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REVIEW

Osteoporosis and Primary Biliary Cholangitis: A Trans-ethnic Mendelian Randomization Analysis

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Abstract

Osteoporosis is a major clinical problem in many autoimmune diseases, including primary biliary cholangitis (PBC), the most common autoimmune liver disease. Osteoporosis is a major cause of fracture and related mortality. However, it remains unclear whether PBC confers a causally risk-increasing effect on osteoporosis. Herein, we aimed to investigate the causal relationship between PBC and osteoporosis and whether the relationship is independent of potential confounders. We performed bidirectional Mendelian randomization (MR) analyses to investigate the association between PBC (8021 cases and 16,489 controls) and osteoporosis in Europeans (the UK Biobank and FinnGen Consortium: 12,787 cases and 726,996 controls). The direct effect of PBC on osteoporosis was estimated using multivariable MR analyses. An independent replication was conducted in East Asians (PBC: 2495 cases and 4283 controls; osteoporosis: 9794 cases and 168,932 controls). Trans-ethnic meta-analysis was performed by pooling the MR estimates of Europeans and East Asians. Inverse-variance weighted analyses revealed that genetic liability to PBC was associated with a higher risk of osteoporosis in Europeans (OR, 1.040; 95% CI, 1.016–1.064; $P=0.001$). Furthermore, the causal effect of PBC on osteoporosis persisted after adjusting for BMI, calcium, lipidemic traits, and sex hormones. The causal relationship was further validated in the East Asians (OR, 1.059; 95% CI, 1.023–1.096; $P=0.001$). Trans-ethnic meta-analysis confirmed that PBC conferred increased risk on osteoporosis (OR, 1.045; 95% CI, 1.025–1.067; $P=8.17\times10^{-6}$). Our data supports a causal effect of PBC on osteoporosis, and the causality is independent of BMI, calcium, triglycerides, and several sex hormones.

Keywords Primary biliary cholangitis · Osteoporosis · Trans-ethnic · Mendelian randomization

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Abbreviations

BMI	Body mass index
BAT	Bioavailable testosterone
CAUSE	Causal analysis using summary effect estimates
CI	Confidence interval
GWAS	Genome-wide association study
IV	Instrumental variable
IVW	Inverse variance weighted
LDSC	Linkage disequilibrium score regression
MR	Mendelian randomization
MVMR	Multivariable Mendelian randomization
OR	Odds ratio
PBC	Primary biliary cholangitis
PRESSO	Pleiotropy residual sum and outlier
SHBG	Sex hormone binding globulin
SNP	Single nucleotide polymorphism
TT	Total testosterone
TG	Triglycerides

Introduction

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease characterized by immune-driven injury to the small- and medium-sized intrahepatic bile ducts [1–3]. It is the most common autoimmune liver disease with increasing global trends in prevalence and incidence [4, 5]. Patients with PBC have variable clinical manifestations, ranging from asymptomatic biochemical cholestasis, liver fibrosis, cirrhosis to liver failure [1, 3]. Beyond the hepatobiliary symptoms, osteoporosis is a common complication of PBC and affects at least one-third of patients [6, 7]. Observational studies also demonstrate a positive association between osteoporosis and the severity of PBC [8–12]. Furthermore, osteoporosis is a leading cause of fracture and related mortality in patients with PBC, thus imposing a challenge for clinical management [10–15]. Although vitamin D and calcium supplementation have been widely recommended for the treatment of osteoporosis in PBC, the efficacy of this intervention lacks high-quality evidence [16].

Prior studies have revealed shared features between PBC and osteoporosis. For instance, both PBC and osteoporosis primarily affect middle-aged or elderly women [10, 17]. In addition, multiple common biochemical features are observed in PBC and osteoporosis, including the decreased levels of serum vitamin D and calcium, dysregulation of lipid profiles, and the alteration of sex hormones [1, 14]. Furthermore, genome-wide association studies (GWAS) have reported several risk genes of PBC which are also associated with osteoporosis (e.g., *RANKL*, *CLDN14*) [18–24]. However, it remains to be elucidated whether PBC is causally related to osteoporosis, since the findings yielded by conventional observational studies may be subject to biases due to confounding or reverse causality.

Mendelian randomization (MR) uses genetic variants, typically single-nucleotide polymorphisms (SNPs), as instruments proxy for exposures to estimate the causal effects on outcomes of interest [23–25]. The growing number of publicly available GWAS datasets has made MR studies feasible, which can be a powerful tool for inferring causality. In this study, we performed two-sample bi-directional MR analyses using summary-level GWAS data to investigate the potential causal relationship between PBC and osteoporosis in Europeans and East Asians. Furthermore, multivariable MR was used to assess whether potential confounding factors including bone mass index (BMI), calcium metabolism, lipidemic traits, and sex hormones may affect the causal effect of PBC on osteoporosis.

Methods

MR Design

We performed two-sample MR analyses with summary-level GWAS data from European populations and replicated the results in East Asian populations and trans-ethnic populations. Multivariable MR analyses were used to evaluate the direct effect of PBC on osteoporosis by conditioning on potential confounding factors. This study was conducted according to Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE) guideline. Ethical approval and participant consent to participate, originally obtained in these patient cohorts, were not required here.

Data Sources

GWAS Datasets of PBC

A diagnosis can be made in a patient with PBC with at least two of the following: (i) ALP > 2 × ULN or GGT > 5 × ULN. (ii) Anti-mitochondrial antibody (AMA) > 1:40. (iii) Florid bile duct lesion on histology [1]. The summary level data of PBC were obtained from the largest international GWAS meta-analysis including five independent European cohorts (a total of 24,510 individuals [8021 cases and 16,489 controls]) and two East Asian cohorts (6778 individuals [2495 cases and 4283 controls]) [26] (Table S1). Standard quality control was performed and minor allele frequency < 0.01 or 0.5% was removed. A total of 5,186,747 variants in Europeans and 5,347,815 variants in East Asians were remained following quality control. The details were previously reported [26].

GWAS Datasets of Osteoporosis

First, we obtained summary statistics of largest GWAS studies, from UK Biobank (UKB) (6484 cases and 401,279 controls) [27] and FinnGen data sets (6303 cases and 325,717 controls) of European ancestry, as well Biobank Japan of East Asian populations (9794 cases and 168,932 controls) [28] (Table S1). The osteoporosis cases met international consensus criteria (ICD-10). Next, we performed genome-wide meta-analysis using GWAS statistics of osteoporosis from UKB and FinnGen cohorts by means of the Ricopili pipeline with fixed-effect model, and a total of 28,015,291 variants were included for the following analyses. Ricopili provided an efficient workflow to process GWAS analytical steps including quality control (QC), imputation, and meta-analysis [29]. In addition, we applied FUMA to summary statistics of the osteoporosis GWAS meta-analysis results [30]. For East Asians, there were 13,436,082 variants in osteoporosis GWAS from Biobank Japan.

GWAS Datasets of Potential Confounders

We selected several potential confounders that may influence the relationship between PBC and osteoporosis, including body mass index (BMI), serum levels of vitamin D, calcium, total cholesterol, triglycerides, as well as sex hormones (estradiol, total testosterone, bioavailable testosterone, sex hormone binding globulin [SHBG]). We drew on GWAS data sources of potential confounders with maximized sample sizes of European ancestry. The GWAS of potential confounders were included as follows: BMI from 806,834 individuals included in a GWAS meta-analysis [31]; serum 25 hydroxyvitamin D [25(OH)D] concentration from 496,946 individuals [32], calcium from 357,831 individuals [33], and total cholesterol and triglycerides (TG) from 1,320,016 individuals [34]. For sex hormones traits, estradiol GWAS data was from 163,985 European individuals [35]; total testosterone (TT) from 425,097 individuals, bioavailable testosterone (BAT) from 382,988, and SHBG from 370,125 individuals of European ancestry [36]. In East Asian, we obtained GWAS data for the following traits: BMI from 163,835 individuals, calcium from 83,980 individuals, total cholesterol from 135,808 individuals, triglycerides from 111,667 individuals [28], total testosterone from 2327 individuals, and SHBG from 2379 individuals in IEU OpenGWAS (https://gwas.mrcieu.ac.uk/). Detailed information (including population, sample size, covariates, minor allele frequency, GWAS model reference panel, and available number of variants) of above GWAS data are shown in Table S1.

Sample Overlap Measurement in Two Sample MR

Sample overlap is a noteworthy problem in two-sample MR, which may incur inflated type 1 error rates due to weak instrument bias [37]. We first carefully checked data source of all the GWAS datasets. Then, we calculated the intercept and corresponding standard error for bivariate LDSC analysis to estimate the sample overlap [38]. According to the tutorial of bivariate LDSC, an intercept within one standard error of zero implies no or negligible sample overlap between two traits. Furthermore, MRlap which makes causal estimates accounting for sample overlap, winner's curse, and weak instruments was also performed to eliminate the underestimated sample overlap effect [39].

Selection of Genetic Instrumental Variants

The instrumental variables (IVs) were extracted based on three core assumptions for valid MR estimates: (1) the genetic variants are closely associated with exposure; (2) the genetic variants have no relationship with any confounder; and (3) the genetic variants affect the outcome exclusively dependent of exposure [25, 40]. We identified SNPs with $P < 5 \times 10^{-8}$ as proxy for exposure trait, except for the East Asian osteoporosis GWAS data. Since no IVs were extracted with P value $< 5 \times 10^{-8}$ in the East Asian osteoporosis GWAS data, SNPs with $P < 1 \times 10^{-5}$ were extracted as IVs for this dataset. The IVs were then clumped for independence with PLINK v 1.9 (LD clumping $r^2 < 0.001$ within 10,000-kb windows, except for $r^2 < 0.01$ within 5000-kb windows in East Asian osteoporosis GWAS data) using 1000 Genomes European/East Asian data as reference panel. Next, we harmonized the effects of genetic instruments on exposure and outcome and exclude palindromic IVs. Additionally, MR Steiger filtering analyses were further performed to identify and exclude IVs that demonstrated stronger association with outcome than exposure [41]. To ensure the statistic strength of instruments, we calculated F statistic of each genetic instruments ($F = \text{beta}^2/\text{se}^2$) and IVs with strong F statistic (> 10) were remained for further MR analyses. Furthermore, we applied PhenoScanner [42, 43] to exclude IVs linked to known confounding factors (including BMI, vitamin D, calcium, lipid traits, sex hormones). The remaining SNPs were applied to conduct MR analyses.

Univariable MR

We performed bidirectional two sample MR analyses with multiple methods including inverse variance weighted (IVW), weighted median, MR-Egger, MR-PRESSO (Mendelian Randomization Pleiotropy RESidual Sum and Outlier), MRlap, and Causal Analysis Using Summary Effect Estimates (CAUSE). IVW method under a multiplicative

random-effects model was used as a primary method. IVW method obtained causal estimates for risk factors by pooling the Wald ratio estimates of individual SNP; in detail, each estimate was calculated with the SNP-outcome association divided by SNP-exposure association [44, 45]. Univariable MR analyses were performed in Europeans and East Asians separately. To strengthen the robustness of the MR estimates, we further performed a trans-ethnic meta-analysis by combining the results of Europeans and East Asians.

Multivariable MR

Regarding the plausible confounders, we performed multivariable MR analyses to estimate the direct effect of PBC on osteoporosis in both Europeans and East Asians. Given the possible relationships between exposures and outcome (e.g., confounder, collider, and mediator) assumed in MVMR analysis [46], we first examined whether each of the potential confounders was causally related to osteoporosis using univariable MR analyses, with IVW as a primary method. Potential confounders which showed causal association with osteoporosis in either forward or reverse direction were retained as exposures for MVMR analyses. Then four models were established in the MVMR sequentially: model one was adjusted for BMI; model two was adjusted for BMI and calcium; model three was adjusted for BMI, calcium, and TG; and model four was adjusted for BMI, calcium, TG, and sex hormone traits (SHBG, BAT, and TT). The selection of genetic instruments in each of the GWAS data was the same as the criteria mentioned above. Both IVW with multiplicative random-effects model and MR-Egger approaches were utilized to make causal estimates and to correct for pleiotropy. Conditional *F* statistic was calculated to assess the strength of genetic instruments.

Sensitivity Analyses

For sensitivity analyses, weighted median, MR-Egger, MR-PRESSO, MRlap, and CAUSE were applied. Weighted median method makes causal estimates from the median of the weighted empirical density function of each SNP effect estimates when at least 50% instrumental variants are valid [47]. In MR-Egger method, the slope coefficient stands for the valid causal estimate in the presence of pleiotropy [48]. The intercept of MR-Egger regression deviating from zero with P value < 0.05 indicated the presence of pleiotropic SNPs. Confined to several methodological limitations including violations of the InSIDE assumption and the influence of outlying variants, MR-Egger method was not an efficient method to estimate the causal effect in our study [49]. The global test in MR-PRESSO method was applied to assess pleiotropy. Moreover, the outliers were evaluated with MR-PRESSO outlier test. MR-PRESSO framework estimates the causal effect with IVW methods after outlier removal [50]. We employed MRlap which makes causal estimates accounting for sample overlap, winner's curse, and weak instruments. In MRlap analyses, the GWAS statistics (including beta, standard error, and *P* value) were standardized based on sample size before conducting MR analyses. MR-CAUSE detects causal relationships accounting for correlated and uncorrelated horizontal pleiotropic effects [51]. In CAUSE analyses, gamma is the effect size of exposure on outcome; eta is the effect size of correlated pleiotropy; Q represents the proportion of variants exhibiting correlated pleiotropy; P is the probability of accepting a sharing model. We used Cochran's Q statistic (estimate from IVW method) and I-squared metrics (I^2) to reveal the heterogeneity due to the multiple instrumental variants, with P value < 0.05 in Cochran's Q and $I^2 > 50\%$ indicating the heterogeneity [44, 52]. Furthermore, the leave-one-out analyses were conducted to detect potential influential SNPs that may bias the association. The scatter plots (SNP-exposure vs. SNP-outcome relationships) and funnel plots were used to present further heterogeneity statistics [53, 54].

Statistical Analyses

All statistical analyses were conducted using PLINK version 1.9 and the packages TwoSampleMR (version 0.5.6), MRPRESSO (version 1.0), Mendelian-Randomization (version 0.7.0), MVMR (version 0.4), MRlap (version 0.0.3.0), CAUSE (version 1.2.0.335), data.table (version 1.14.8), dplyr (version 1.1.2), meta (version 6.5–0), ggplot2 (version 3.4.2), and forestplot (version 3.1.1) of the free available statistical software R (version 4.2.0; R Foundation for Statistical Computing).

Results

Basic Characteristics

An overview of the current MR study is reflected in Fig. 1. Detailed GWAS summary-level data is summarized in Table S1. Potential confounders for multivariable MR included BMI, serum levels of vitamin D, calcium, total cholesterol, triglycerides, as well as sex hormones (estradiol, total testosterone, bioavailable testosterone, sex hormone binding globulin [SHBG]). The number of IVs ranged from 4 to 37 in univariable MR analyses (Tables S2–S6). Steiger filtering test was used to remove the genetic variants that have stronger association with outcome than exposure. The IVs with F statistics larger than 10 were used in the analysis. PhenoScanner was employed to exclude IVs linked to potential confounding factors. We found that there was

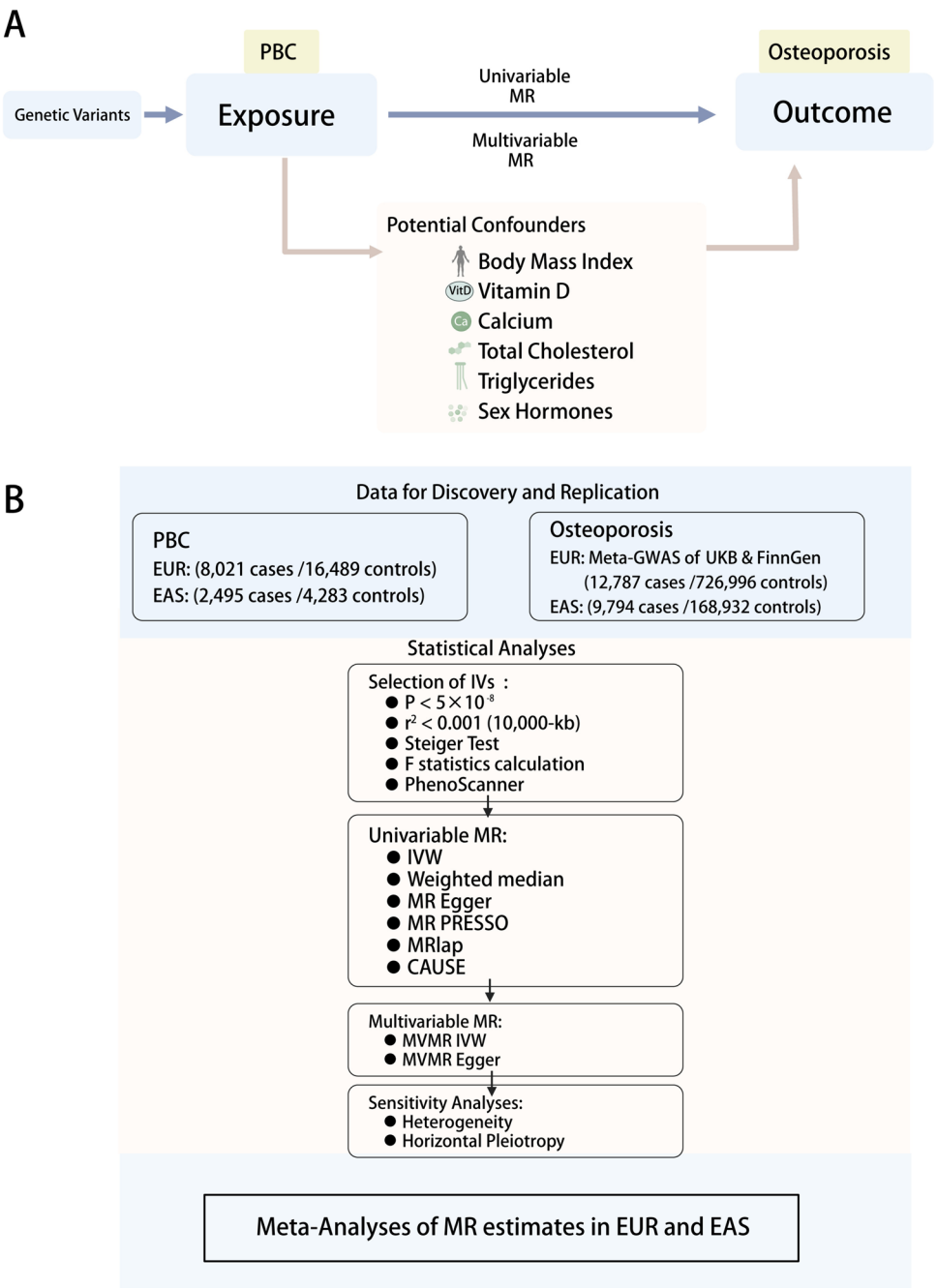

Fig. 1 Overview of this MR study. **A** We used genetic variants as proxy for exposure to assess the causal associations between PBC (exposure) and osteoporosis (outcome) by univariable MR methods. Additionally, we performed multivariable MR analyses to estimate the direct effect of PBC on osteoporosis adjusting for candidate confounders. **B** We performed MR analyses in European and East Asian populations and pooled meta-analysis estimates were then evaluated. PBC, primary biliary cholangitis; MR, Mendelian randomization

no sample overlap between PBC and osteoporosis in Europeans (intercept between PBC and osteoporosis: − 0.0003; standard error of intercept: 0.007). In East Asians, however, the bivariate LDSC intercept between PBC and osteoporosis (− 0.008) was slightly higher than standard error of intercept (0.005), suggesting mild sample overlap.

Meta-Analysis of European Osteoporosis GWAS Data

To increase the statistical power of MR analysis, we combined two large-scale biobank-based GWAS summary data of osteoporosis from European ancestry (the UK Biobank and the FinnGen Consortium). Following quality control, the European cohort of osteoporosis (OSEUR) consisted of 28,015,291 variants across 12,787 cases and 726,996 controls, and the genomic control (GC) inflation factor λ was 1.077 (Figs. S1 and S2). This meta-analysis identified a total of 24 lead SNPs from 43 independent significant SNPs across 18 genomic loci at genome-wide significance ($P < 5 \times 10^{-8}$), including the MHC region and 17 non-MHC risk loci. The details of the risk loci and mapped genes are listed in Supplementary Table S7.

Table 1 UVMR estimates for causal effect of primary biliary cholangitis on osteoporosis and vice versa

Exposure	Outcome	Ancestry	Method	SNPs	OR (95%CI)	Pval
Primary biliary cholangitis	**Osteoporosis**	**EUR**	IVW	37	1.040 (1.016–1.064)	**0.001**
			Weighted median		1.040 (1.006–1.074)	**0.019**
			MR-Egger		1.056 (0.993–1.123)	0.091
			MR-PRESSO		1.040 (1.017–1.063)	**0.002**
		EAS	IVW	13	1.059 (1.023–1.096)	**0.001**
			Weighted median		1.056 (1.013–1.101)	**0.010**
			MR-Egger		1.103 (0.984–1.235)	0.119
			MR-PRESSO		1.059 (1.024–1.094)	**0.007**
Osteoporosis	**Primary biliary cholangitis**	**EUR**	IVW	4	1.092 (0.872–1.368)	0.444
			Weighted median		1.084 (0.838–1.401)	0.539
			MR-Egger		0.918 (0.165–5.112)	0.931
			MR-PRESSO		1.092 (0.942–1.242)	0.334
		EAS	IVW	9	1.346 (0.932–1.945)	0.113
			Weighted median		1.517 (0.962–2.391)	0.073
			MR-Egger		1.325 (0.310–5.669)	0.716
			MR-PRESSO		1.030 (0.872–1.189)	**0.006**

EAS East-Asian, *EUR* European, *IVW* inverse-variance weighted, *MR-PRESSO* Mendelian Randomization Pleiotropy RESidual Sum and Outlier, *SNP* single-nucleotide polymorphism, *OR* odds ratio, *CI* confidence interval

Causal Relationship Between PBC and Osteoporosis Using Univariable MR Analyses

Using univariable MR analyses with the IVW approach, we noted that genetic liability to PBC was associated with higher risk of osteoporosis in Europeans (OR, 1.040; 95% CI, 1.016–1.064; $P = 0.001$) (Table 1 and Figs. 2, S4, and S5). In sensitivity analyses, there was no evidence of horizontal pleiotropy based on MR-Egger regression model ($\text{Egger}_{\text{intercept}} = -0.004$; $P = 0.6$) and MR-PRESSO global test (RSSobs, 53.676; $P = 0.071$) (Table S8). Meanwhile, no heterogeneity was observed ($P_{\text{Cochran's Q}} = 0.061$, $I^2 = 0.279$) (Table S8). No outlier was detected in the analyses by MR-PRESSO outlier test. Consistent results were also found when weighted median method was performed (OR, 1.040; 95% CI, 1.006–1.074; $P = 0.019$) (Table 1). After accounting for potential sample overlap, the causal relationship between PBC and osteoporosis persisted using MRlap analysis (OR, 1.014; 95% CI, 1.001–1.027; $P = 0.03$) (Table S10). Furthermore, leave-one-out analysis indicated that no single SNP drove the causal association (Fig. S3). Next, we investigated the effect of osteoporosis on PBC by performing reverse MR analyses. However, there was no indication of causal effect in the reverse direction among European populations (IVW: OR, 1.092; 95% CI, 0.872–1.368; $P = 0.444$) (Table 1 and Figs. 2, S7, and S8). CAUSE indicated no evidence for a causal effect PBC on osteoporosis (OR, 1.030; 95% CI, 1.000–1.051; $P = 0.15$), and vice versa (OR, 0.980; 95% CI, 0.869–1.094; $P = 1.0$) (Table S9).

Causal Estimates of PBC on Osteoporosis After Adjusting for Potential Confounders Using Multivariable MR Analyses

Considering that several risk factors were associated with both PBC and osteoporosis and may potentially bias the MR estimates, we performed multivariable MR analyses

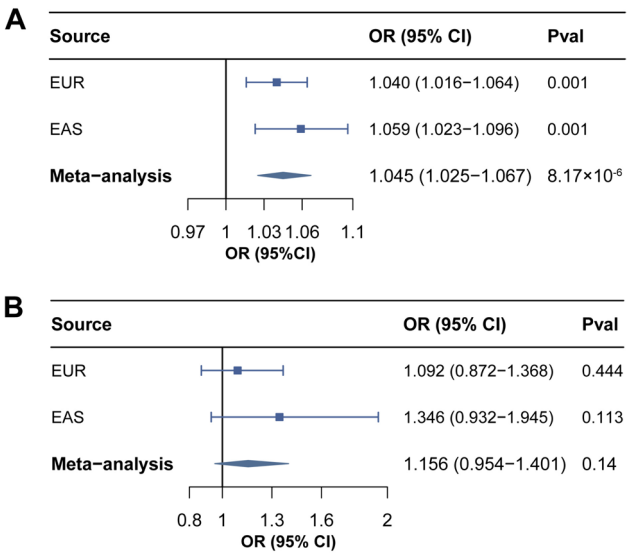

Fig. 2 Causal relationship between PBC and osteoporosis using IVW method. **A** Causal estimates for effect of PBC on osteoporosis in Europeans, East Asians, and pooled trans-ethnic meta-analysis. **B** Causal estimates for effect of osteoporosis on PBC in Europeans, East Asians, and pooled trans-ethnic meta-analysis. OR, odds ratio; CI, confidence interval; Pval, *P* value; EUR, European; EAS, East Asian

to evaluate the direct effect of PBC on osteoporosis with potential confounders adjusted. Among Europeans, genetically determined BMI, serum calcium, triglycerides, total testosterone, BAT, and SHBG were causally associated with osteoporosis in univariable MR analyses and were included in the following MVMR analysis (Tables S11 and S12). PBC remained positively related to osteoporosis after accounting for the potential confounders in different models, with conditional *F* statistics surpassing 10 (Fig. 3 and Table S13). All instrumental variables used in multivariable MR are reported in Supplementary Tables S14–S18. The causal estimates by the multivariable IVW were further confirmed by the MVMR Egger method, except for the model with BMI and calcium adjusted (Table S13). In particular, a larger effect size was observed after adjusting for potential confounders compared with the univariable MR analysis (Table S13). These data confirmed the robust relationship between PBC and osteoporosis.

Validation of Causal Association Between PBC and Osteoporosis in East Asians

We further replicated the causal relationship between PBC and osteoporosis in East Asians. In line with the findings in European populations, PBC conferred increased risk on osteoporosis in East Asian datasets (IVW: OR, 1.059; 95% CI, 1.023–1.096; $P=0.001$) (Table 1 and Figs. 2, S4, and S5). MR-Egger regression ($P_{intercept}=0.478$) and MR-PRESSO global test (RSSobs, 21.15; $P=0.132$) indicated no pleiotropy (Table S8). No outlier was found. Moreover, none of influential SNPs was detected in leave-one-out analysis (Fig. S3), suggesting the association was not driven by any individual SNPs. In addition, there was no evidence for heterogeneity (Cochran's Q statistic = 17.718, $P_{Cochran's\ Q}=0.125$, $I^2=0.323$) (Table S8). The estimates from weighted median, MR-PRESSO, and MRlap method were consistent with those from IVW method (weighted median: OR, 1.056; 95% CI, 1.013–1.101; $P=0.01$; MR-PRESSO: OR, 1.059; 95% CI, 1.024–1.094; $P=0.007$; MRlap: OR, 1.058; 95% CI, 1.032–1.084; $P=1.49\times10^{-5}$). There was no evidence of causal effect of PBC on osteoporosis in CAUSE method (OR, 1.02; 95% CI, 0.99–1.051; $P=0.44$) (Table S9).

In the reverse direction, IVW analyses showed that osteoporosis was not associated with an increased risk of PBC (OR, 1.346; 95% CI, 0.932–1.945; $P=0.113$) (Table 1 and Figs. 2, S7, and S8). The estimates from weighted median, MRlap and CAUSE were in line with those from IVW method (weighted median: OR, 1.517; 95% CI, 0.962–2.391; $P=0.073$; MRlap: OR, 2.137; 95% CI, 1.063–3.211; $P=0.166$; CAUSE: OR, 1.377; 95% CI, 0.763–2.509; $P=0.53$) (Table 1, S9, and S10). However, there was significant association when MR-PRESSO method was performed (OR, 1.030; 95% CI, 0.872–1.189; $P=0.006$) (Table 1). MR-Egger method showed a nonsignificant intercept ($P_{intercept}=0.983$), and MR-PRESSO global test indicated no pleiotropy (RSSobs, 1.797; $P=0.995$) (Table S8). In addition, no influential SNP was detected to drive the causal association in leave-one-out analysis (Fig. S3). Heterogeneity was not found as well (Cochrans' $Q=1.481$; $P_{Cochran's\ Q}=0.993$; $I^2=0$) (Table S8). In East Asians, we observed strong evidence of causal effect of PBC on osteoporosis, while the evidence for the reverse causation was less compelling.

In East Asians, among the potential confounders, only BMI had a causal effect on osteoporosis (IVW: OR, 0.811; 95% CI, 0.667–0.987; $P=0.036$) (Table S12). Therefore, BMI was retained as exposure to be adjusted in the following MVMR analyses. As a result, PBC was still causally related to osteoporosis after conditioning on BMI (Fig. 3 and Table S13).

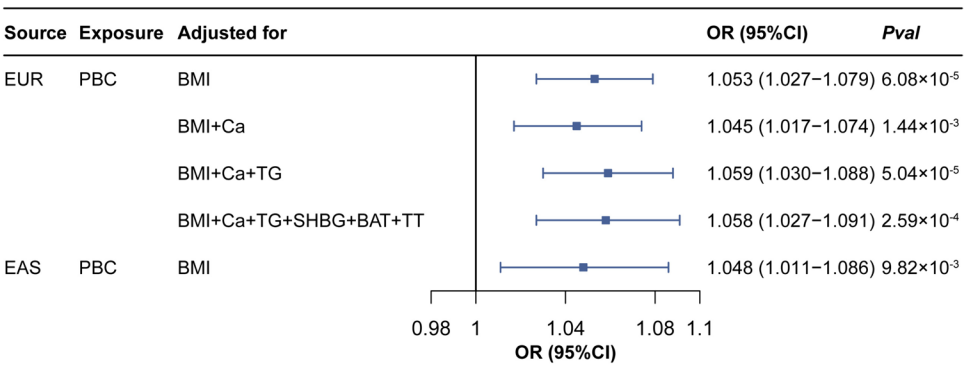

Fig. 3 Causal effect of PBC adjusted for potential confounders on osteoporosis using IVW method in multivariable MR. We performed multivariable MR analyses to estimate the direct causal effect of PBC on osteoporosis in Europeans and East Asians after adjusting for potential confounders. BMI, body mass index; TG, triglycerides; SHBG, sex hormone binding globulin; BAT, bioavailable testosterone; TT, total testosterone; OR, odds ratio; CI, confidence interval; Pval, *P* value; EUR, European; EAS, East Asian

Meta-Analysis of Combined Results from Europeans and East Asians

To increase the statistical power, we further integrated the MR results from Europeans and East Asians. Estimates from the European and the East Asian were combined using the fixed-effects meta-analysis method, owing to no indication of heterogeneity in trans-ethnic meta-analysis ($I^2 = 0$). The pooled meta-analysis further supported that genetically predicted PBC increased the risk of osteoporosis (IVW: OR, 1.045; 95% CI, 1.025–1.067; $P = 8.17 \times 10^{-6}$). However, there was no evidence indicating the causal effect of osteoporosis on PBC in the reverse direction (IVW: OR, 1.156; 95% CI, 0.954–1.401; $P = 0.14$) (Fig. 2).

Discussion

We report herein that genetically predicted PBC was positively associated with osteoporosis among European and East Asian populations. Furthermore, multivariable MR analyses demonstrated that the causal association between PBC and osteoporosis was independent of potential confounding factors, such as BMI, calcium, triglyceride, total testosterone, bioavailable testosterone, and SHBG. These results are supported by a set of sensitivity analyses.

Previous observational studies have discussed the association between osteoporosis and PBC [8–12]. Several large-scale cohort studies have demonstrated that osteoporosis risk was three- to four-fold greater in patients with PBC than general population in both Europeans and Asians [8, 10]. In addition, PBC patients with osteoporosis were found to have worse liver function and more advanced stage of liver disease [9, 11]. Very recently, a nationwide cohort study from Sweden (3980 cases of PBC and 37,196 matched controls) reported 1.9-fold increased risk of osteoporotic fracture in patients with PBC [12], leading to twofold higher 30-day and 1-year mortality following fracture in these individuals [12]. However, these observational studies failed to make causal inferences partially due to unmeasured confounding and reverse causation [24, 40]. MR is a practical method for estimating causality. Given that the genetic instruments are received randomly at conception, the limitations in observational studies could be overcome in MR. A recent two-sample univariable MR study found that PBC was not causally associated with bone mineral density in Europeans [22]. However, this study was limited by small sample size and did not account for confounding factors. By utilizing the largest PBC GWAS datasets and adjusting for potential confounders, our current findings extend prior research by demonstrating causal relationship between genetically predicted PBC and increased risk of osteoporosis in different ancestries, which provide clues for clinicians to intervene on PBC in time.

While our study suggested the causal effect of PBC on osteoporosis, the biological mechanism underlying the correlation remains to be elucidated. Osteoporosis is a disease characterized by decreased bone mass or bone density and deterioration of bone micro-architecture [55]. Bone remodeling is regulated by the coupling of bone formation and bone resorption in osteoblasts and osteoclasts [56]. Different from postmenopausal osteoporosis with increased bone resorption, osteoporosis in patients with PBC is characterized by reduced bone formation [57]. It has been reported that unconjugated bilirubin and serum from jaundiced patients with cholestatic liver disease significantly decreased osteoblast viability, differentiation, and mineralization [58].

Intriguingly, common molecular mechanisms exist between PBC and osteoporosis. PBC is characterized by immune-mediated injury of small- and medium-sized intrahepatic bile ducts. The dysfunction of immune system is also associated with osteoporosis [52]. Inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-7, IL-17, and prostaglandin E2 (PGE2) can induce the expression of M-CSF and RANKL, stimulating osteoclasts and causing bone loss [59]. In addition, hormonal imbalance may also be a shared molecular mechanism between the two diseases. For example, hormones play a key role in regulating bone density and bone metabolisms [60]. PBC patients often have disruptions in bile acid metabolism, which may affect the absorption and utilization of vitamin D, thus impacting bone density and bone metabolism. Interestingly, serum from jaundiced patients up-regulated the gene expression of *RANKL/OPG* (receptor activator of nuclear factor-κB ligand/osteoprotegerin), which is a PBC-susceptible gene and plays an important role in osteoclastogenesis [58]. These findings at genetic level are worth to be further investigated in the future.

In addition, the pathophysiology of osteoporosis is multifactorial, such as vitamin D and calcium metabolism, hormone dysregulation, cytokine release, and growth factors (i.e., deficiency of insulin-like growth factor 1 [IGF-1]) [61]. Notably, many of these factors have also been widely discussed in PBC, especially calcium metabolism. Patients with PBC and other chronic liver diseases are vulnerable to deficiency of vitamin D, [62] which is indispensable regarding calcium metabolism and bone synthesis. Accordingly, vitamin D and calcium supplementation have been widely recommended as the first-line therapy for osteoporosis in PBC [1]. In multivariable MR analyses, we investigated several potential factors including calcium which may affect the causality between PBC and osteoporosis. Our study suggested that the causal relationship between PBC and osteoporosis was not influenced by calcium. Therefore, deficiency of calcium may not explain the high risk of osteoporosis in patients with PBC.

A primary strength of our study is that our study uses trans-ethnic GWAS datasets to estimate the causal association between PBC and osteoporosis, providing generalization to both Europeans and East Asians. Additionally, we estimated the potential effect of common risk factors in the causal relationship between PBC and osteoporosis, strengthening the robustness of the causality. Moreover, we have utilized multiple analyses to eliminate the effect derived from potential sample overlap, which contributes to inflating the weak instrument bias.

Several limitations should be acknowledged in this study. First, we performed MR analyses with summary-level GWAS data, which limited the sub-group exploration such as gender and age stratification. Second, we were unable to differentiate the causality according to the severity of PBC, owing to the absence of identified PBC severity-associated SNPs at present. Third, a noteworthy problem of MR analysis is the potential horizontal pleiotropy. We performed MR-CAUSE to estimate the causal relationship between PBC and osteoporosis [51]. However, there was no evidence for causal association between PBC and osteoporosis using CAUSE. We have to acknowledge the robustness of CAUSE, especially for its accounting for uncorrelated pleiotropy. However, MR-CAUSE method relies on the assumption that the effects of genetic variants on the mediator variable are independent of the moderated risk factors, which may not hold true in all cases. In addition to CAUSE, MVMR is also a developed method to estimate the independent direct effects of multiple, potentially related exposures on outcome, which can account for the pleiotropic pathway. Although no evident horizontal pleiotropy was detected in this study, other potential risk factors that may bias the effect should be considered in the future studies. Further studies are required to examine the effect when larger GWAS data are available.

Our data indicates that genetically predicted PBC was associated with an increased risk of osteoporosis among European and East Asian populations. Furthermore, this causal effect was independent of several potential risk factors, such as calcium, triglycerides, total testosterone, bioavailable testosterone, and SHGB. Our study helps to raise awareness that novel therapeutic interventions in addition to vitamin D and calcium supplementation should be developed to manage osteoporosis in PBC. Further well-designed studies are needed to investigate the underlying biological mechanism of osteoporosis in PBC.

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Author Contribution Dr. Tang and Dr. Ma had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Ruqi Tang, Xiong Ma, Zhiqiang Li, and M. Eric Gershwin. Acquisition, analysis, or interpretation of data: Yi Wu, Qiwei Qian, Minoru Nakamura, Qiaoyan Liu, Rui Wang, Xiting Pu, Yao Li, and Huayang Zhang. Drafting of the manuscript: Yi Wu and Qiwei Qian. Critical review of the manuscript for important intellectual content: Ruqi Tang, Xiong Ma, Zhiqiang Li, and M. Eric Gershwin. Statistical analysis: Yi Wu, Qiwei Qian, and Zhiqiang Li. Obtained funding: Xiong Ma and Ruqi Tang. Administrative, technical, or material support: Zhengrui You, Qi Miao, Xiao Xiao, Min Lian, and Qixia Wang. Supervision: Ruqi Tang and Xiong Ma.

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Data Availability See Table S1.

Declarations

Ethical Approval Ethical approval and participant consent to participate, originally obtained in these patient cohorts, were not required here.

Competing Interests The authors declare no competing interests.

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