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RESEARCH ARTICLE

# Genetic link between primary biliary cholangitis and connective tissue diseases in European populations: A two-sample Mendelian randomization study

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## Abstract

### Background

An association between primary biliary cholangitis (PBC) and connective tissue diseases (CTDs) [rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), systemic sclerosis (SSc)] has been found in observational studies. However, the direction causality is unclear. The aim of this study was to assess the causality between PBC and CTDs and to promote early screening, pre-emptive therapy, and accurate stratification.

### Methods

A two-sample Mendelian randomization (MR) analysis was performed to assess the causal relationship between PBC [Genome-Wide Association Study (GWAS) meta-analysis, 8021 cases/16498 controls], and SLE (GWAS meta-analysis, 8021 cases/16489 controls), RA (FinnGen, 6236 cases/14727 controls), SS(FinnGen, 2495 cases/365533 controls), SSc (FinnGen, 302 cases/213145 controls). Inverse variance weighting (IVW) was used as the primary analysis method, supplemented by four sensitivity analyses to assess the robustness of the results.

### Results

The IVW revealed that genetically predicted PBC increased the risk of SLE [odd's ratio (OR) = 1.43, 95% confidence interval (CI) 1.30–1.58, P < 0.001]), RA (OR = 1.09, 95%CI1.04–1.14, P<0.001), and SS (OR = 1.18, 95%CI1.12–1.24, P<0.001), but not that of SSc. In addition, no association was observed between CTDs as an exposure and PBC. Sensitivity analyses did not reveal horizontal pleiotropy.

## Conclusions

Our study provided new genetic evidence for a causal relationship between PBC and CTDs. PBC increased the risk of SLE, RA, and SS. Our findings highlighted the importance of active screening and intervention for CTDs in patients with PBC.

## 1 Introduction

Primary biliary cholangitis(PBC) is a chronic, inflammatory, immune-mediated liver injury [1]. Until 2016, it was called primary cirrhosis [2, 3]. PBC is a multi-causal disease in which the immune system triggers an attack by targeting and destroying epithelial cells of the bile duct [3]. Although the liver is the main target organ of PBC, PBC may affect multiple systems, such as arthritis [4], interstitial lung disease [5, 6], pulmonary hypertension [7] and nephritis [8]. These coexisting symptoms often make the diagnosis and treatment more difficult, and can even alter the course, prognosis and outcome of PBC.

PBC is distinguished from connective tissue diseases(CTDs). However, in observational studies, a combination of CTDs was observed in patients with chronic biliary cholangitis, with the most frequently reported CTDs as Sjögren's syndrome(SS), A recent meta-analysis reported that 35% [(95% confidence interval (CI): 28%–-42%]) of patients with PBC had combined SS [9], This may be related to the fact that PBC and Sjogren's syndrome share common epithelial cell immune changes in epithelial cells. Certainly, A previous study that report the clinical features of 34 patients with PBC combined with systemic lupus erythematosus (SLE) and distinguish liver injury in SLE from PBC [10]. In addition, one study noted that studies of extrahepatic manifestations of PBC have focused on rheumatic diseases and reported the prevalence of various rheumatic diseases in 361 patients [11]. In addition to the overlap between the diseases, it noted that CTDs such as SLE, systemic sclerosis (SSC), SS, and inflammatory myopathy (IM)have a higher rate of positivity for the AMA signature antibody (anti-mitochondrial antibody) than the normal population [12]. A large number of clinical studies are available on PBC and CTDs, and some research has been done on animal models and immune mechanisms of PBC [13], However, this is not matched by genetic epidemiological studies of PBC and extrahepatic CTDs. In addition, early identification of autoimmune diseases associated with PBC is recommended in the guidelines for its management [1], Therefore, genetic epidemiologic studies of PBC and extrahepatic CTDs are necessary. Exploring the relationship between PBC and extrahepatic CTDs can help to enhance the understanding of the pathogenesis of PBC and CTDs and to develop appropriate therapeutic approaches.

Although traditional case-control or cross-sectional studies have proposed a relationship between PBC and CTDs, most are observational designs with small sample sizes [14, 15], therefore, they exhibit the limitations of lack of valid inferences of effects, and susceptibility to multiple confounding factors. At the same time, due to the lower prevalence of PBC, there is a lack of cohort studies with larger numbers of patients. With the continued development of new statistical methods, genome-wide association study (GWAS) data, epigenetics and various genomics, Mendelian randomization (MR) studies are more widely used to explore causal relationships between complex exposures and disease. Three basic assumptions need to be met when performing MR analyses: genetic variation is 1. associated with exposure, 2. not associated with confounders, and 3. not associated with outcome. The rationale of MR lies in equal, random and independent genetic variation during meiosis and uses genetic variation as an instrumental variable. The properties of MR minimize confounding factors and reverse causality, and it is second only to randomized control trials (RCTs) in terms of level of evidence.

Therefore, in this study, the correlation between PBC and various CTDs was assessed by two-sample MR [16]. This study provides provided insights early screening, early treatment, and disease stratification in patients with PBC.

## 2 Methods

### 2.1 Two-sample MR

A two-sample MR Study was conducted to assess whether there is a causal relationship between the genetic susceptibility of PBC and CTDs (including RA, SLE, SS, SSc). Multiple single nucleotide polymorphisms (SNPs) were defined as instrumental variables (IVs). The three key assumptions of the MR Analysis are as follows (Fig 1): 1. IVs are strongly related to exposure factors; 2. IVs are independent of any confounding factors; and 3. The selected IVs is not directly related to the outcome variable.

### 2.2 Data sources

The GWAS data is a genome-wide association study of the largest European population and provides the basis for use in studies of PBC, SLE, RA, and SSc (https://www.ebi.ac.uk/gwas/). Data for the PBC were derived from a meta-analysis of GWAS, and the data were derived from the cohorts of three samples: 1. the Canada-UK cohort included 4,615 cases and 9,213 controls, 2. the Italian cohort included 255 cases and 579 controls, and 3. the U.S. cohort including 891 cases and 621 controls. The summary statistics for SLE (5,201 patients and 9066 controls) was obtained from the GWAS catalog(https://www.ebi.ac.uk/gwas/). Summary-level statistical data for RA, SS, SSc were obtained from the FinnGen GWAS results. The GWAS data used in this study is given in Table 1.

### 2.3 Selection of IVs

For genetical instruments of PBC and CTDs, we used single nucleotide polymorphisms (SNPs) that were strongly associated with each exposure ($p < 5 \times 10^{-8}$) as instruments. We screened LD independent SNPs based on R2 = 0.001, window size = 10,000 kb. Phenotypes associated with each genetic variant were examined using the PhenoScanner software. SNPs
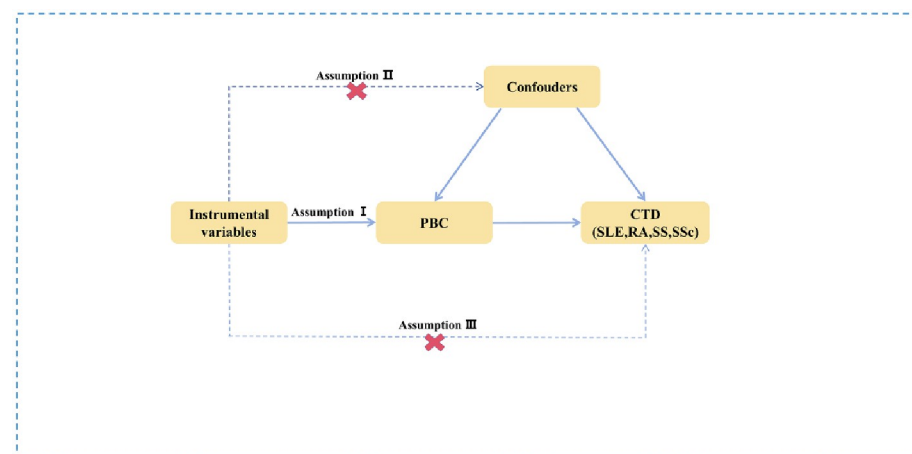


**Fig 1. Diagram of the two-sample Mendelian randomization (MR) study for the association between PBC and CTDs.** PBC, primary biliary cholangitis; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SS, Sjögren's syndrome; SSc, systemic sclerosis.

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**Table 1. Genome-wide association study (GWAS) datasets used in the study.**

| Phenotypes | Source | Phenotypic code | Cases/controls | Ancestry |
|---|---|---|---|---|
| PBC | GWAS meta-analysis [39] | GCST90061440 | 8021/16489 | European |
| SLE | US, Italy, Netherlands, Turkey, Spain [40] | GCST003156 | 5201/9066 | European |
| RA | UK, Finland | ebi-a-GCST90018910 | 8255/409001 | European |
| SS | FinnGen | R9_M13_SJOGREN | 2495/365533 | European |
| SSc | UK | GCST90044535 | 104/456,244 | European |

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overlapped with the risk of outcome were eliminated to prevent potential multiple effects. And "harmonise_data" code function was used to remove the palindromic SNPs in the MR analysis.

## 2.4 Statistical analyses

The "TwoSampleMR" packages of the R software (version 4.2.2) were used to perform MR analysis. Several MR methods were used to infer causal relationships between PBC and CTDs (SLE, RA, SS, and SSc). These methods included inverse variance weighting (IVW), MR-Egger, median weighting, and Weight mode. IVW method is regarded as the most important method in the study. To assess the association between PBC and the four CTDs, MR Analysis was performed. IVW was considered to be the primary MR method, and $p < 0.05$ and consistent direction of the four MR methods were considered significant. When the heterogeneity test shows heterogeneity, multiplicative random effects will be used, otherwise, a fixed effects model will be used.

## 2.5 Sensitivity analyses

We performed "leave-one-out" sensitivity analyses to explore the possibility that causality is driven by a single SNP. We used Cochran's Q statistic to estimate heterogeneity, in which case we used the random effects IVW test to provide a more conservative and robust estimates.

## 3. Results

### 3.1. IVs

SNPs associated with these diseases were selected as IVs based on the established quality control criteria. Each of these SNPS was associated with exposure and not with outcome. The F-statistics of these SNPs were above the threshold of 10, indicating that they strongly represented these diseases in the MR analysis.

### 3.2. Impact of PBC on CTD

Genetic variants of PBC were associated with SLE, RA, and SS (Fig 2). According to IVW method, the presence of PBC increased the risk of developing SLE by 43% (OR = 1.43, 95%CI 1.30–1.58, P<0.001), increased the risk of RA by 12% (OR = 1.12, 95%CI1.06–1.17, P<0.001), as well as increased the risk of SS by 18% (OR = 1.18, 95% CI1.12–1.24, P<0.001) and increased the risk of SSc by 25% (OR = 1.25, 95% CI1.08–1.45, P<0.001). As heterogeneity was detected during the analysis of the RA and SSc database, we used the IVW method(multiplicative random effects). Meanwhile, a leave-one-out method was performed to verify the robustness of the MR analysis and ensure the reliability of the results. MR-egger revealed no significant horizontal pleiotropy between PBC and CTDs, and the results can be seen in
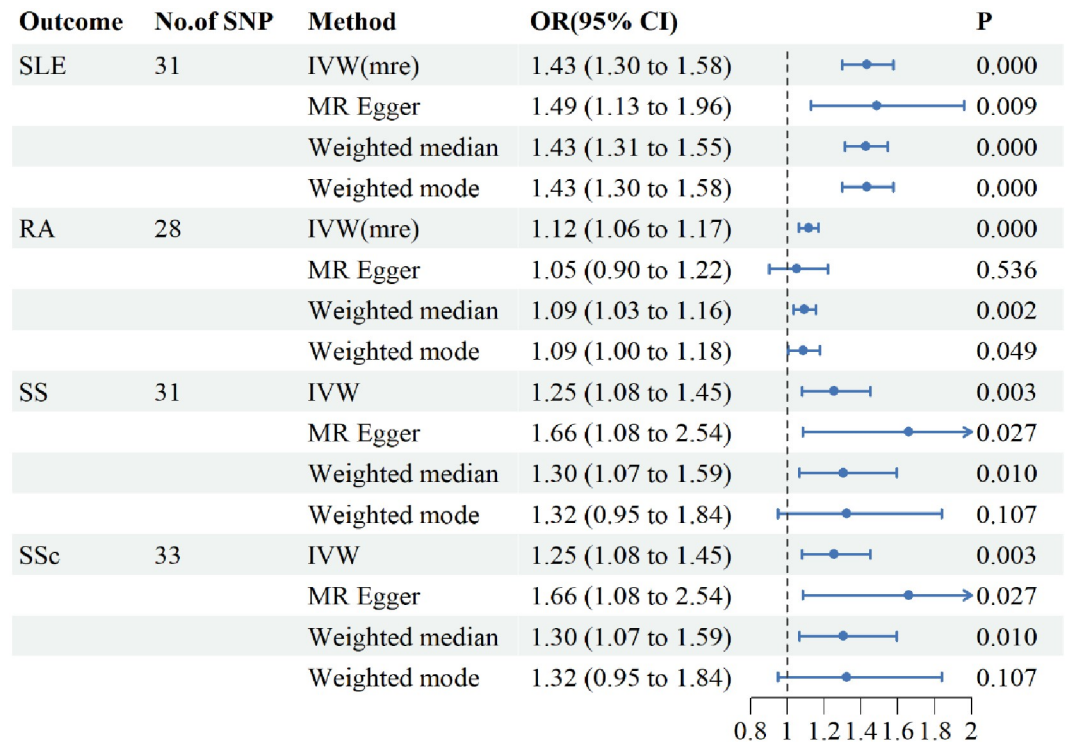
| Outcome | No.of SNP | Method | OR(95% CI) | | P |
|---------|-----------|--------|------------|---|---|
| SLE | 31 | IVW(mre) | 1.43 (1.30 to 1.58) | | 0.000 |
| | | MR Egger | 1.49 (1.13 to 1.96) | | 0.009 |
| | | Weighted median | 1.43 (1.31 to 1.55) | | 0.000 |
| | | Weighted mode | 1.43 (1.30 to 1.58) | | 0.000 |
| RA | 28 | IVW(mre) | 1.12 (1.06 to 1.17) | | 0.000 |
| | | MR Egger | 1.05 (0.90 to 1.22) | | 0.536 |
| | | Weighted median | 1.09 (1.03 to 1.16) | | 0.002 |
| | | Weighted mode | 1.09 (1.00 to 1.18) | | 0.049 |
| SS | 31 | IVW | 1.25 (1.08 to 1.45) | | 0.003 |
| | | MR Egger | 1.66 (1.08 to 2.54) | | 0.027 |
| | | Weighted median | 1.30 (1.07 to 1.59) | | 0.010 |
| | | Weighted mode | 1.32 (0.95 to 1.84) | | 0.107 |
| SSc | 33 | IVW | 1.25 (1.08 to 1.45) | | 0.003 |
| | | MR Egger | 1.66 (1.08 to 2.54) | | 0.027 |
| | | Weighted median | 1.30 (1.07 to 1.59) | | 0.010 |
| | | Weighted mode | 1.32 (0.95 to 1.84) | | 0.107 |

0.8 1 1.2 1.4 1.6 1.8 2

**Fig 2. Causal effects of PBC on the risk of CTDs assessed by different Mendelian randomization methods.** PBC for exposure, CTDs for outcome. PBC, primary biliary cholangitis; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SS, Sjögren's syndrome; SSc, systemic sclerosis. SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval. IVW, inverse-variance weighted; IVW(mre), inverse-variance weighted(multiplicative random effects).

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Table 2. All included SNPs were tested for heterogeneity, and a random effects model was used if heterogeneity existed (Table 2).

## 3.3. Impact of CTD on PBC

Similarly, we explored the effect of CTDs on PBC by using CTDs as exposure and PBC as outcome. Analysis of the results from IVW indicated that there was no evidence to support a causal relationship between CTDs (SLE, RA, SS, and SSc) and PBC (Fig 3), MR-egger method was used to assess pleiotropy, indicated that the presence of horizontal pleiotropy was unlikely

**Table 2. Heterogeneity and pleiotropy tests for the associations between PBC and CTD.**

| MR analysis | Heterogeneity test | | Pleiotropy test | | |
|-------------|--------|--------|-----------------|-----|-----|
| | Q | Q-pval | Egger_intercept | se | p |
| PBC-SLE | 111.110 | <0.001 | -0.009 | 0.032 | 0.776 |
| PBC-RA | 125.150 | <0.001 | 0.006 | 0.015 | 0.708 |
| PBC-SS | 27.007 | 0.571 | 0.001 | 0.019 | 0.941 |
| PBC-SSc | 34.035 | 0.653 | 0.092 | 0.073 | 0.216 |
| SLE-PBC | 46.706 | <0.001 | 0.0509 | 0.034 | 0.162 |
| RA-PBC | 21.723 | 0.001 | -0.041 | 0.050 | 0.442 |
| SS-PBC | 3.188 | 0.074 | NA | NA | NA |
| SSc-PBC | 0.869 | 0.833 | -0.068 | 0.044 | 0.219 |

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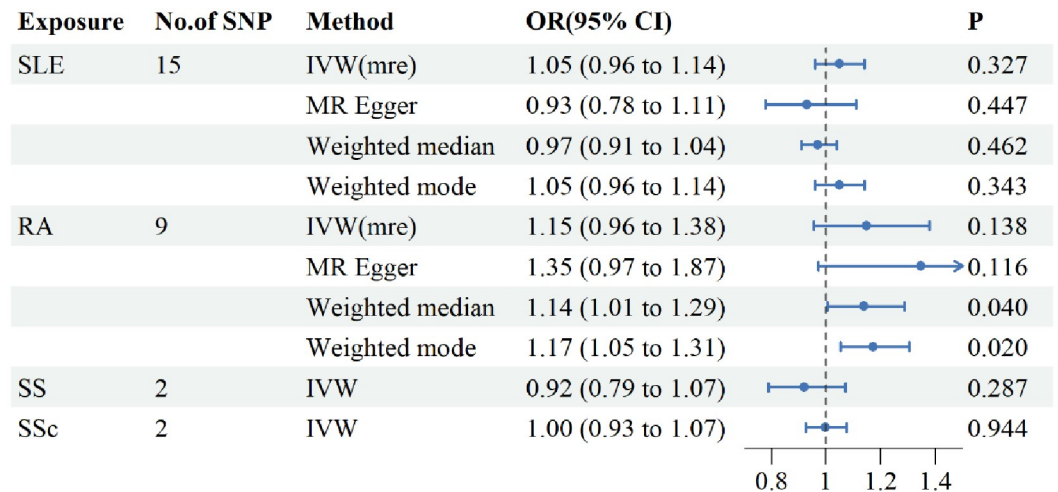| Exposure | No.of SNP | Method | OR(95% CI) | | P |
|---|---|---|---|---|---|
| SLE | 15 | IVW(mre) | 1.05 (0.96 to 1.14) | | 0.327 |
| | | MR Egger | 0.93 (0.78 to 1.11) | | 0.447 |
| | | Weighted median | 0.97 (0.91 to 1.04) | | 0.462 |
| | | Weighted mode | 1.05 (0.96 to 1.14) | | 0.343 |
| RA | 9 | IVW(mre) | 1.15 (0.96 to 1.38) | | 0.138 |
| | | MR Egger | 1.35 (0.97 to 1.87) | | 0.116 |
| | | Weighted median | 1.14 (1.01 to 1.29) | | 0.040 |
| | | Weighted mode | 1.17 (1.05 to 1.31) | | 0.020 |
| SS | 2 | IVW | 0.92 (0.79 to 1.07) | | 0.287 |
| SSc | 2 | IVW | 1.00 (0.93 to 1.07) | | 0.944 |

0.8  1  1.2  1.4

**Fig 3. Causal effects of CTDs on the risk of PBC assessed by inverse Mendelian randomization method.** CTDs for exposure, PBC for outcome. PBC, primary biliary cholangitis; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SS, Sjögren's syndrome; SSc, systemic sclerosis. SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval. IVW, inverse-variance weighted; IVW(mre), inverse-variance weighted(multiplicative random effects).

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to bias the causal relationship between CTDs and RA. Additionally, the "leave-one-out" method ensured the robustness of the results. In conclusion, there is no evidence that genetically predicted CTDs do not lead to an increased risk of PBC.

## 4 Discussion

To the best of our knowledge, this is the first study to validate the causal relationship between PBC and SLE, SS and SSc using MR and large-scale GWAS. Our study reported that PBC increased the risk of SLE, RA, and SS, suggesting an important role of PBC in the pathogenesis of these three CTDs. However, no inverse relationship was observed, i.e., CTDs did not increased increase the risk of PBC. Moreover, PBC did not increase the risk of SSc.

Our study reported that genetically predicted PBC increases the risk of developing SLE. PBC predominantly affects middle-aged and older women, whereas SLE is most often seen in women of childbearing age. Therefore it seems likely that the coexistence of PBC with SLE is low. However, PBC combined with SLE has been reported at a rate of 1.25–3.6% in PBC [14, 17–19] and 1.4–7.5% in SLE [20–22], which seems to indicate the existence of a similar genetic susceptibility to SLE and PBC [23]. Although the pathophysiologic mechanisms underlying the association between PBC and SLE are not fully understood, our study enhanced the understanding by revealing a causal relationship between the two diseases. It is reported that although hepatic dysfunction is the main cause of death in PBC, the cause of death in patients with PBC-SLE is mainly the disease activity or SLE-related complications. The lower survival rate in patients with SLE-PBC is the delayed diagnosis of SLE and more accumulated damage at the baseline [24]. This suggested the importance of early screening of potential SLE among PBC patients with PBC.

The MR results suggest that PBC increases the potential risk of RA. Both RA and PBC are prevalent in middle-aged women. In several large cohort studies, the prevalence of PBC in combination with RA ranged from 1.8% to 13% [11, 14, 18, 25, 26], and RA combined with PBC was also reported [21, 27]. Meanwhile, a genetic study reported that RA and PBC share HLA-DQB1, STAT4, IRF5, MMEL1 and CTLA4 genes [28]. A recent study exploring the

genetic link between RA and three autoimmune liver diseases found that PBC increased the risk of RA, and our study found the same result. This suggested that serological antibodies to RA should be screened in patients with PBC with arthralgia and presence of synovitis should be monitored in the ultrasound of joints. If synovitis is detected in a PBC patient with arthralgia, early intervention is essential.

The MR analysis reported that PBC increases the risk of SS. SS is the CTD that is most commonly associated with PBC, with a prevalence of 10%-66%[6, 9, 17, 29]. It has been suggested that these two diseases exhibit a similar immune response in the face of infection, exhibiting inflammation the infiltration of CD4+ T-cells under cytophagy by the antigen IGAs [30]. Moreover, they share similar symptoms, e.g., fatigue and itching, and a similar genetic and pathogenic [30, 31]. A cohort study reported that patients with PBC with comorbid SS were more likely to have fever and an elevated ESR [25]. This indicated that in patients with PBC, antibodies to SS should not be screened focusing on SS-specific manifestations. Glucocorticoids and immunosuppressants are beneficial if PBC is combined with SS.

Our study reported that genetically predicted PBC increases the risk of developing SSc. Several studies have reported PBC combined with SSc [14, 32, 33], It is the first reported CTD in patients with PBC [34]. SSc exhibit similar outcomes as PBC: sclerosis and cirrhosis. A recent study noted that SSc was the most frequent comorbidity in PBC and this rate may still be underestimated [35]. This suggests that we should pay more attention to PBC patients with skin manifestations such as Raynaud's phenomenon, finger tightness, etc.

Over the past decades, GWAS has successfully identified more than 100 susceptibility loci in the human genome that are closely linked to the HLA locus [36, 37] However, identifying the underlying genetic and environmental factors in autoimmune diseases is not straightforward. Autoimmune disease is a multifactorial disease with multiple influences that can interact with each other [38]. The limitations of observational studies do not allow definitive conclusions to be drawn about the etiology of autoimmune diseases. RCT is the gold standard for assessing causality, however, environmental factors affecting autoimmune diseases are difficult to accurately measure and intervene in. All these issues can be addressed with MR studies of the random distribution of genetic variants during meiosis. In this study, we explored the link between PBC and CTDs with the aim of deepening our understanding of PBC with comorbidity of CTDs. The study can contribute to early diagnosis, accurate stratification, and preemptive treatment, thereby improving the progress of patients.

Our study has some limitations. First, MR must satisfy three major assumptions. Due to the limitation of MR analysis, the second and third assumptions could not be accurately verified. Therefore, we firmly adopted the MR-egger method for horizontal pleiotropy. Second, our study included a European population, which limits the applicability of our findings to other populations. Our study data were derived from several large-scale GWAS, and subgroup analyses were not feasible in the absence of specific demographic data, laboratory indicators, and clinical symptoms. In addition, MR analysis only provided direction for the relationship between PBC and CTD, and the underlying pathological mechanisms should be further investigated.

## 5 Conclusions

To the best of our knowledge, this is the first study to investigated the genetic link between PBC and CTDs and to report that PBC increases the risk of multiple CTDs. Reverse MR indicated that insufficient evidence is available to support that CTDs increase the risk of PBC. To further elucidate the link between PBC and CTD genes, multicenter cohort studies should be conducted in future, using the GWAS database with a larger sample size, and the similar pathogenesis of the two should be explored.

## Supporting information

**S1 File.**
(DOCX)

**S2 File.**
(DOCX)

**S3 File.**
(DOCX)

**S4 File.**
(XLSX)

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## Author Contributions

**Conceptualization:** Zhekang Liu.

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**Investigation:** Yijia Shao.

**Software:** Zhekang Liu, Yijia Shao.

**Supervision:** Zhekang Liu.

**Writing – original draft:** Zhekang Liu.

**Writing – review & editing:** Zhekang Liu, Yijia Shao, Xinwang Duan.

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