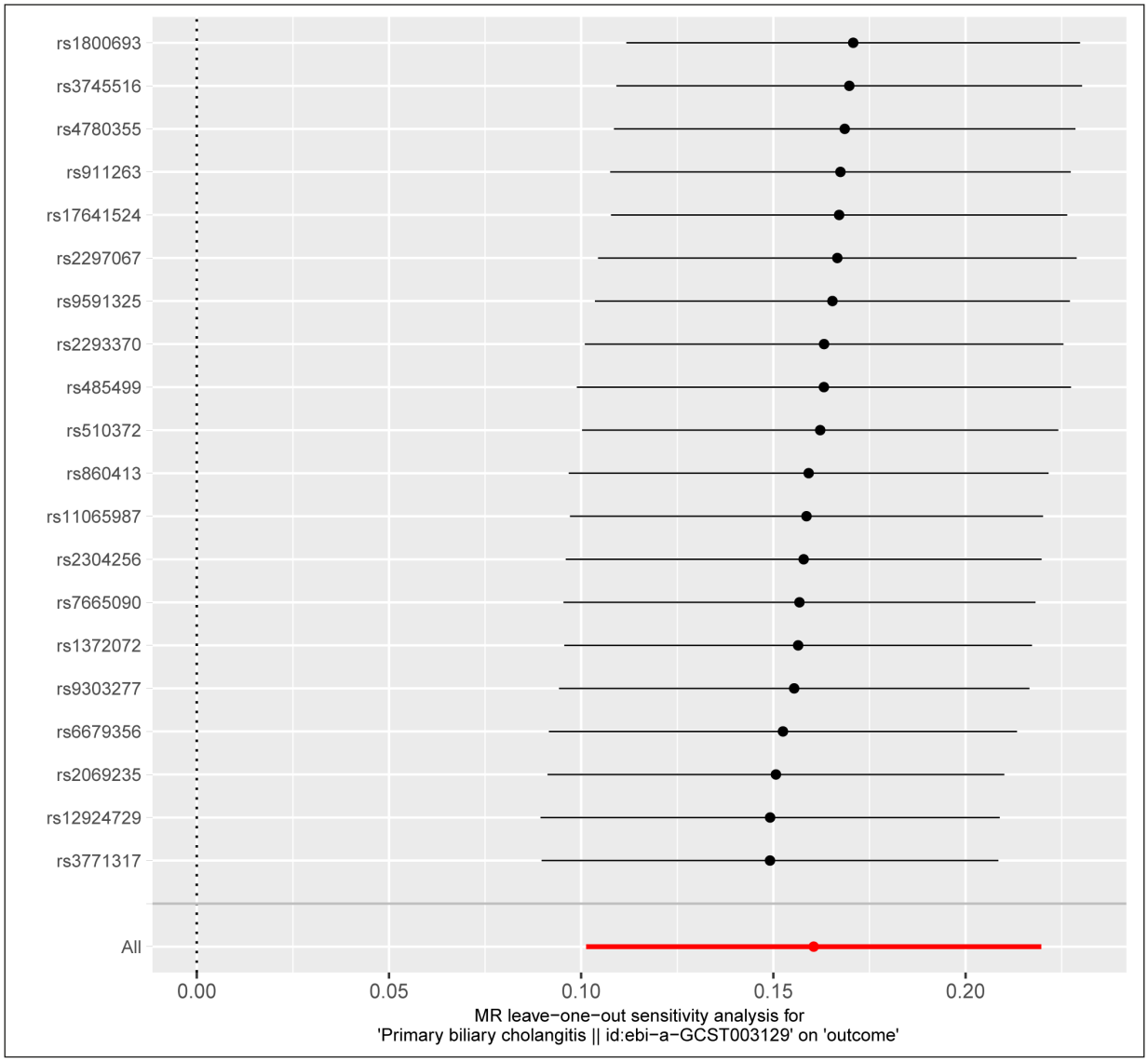

Figure 3. Leave-one-out sensitivity analysis of the effect of PBC on SjS. The leave-one-out sensitivity analysis showed that no single SNP had a large effect on the overall results.

MR-Egger intercept test did not indicate horizontal pleiotropy in the present study ($p=0.570$) and the leave-one-out sensitivity analysis showed that no single SNP had a large effect on the overall results (Figure 5). Re-performing the leave-one-out sensitivity analysis did not show any SNP outliers, suggesting that our results were stable.

Table II. MR results of SjS on PBC.

Method	β	SE	OR (95% CI)	*p*-value
IVW	0.552	0.156	1.737 (1.280-2.357)	$p<0.001$
WME	1.398	0.113	1.398 (1.120-1.746)	0.003
MR-Egger	0.989	0.736	2.687 (0.635-11.372)	0.237

MR, Mendelian randomization analysis; PBC, primary biliary cholangitis; SjS, Sjogren's syndrome; IVW, inverse variance weighting; WME, weighted median.

Discussion

A 2005 nationwide epidemiologic survey[26] of 1,032 PBC patients showed that 1/3 of PBC patients were comorbid with another autoimmune disease, most commonly SjS, autoimmune thyroid disease, scleroderma, and systemic lupus erythematosus. These extrahepatic complications can alter the disease progression and prognosis of

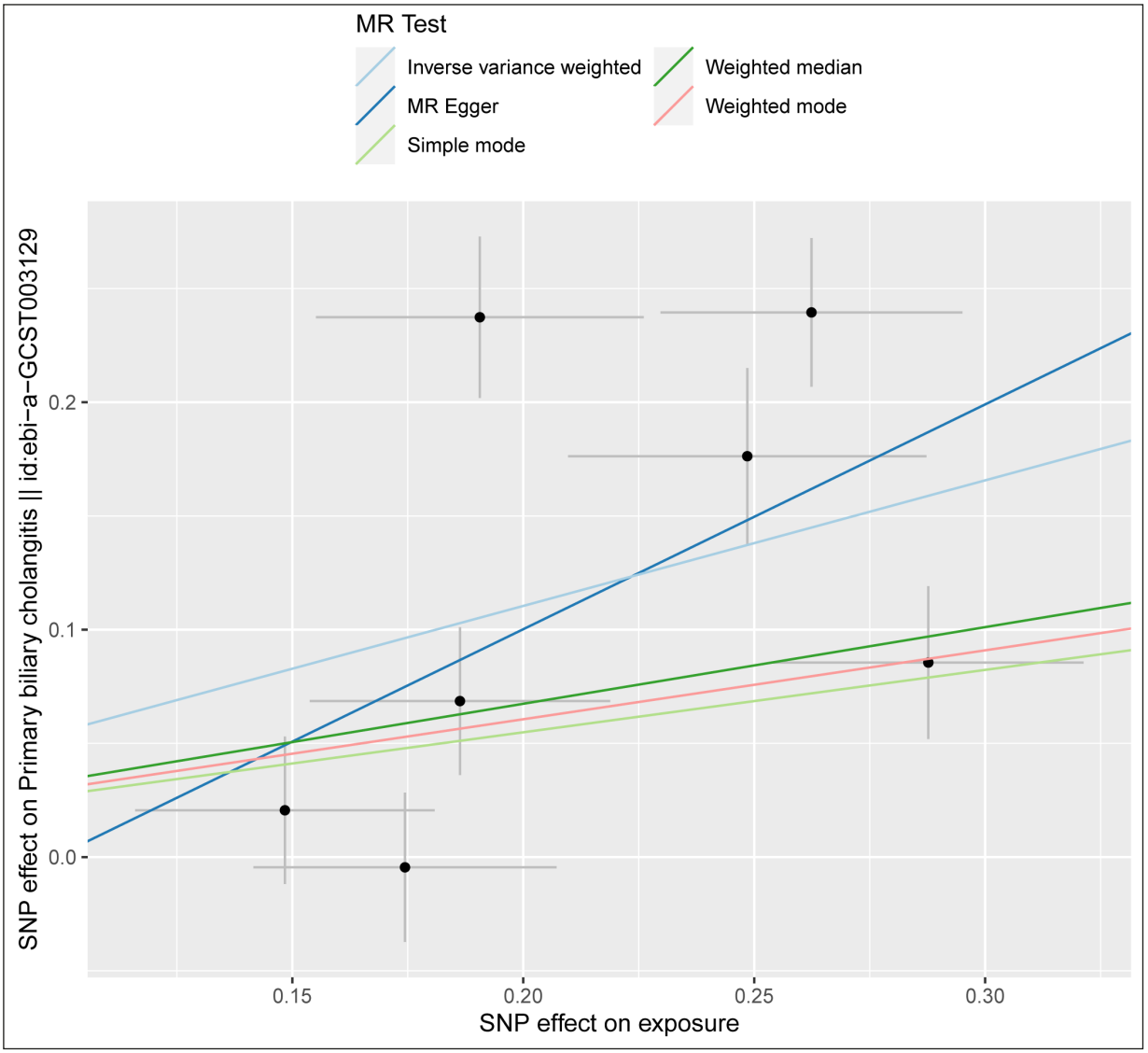

Figure 4. Scatter plot of the causal effect of SjS on PBC. The weighted median method showed that SjS increased the risk of PBC (OR=1.398, 95% CI: 1.120 to 1.746, p=0.003).

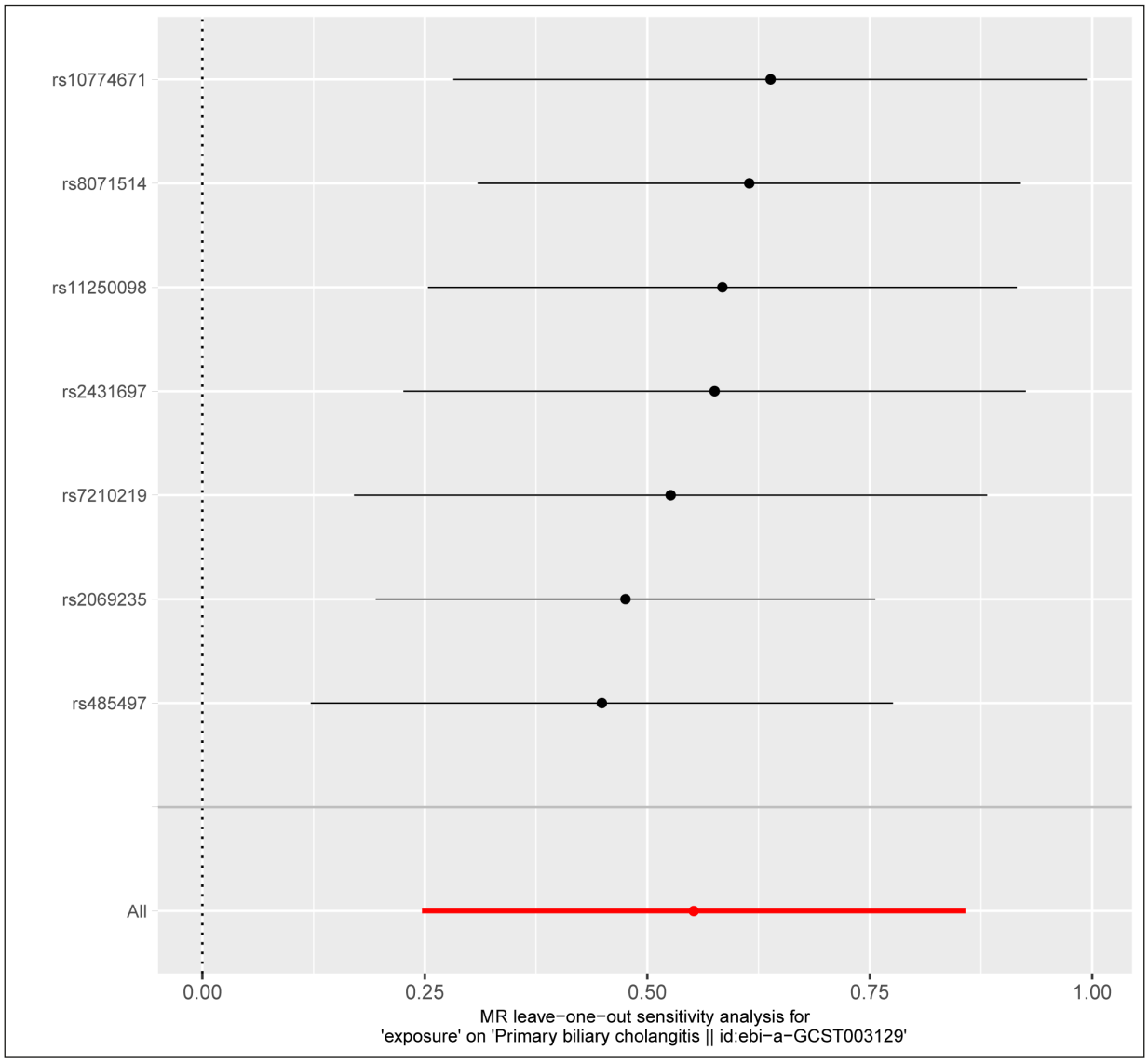

Figure 5. Leave-one-out sensitivity analysis of the effect of SjS on PBC. The leave-one-out sensitivity analysis showed that no single SNP had a large effect on the overall results.

patients with PBC, making it more difficult to diagnose and treat. Current data[27] on concomitant PBC/SjS vary widely, with the prevalence of SjS or combined SjS ranging from 3.5% to 73% in patients with PBC. A meta-analysis[28] combining 17 studies, including 13,802 patients, calculated the prevalence of SjS in PBC to be 35%. SjS, as well as PBC, is one of the most common extrahepatic diseases; it is characterized by the destruction of exocrine glands, with typical symptoms of dry eyes and dry mouth[29].

This is the first bidirectional MR analysis to evaluate the causal effect between PBC and SjS. In this study, MR analysis proved accurately that PBC can increase the risk of SjS. A causal relationship was also found between SjS and PBC. There is an interaction between SjS and PBC.

A common immunopathogenic mechanism due to genetic and environmental factors may be the main cause of combined SjS in PBC. Both SjS and PBC are characterized by the progressive destruction of epithelial tissues. Environmental triggers cause apoptosis of salivary and biliary epithelial cells, resulting in direct exposure of autoantigenic components to apoptotic vesicles without post-translational modification, which in turn elicits immune attack by the body. A variety of cytokines, HLA class II molecules, and adhesion

molecules expressed by salivary and biliary epithelial cells are involved in this immunopathogenic process[30,31]. However, the underlying mechanisms behind the relationship between PBC and SjS are complex and deserve further investigation[32]. Our study found that PBC can significantly increase the risk of developing SjS. This may be related to the common pathogenesis of PBC and SiS.

The number of clinical studies on whether SjS increases the risk of PBC is low. Hatzis et al[10] retrospectively studied the clinical, biochemical, immunologic, and hepatic histologic data of 410 patients with SjS, of which 36 patients had manifestations of cholestasis, and 27 patients were diagnosed with PBC, and the majority of these patients had stage I PBC lesions from the hepatic pathology. However, the study may have underestimated the prevalence of PBC because liver biopsy was not performed in 8 patients with anti-mitochondrial antibody (AMA)-negative cholestasis of unknown etiology, which may have missed some AMA-negative PBC cases[10]. More thoughtfully, the lack of AMA results and liver histology in SjS patients who did not develop cholestasis may have led to the underdiagnosis of some patients with PBC. The prevalence of PBC in the SjS population may be much higher. Another study[33] claimed that up to 20% of SjS patients had liver injury, and some of them were combined with PBC. This study provides evidence to support a causal relationship between SjS and PBC. Although the MR-PRESSO was found to have horizontal pleiotropy in the sensitivity analysis section, the results were consistent with the previous analysis by eliminating the outliers and then performing the MR analysis again.

Our results can inspire the diagnosis of PBC and the management of patients with SjS. For clinicians, it makes sense to monitor diagnostic markers of PBC in patients with SjS, especially when there are risk factors for PBC. In addition, specific drugs used to treat SjS may lead to liver and autoimmune hepatitis damage. Therefore, clinicians treating SjS should be cautious about using medications even if they do not elevate diagnostic markers for PBC. Both PBC and SjS are pathogenetically manifested as autoimmune epithelial cell inflammation. Under the microscope, the chronic inflammation of the microscopic bile duct in PBC is consistent with the pathological injury of the salivary glands in SjS, which is the same disease occurring in two different sites[9]. In terms of the temporal sequence of the onset of symptoms of both SjS and PBC, many patients first have symptoms of dry mouth and dry eyes before cholestasis, but because liver biopsy is rarely performed in patients without cholestasis in clinical practice, it is difficult to assess the temporal sequence of SjS and PBC, whether SjS and PBC appear at the same time, or whether SjS or PBC are present first.

In this study, MR analysis fulfills three assumptions. Assumption I was verified by selecting 20 and 7 SNPs in GWAS that were strongly associated with PBC and SjS, respectively. Assumptions II and III were not associated with any potential confounders. The LD between SNPs was evaluated and screened, and we found no SNPs at $R^2>0.05$. For assumptions II and III, the results were influenced only by exposure. Heterogeneity and sensitivity analyses have been performed to detect and eliminate any potential pleiotropy and to ensure that our MR estimates are robust and reliable, with no significant bias from other sources of pleiotropy.

The strength of this study is the use of MR methods to minimize residual confounding and reverse causation in traditional observational studies. However, there are still some limitations that cannot be addressed. First, this study should not suffer from weak instrumental variable bias because the F-statistics were all >10; however, there were too few IVs for SjS that could be included. At the same time, this requires MR studies on populations with larger sample sizes. Second, the data obtained were GWAS pooled data without specific individual information for subgroup analysis. Thirdly, since a population of European origin was used, we should be cautious about generalizing the findings to other populations.

Conclusions

In conclusion, this study supports a bidirectional causal relationship between PBC and SjS. Based on our findings, it is reasonable to consider promoting routine PBC screening in patients with SjS. In addition, appropriate management of PBC is essential to reduce the risk of SjS.

Conflict of Interest

None. The funding organizations are public institutions and had no role in the design and conduct of the study, the collection, management, and analysis of the data, or the preparation, review, and approval of the manuscript.

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Authors' Contributions
Study concept and design: Qiang Li and Liang Chen. Analyzed data and drafted the manuscript: Neng Wang, Yu Zhou, and Hong Li. Critical revision of the manuscript: Qiang Li.

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Ethics Approval
This study reanalyzed previously collected and published data and, therefore, did not require additional ethical approval.

Availability of Data and Materials
We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes without breaching participant confidentiality. The supporting data can be accessed from the corresponding authors. Data were collected from GWAS Catalog (https://www.ebi.ac.uk/gwas/) and the FinnGen database (https://www.finngen.fi/fi).

Informed Consent
This study did not involve any human subjects; therefore, consent to participate was not applicable.

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