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Mendelian randomization: an approach for precision medicine and public health

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Mendelian randomization: an approach for precision medicine and public health

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Editorial: Mendelian randomization: an approach for precision medicine and public health

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KEYWORDS

mendelian randomization, genetic variants, causal inference, lifestyle factors, immune dysfunction

Editorial on the Research Topic
Mendelian randomization: an approach for precision medicine and public health

Mendelian randomization (MR) is an approach that leverages genetic variants as instrumental variables to infer causal relationships between exposures and health outcomes. By minimizing confounding and reverse causality, MR offers advantages over traditional observational studies to guide clinical decision-making and public health interventions. This Research Topic in *Frontiers in Genetics* showcases the versatility of MR, featuring studies that explore lifestyle factors, immune dysregulation in disease pathogenesis, and beyond.

Lifestyle factors significantly influence disease risk, yet observational studies often struggle to disentangle causation from correlation. This Research Topic includes several investigations that clarify these relationships using MR. Zhang et al. reported the association between coffee consumption and osteoarthritis risk, with body mass index (BMI) playing a significant mediating role (Zhang et al.). Their findings suggest that maintaining healthy BMI levels and choosing caffeinated over decaffeinated coffee varieties may help reduce osteoarthritis risk. Besides, Wang et al. explored the causal effects of smoking and alcohol consumption on upper urinary calculi, Liang et al. investigated tea intake and gout risk, and Zhou et al. identified positive causal effects of hypertension, BMI, waist-hip ratio adjusted for BMI and tobacco use to aortic aneurysm (Wang et al.; Liang et al.; Zhou et al.). All these studies exemplify how MR can validate or refute observational associations between lifestyle factors and diseases, offering insights into whether lifestyle habits directly contribute to joint health and reinforcing the importance of targeted public health campaigns to reduce disease risks.

The immune system's role in disease pathogenesis is a recurring theme in this Research Topic. Utilizing MR approaches, Shi et al. discovered causal links between immune cell phenotypes (e.g., CD45 on HLA DR + NK cells) and cerebrovascular anomalies (Shi et al.). Inflammatory bowel diseases include ulcerative colitis and Crohn's disease. Several studies investigated the causal links between inflammatory bowel disease and other diseases. Specifically, Xiao et al. investigated the causal links between inflammatory bowel disease and IgA nephropathy, offering mechanistic insights for comorbid conditions. However, no

significant causal link was observed between inflammatory bowel disease and type 2 diabetes mellitus in Xiao et al., Tang et al.

MR's applications extend far beyond lifestyle factors and immune dysregulation in disease pathogenesis, as demonstrated by the diverse range of studies in this Research Topic. For example, aging was a recurring theme in this Research Topic. Chen et al. reported earlier age at menarche may have a causal effect on the high risk of ischemic heart disease (Chen et al.). Xie et al. revealed that obstructive sleep apnea may accelerate telomere shortening, suggesting a biological pathway connecting sleep disorders to cellular aging (Xie et al.). Besides, the causality of biomarkers to disease outcomes remains an active area of investigation. Zhu et al. reported a causal relation rather than reversed causality between both hepatic enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) and spontaneous abortion, highlighting potential biomarkers to pregnancy complications (Zhu et al.). Guan et al. investigated the causal association between serum bilirubin and heart failure, yet not reaching statistical significance (Guan et al.). Zou et al. identified causal genes related with endoplasmic reticulum stress in inflammatory bowel disease (i.e., ulcerative colitis and Crohn's disease) suggesting new therapeutic targets for clinical practice (Zou et al.). In addition, emerging evidence highlights important disease comorbidities across multiple conditions. Liu et al. reported an increased risk of heart failure in individuals with ankylosing spondylitis (Liu et al.). However, neither the causal relationship between ankylosing spondylitis and other cardiovascular diseases (such as atrial fibrillation, coronary artery disease), nor the reverse causality between ankylosing spondylitis and mentioned cardiovascular diseases, reached statistical significance. Shen et al. reported a bidirectional causal relationship between gastroesophageal reflux disease and chronic obstructive pulmonary disease (COPD), and COPD was also found to increase the risk of irritable bowel syndrome and constipation (Shen et al.). Shu et al. demonstrated a putative causal link of insomnia on low back pain and a null causal effect of low back pain on insomnia (Shu et al.). All these findings may stimulate new strategies for patient management in clinical practice, benefiting public health.

Collectively, this Research Topic highlights MR's pivotal role in advancing precision medicine and public health. Continuous innovation in MR methodologies and interdisciplinary collaborations are encouraged to translate these insights into meaningful improvements for global health.

Author contributions

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Association of cardiovascular risk factors and lifestyle behaviors with aortic aneurysm: A Mendelian randomization study

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Objective: To examine the causality between hypertension, diabetes, other cardiovascular risk factors, lifestyle behaviors, and the aortic aneurysm among patients of European ancestry.

Methods: We performed two-sample Mendelian randomization (MR) analysis to investigate the causality of 12 modifiable risk factors with aortic aneurysm, including hypertension, body mass index (BMI), waist–hip ratio (WHR), diabetes, tobacco smoking, alcohol and coffee consumption, physical activity, and sleep duration. Genome-wide significant genetic instruments ($p < 5 \times 10^{-8}$) for risk factors were extracted from European-descent genome-wide association studies, whereas aortic aneurysm genetic instruments were selected from the UK Biobank and FinnGen cohort. The inverse-variance weighted MR was used as the main analysis, and MR-Egger (MRE), weighted median MR, MR pleiotropy residual sum and outlier, and Phenoscanner searching were performed as sensitivity analyses. Furthermore, we calculated MRE intercept to detect pleiotropy and Cochran's Q statistics to assess heterogeneity and conducted bidirectional MR and MR Steiger tests to exclude the possibility of reverse causality.

Results: We observed significantly higher risks for the aortic aneurysm in hypertension [pooled OR: 4.30 (95% CI 2.84–6.52)], BMI [OR: 1.58 (95% CI 1.37–1.81)], WHR [OR: 1.51 (95% CI 1.21–1.88)], WHR adjusted for BMI (WHRadjBMI) [OR: 1.35 (95% CI 1.12–1.63)], age of smoking initiation [OR: 1.63 (95% CI 1.18–2.26)], and tobacco use (initiation, cessation, and heaviness) [OR: 2.88 (95% CI 1.85–2.26)]. In sensitivity analysis, the causal effects of hypertension, BMI, WHRadjBMI, and tobacco use (initiation, cessation, and heaviness) remained robust.

Conclusion: There was a positive causal relationship between hypertension, BMI, WHR, and WHRadjBMI and aortic aneurysm.

KEYWORDS

aortic aneurysm, diabetes mellitus, body mass index, hypertension, Mendelian randomization

Introduction

Aortic aneurysms, clinically featured by permanent degradation and dilation of the aorta, are common macrovascular disorders. They could be classified according to lesion sites such as thoracic aortic aneurysm (TAA) and abdominal aortic aneurysm (AAA) (Quintana and Taylor, 2019). From previous published observational studies, several risks and protective factors for AAA have been identified, such as hypertension (Rapsomaniki et al., 2014; Kobeissi et al., 2019), history of smoking (Sode et al., 2013; Altobelli et al., 2018), body mass index (BMI) and body fat distribution (Cronin et al., 2013; Stackelberg et al., 2013), diabetes mellitus (Raffort et al., 2018), and other metabolic syndrome-related traits or lifestyles (Forsdahl et al., 2009; Kubota et al., 2018). When it comes to TAA, smoking, hypertension, and diabetes mellitus were shared (Quintana and Taylor, 2019; D'Cruz et al., 2019; Senser et al., 2021). Nevertheless, observational studies could not investigate causality because of potential confounders and reverse causation bias (Lawlor et al., 2008; Evans and Davey Smith, 2015; J ansen et al., 2014). Further research was still needed to investigate the causal role of the risk factors above, which could help better understand the mechanisms underlying aortic aneurysm development.

The Mendelian randomization (MR) design is a genetic instrumental variable analysis utilizing single nucleotide polymorphisms (SNPs) as genetic instruments to estimate the causal effect of a risk factor (exposure) on an outcome (risk for aortic aneurysm), bypassing the influence of confounding and reverse causality. Previous MR studies have investigated the causal effects of certain metabolic syndrome-related traits and lifestyle behaviors, including type 2 diabetes (T2DM) (van 't Hof et al., 2017), BMI (Larsson et al., 2020a), smoking (Larsson et al., 2020b), and alcohol consumption (Larsson et al., 2020c) on the risk of aortic aneurysm. Tobacco smoking and alcohol consumption were considered harmful while no significant association was found between T2DM, BMI, and risk of aortic aneurysm. For other risk factors reported in the literature, including type 1 diabetes (T1DM), hypertension, and lifestyle behaviors including coffee consumption, and physical activity, the causality with risk of the aortic aneurysm has not yet been investigated, or no definitive conclusion was reached. Sleep duration was also taken into consideration because of a recent MR study that revealed a harmful effect of insomnia on intracranial aneurysms (Karhunen et al., 2021). Despite that part of the factors were already evaluated in previous studies, no relevant research systematically investigated the causal relationship between known cardiovascular risk factors and lifestyle behaviors in aortic aneurysm development. In addition, we obtained different conclusions on some factors, such as BMI.

In this study, we performed MR analysis to investigate the causal effects of 12 cardiovascular risk factors and lifestyle behaviors on the aortic aneurysm. It is hoped that this work might complement the existing evidence for the pathogenesis and primary prevention of aortic aneurysms.

Materials and methods

Two-sample Mendelian randomization

We performed two-sample MR analyses with the ***TwosampleMR*** package (version 0.5.6) in this study (Burgess and Thompson, 2015; Hemani et al., 2018). MR uses SNPs as genetic instruments for the causal inferences about the effect of exposure on an outcome (risk for aortic aneurysm). Genetic instruments in MR should satisfy three assumptions: (Quintana and Taylor, 2019) the SNP was associated with the exposure; (Rapsomaniki et al., 2014) the SNP was not associated with confounders that can affect the causal effect of exposure on the outcome, and (Kobeissi et al., 2019) the SNP was associated with the outcome (risk for aortic aneurysm) only through the exposure. We used publicly available data for this MR study, and the access to these data was described in each of the subsequent GWASs (Table 1).

Exposure

Based on previous studies, we selected two categories of phenotypes that have been associated with aortic aneurysm: (Quintana and Taylor, 2019) metabolic syndrome-related traits: diabetes (both T2DM and T1DM), and several other metabolic syndrome-related traits, including hypertension, BMI, waist–hip ratio (WHR), and WHR adjusted for BMI (WHRadjBMI); and (Rapsomaniki et al., 2014) lifestyle behaviors: age of smoking initiation, tobacco use (initiation, cessation, and heaviness), alcohol consumption, coffee consumption, physical activity, and sleep duration.

We used genome-wide association studies (GWASs) with large sample sizes and cohorts completely or mainly composed of individuals of European ancestry as data sources for the genetic instruments of the phenotypes. First, we included SNPs associated with each selected trait at the genome-wide significance threshold ($p < 5 \times 10^{-8}$). If linkage disequilibrium (LD) was present ($r^2 > 0.001$), we only used the SNP with the strongest association to ensure the independence assumption. We also calculated the F statistics for each SNP and used only SNP with F statistics larger than 10 to avoid weak instrument bias (Burgess and Thompson, 2011). Then, for any unavailable SNPs in GWAS for aortic aneurysms, we used SNPs with LD of at least $r^2 > 0.80$ as an alternative (Peters et al., 2021). At last, we included 210/204 SNPs (UK Biobank/FinnGen) for hypertension

TABLE 1 Overview of the data sources of the instrumental variables used in the MR study (UK Biobank/FinnGen).

Risk factors	SNP	SNP available	Outliers[a]	Potential confounders[b]	Sample size	Ancestry	Units	PVE	Overlap[c]
Hypertension Elsworth et al. (2019)	216	210/204	0/0	55/54	4,62,933	European	1 SD increase in hypertension	2.8%/2.8%	60%–70%/none
BMI Yengo et al. (2018)	490	477/467	0/2	177/173	6,81,275	European	1 SD increase in BMI	4.8%/4.7%	85%/none
WHR Pulit et al. (2019)	206	202/199	1/2	129/127	6,94,649	European	1 SD increase in WHR	2.2%/2.2%	85%/none
WHR adjusted for BMI Pulit et al. (2019)	206	202/198	1/2	64/65	6,97,734	European	1 SD increase in WHR adjusted for BMI	2.8%/2.8%	87%/none
T2DM Xue et al. (2018)	118	115/114	0/0	50/49	6,55,666	European	Odds of T2DM	13.9%/13.8%	79%/none
T1DM Onengut-Gumuscu et al. (2015)	36	36/36	0/0	4/4	29,652	European	Odds of T1DM	17.9%/17.9%	None/none
Age of smoking initiation Liu et al. (2019)	202	194/193	0/0	45/44	12,32,091	European	Ever smoked regularly compared with never smoked	0.6%/0.6%	≈30%–35%/none
Tobacco use (initiation, cessation, and heaviness) Wootton et al. (2020)	124	118/116	0/0	39/39	4,62,690	European	1 SD increase in CSI (comprehensive smoking index)	1.1%/1.1%	Full/none
Alcohol consumption Liu et al. (2019)	71	68/68	0/0	25/25	9,41,280	European	1 SD increase in log transformed alcoholic drinks/week	0.5%/0.5%	≈30%–35%/none
Coffee consumption Cornelis et al. (2015)	4	4/4	0/0	3/3	1,21,824	Mixed	1 cup increase of coffee consumed/day	0.5%/0.5%	None/none
Physical activity Klimentidis et al. (2018)	19	19/19	0/1	4/4	3,77,234	European	1 SD increase in moderate to vigorous physical activity (MVPA)/MET-min/week	0.2%/0.2%	Full/none
Sleep duration Doherty et al. (2018)	12	12/12	0/0	3/3	91,105	European	1 SD increase in sleep duration	0.5%/0.5%	Full/none

[a]Outliers: outlier SNPs detected by MR-PRESSO (UK Biobank/FinnGen).
[b]Potential Confounders: SNPs associated with other risk factors of aortic aneurysm (UK Biobank/FinnGen) reported in previous GWAS using Phenoscanner.
[c]Overlap: The estimated overlap of UK Biobank/FinnGen with the risk factor GWASs.
Abbreviations: BMI, body mass index; PVE, proportion of variance explained; SD, standard deviation; SNP, single nucleotide polymorphism; T1DM, type 1 diabetes; T2DM, type 2 diabetes; WHR, waist–hip ratio.

(Elsworth et al., 2019), 477/467 for BMI (Yengo et al., 2018), 202/199 for WHR (Pulit et al., 2019), 202/198 for WHRadjBMI (Pulit et al., 2019), 115/114 for type 2 diabetes (Xue et al., 2018), 36/36 for type 1 diabetes (Onengut-Gumuscu et al., 2015), 194/193 for age of smoking initiation (Liu et al., 2019), 118/116 for tobacco use (initiation, cessation, and heaviness) (Wootton et al., 2020), 68/68 for alcohol consumption (Liu et al., 2019), 4/4 for coffee consumption (Cornelis et al., 2015), 19/19 for physical activity (Klimentidis et al., 2018), and 12/12 for sleep duration (Doherty et al., 2018) (Table 1; Supplementary Table S1). We calculated the proportion of the explained variance (PVE) of the exposure by the genetic instrument using an already published formula PVE = $beta^2/(beta^2 + N*se^2)$ (Shim et al., 2015), where beta is the effect size, se is the standard error, and N is the sample size of each instrument. We then summed them up to represent the proportion of variance explained by all SNPs used, which ranged from 0.2% for physical activity to 17.9% for T1DM.

Outcome selection

We obtained GWAS summary statistics of aortic aneurysms from two publicly available cohorts, namely, the UK Biobank and the FinnGen cohorts. The UK Biobank is a UK cohort study for the general population, including 1,374 aortic aneurysms (Phecode 442.1) patients and 4,00,595 controls until 2017 (Sudlow et al., 2015). The GWAS in UK Biobank aortic aneurysm was conducted with SAIGE on 28 million imputed variants (Zhou et al., 2018). The FinnGen study uses Finnish nationwide cohorts and biobanks and then combines genomic information, including aortic aneurysm status. GWAS summary statistics estimated with the SAIGE algorithm were obtained from the FinnGen R6 release, including 3,658 individuals with aortic aneurysms and 2,44,907 controls, with 1,69,62,023 genotyped SNPs (FinnGen, 2022). Sample overlapping in several exposure GWASs with aortic aneurysms was substantial in the UK Biobank cohort but very

limited in the FinnGen cohort (Table 1). We also calculated the power using a web-based application (https://sb452.shinyapps.io/power/) (Burgess, 2014; FinnGen, 2022).

Statistical analysis

We used the inverse-variance weighted (IVW) method for the main analyses under the random-effects model for each trait. The IVW method combines the Wald ratio estimates of each SNP (the beta coefficients for the SNP-aortic aneurysm association divided by the beta-coefficient for the SNP-exposure association) and calculates the weighted average of the Wald ratio estimates as the causal estimate. The IVW is the most commonly used and has the greatest statistical power but might be biased when the assumptions of MR were violated (Burgess et al., 2013). The UK Biobank and FinnGen cohort estimates were then pooled with a fixed-effect meta-analysis.

We then conducted several sensitivity analyses. First, we utilized the MR-Egger (MRE) regression to assess directional pleiotropy. The MRE method uses the average pleiotropic effects as the intercept but is less efficient and sensitive to outliers (Bowden et al., 2015). Second, we used the weighted median (WM) approach as sensitivity analysis, which provides valid estimates when more than half of the SNPs satisfy the instrumental variable assumptions. Third, we applied the MR pleiotropy residual sum and outlier (MR-PRESSO) method to exclude outlier SNPs ($p < 0.10$) that are potentially horizontally pleiotropic and to check whether the causal estimate changed with the exclusion of the outlying SNPs. Fourth, we performed a look-up of previously reported genome-wide significant association *via* R ***phenoscanner*** packages for any SNPs used (Staley et al., 2016; Kamat et al., 2019). The association was considered as potential pleiotropy and documented when satisfying the following criteria: (Quintana and Taylor, 2019) the association was genome-wide significant ($p < 5 \times 10^{-8}$); (Rapsomaniki et al., 2014) the SNPs associated with either lipid metabolism or any exposure explored in our study; and (Kobeissi et al., 2019) the GWAS was conducted in a population of European ancestry. Then, we excluded identified pleiotropic SNPs and performed MR IVW again to test the robustness of the causal effects.

Several additional tests were conducted to detect pleiotropy, including the MRE intercept (Burgess and Thompson, 2017) and Cochran's Q statistics of MR IVW (Greco et al., 2015), to assess between-instrument heterogeneity. We also performed bidirectional MR (Davey Smith and Hemani, 2014) and MR Steiger tests (Hemani et al., 2017) to exclude the possibility of reverse causality. For bidirectional MR, genetic instruments were selected from the largest GWAS to date for aortic aneurysms following the same inclusion criteria described above. We also performed bidirectional MR (Davey Smith and Hemani, 2014) and MR Steiger tests (Hemani et al., 2017) to exclude the possibility of reverse causality. For bidirectional MR, genetic instruments were selected from the largest GWAS to date for aortic aneurysms following the same inclusion criteria described above (Klarin et al., 2020). Since summary statistics for coffee consumption GWAS were unavailable (Cornelis et al., 2015), we used "filtered coffee intake" reported in UK Biobank as an alternative (Elsworth et al., 2019). The remaining 11 risk factors for aortic aneurysms used the same GWASs mentioned above (Onengut-Gumuscu et al., 2015; Doherty et al., 2018; Klimentidis et al., 2018; Xue et al., 2018; Yengo et al., 2018; Elsworth et al., 2019; Liu et al., 2019; Pulit et al., 2019; Wootton et al., 2020). At last, we performed two-sample MR on each SNP individually and leave-one-out analyses.

All statistical tests are two-tailed, and a p-value smaller than 0.05 was considered statistically significant. To account for multiple testing in our primary analyses of aortic aneurysms concerning the 12 risk factors, we used the Benjamini–Hochberg method to calculate a multiple testing–adjusted p-value (Benjamini and Hochberg, 1995). All the statistical analyses were conducted using R (version 4.0.5).

Results

Metabolic syndrome and lifestyle-related risk factors and aortic aneurysm: Main results

In the pooled analysis, we observed significant causal effects of following modifiable risk factors on aortic aneurysm (Figure 1): hypertension [pooled OR: 4.30 (95% CI 2.84–6.52), p-adjusted = 7.02×10^{-11}], BMI [OR per one SD increase: 1.58 (95% CI 1.37–1.81), p-adjusted = 5.78×10^{-10}], WHR [OR per one SD increase: 1.51 (95% CI 1.21–1.88), p-adjusted = 8.16×10^{-4}], WHRadjBMI [OR per one SD increase: 1.35 (95% CI 1.12–1.63), p-adjusted = 0.004], age of smoking initiation [OR: 1.63 (95% CI 1.18–2.26), p-adjusted = 0.006], and tobacco use (initiation, cessation, and heaviness) [OR: 2.88 (95% CI 1.85–2.26), p-adjusted = 1.21×10^{-5}]. No significant causal effects were observed for T2DM, T1DM, alcohol consumption, coffee consumption, physical activity, and sleep duration (Figure 1). In addition, there were no obvious differences between the causal effects estimated from UK Biobank and FinnGen for any risk factors explored. For pooled analysis, only five risk factors, namely, hypertension, BMI, WHR, WHRadjBMI, and tobacco use, had at least 75% power to reveal statistically significant causal relationships (Supplementary Table S2).

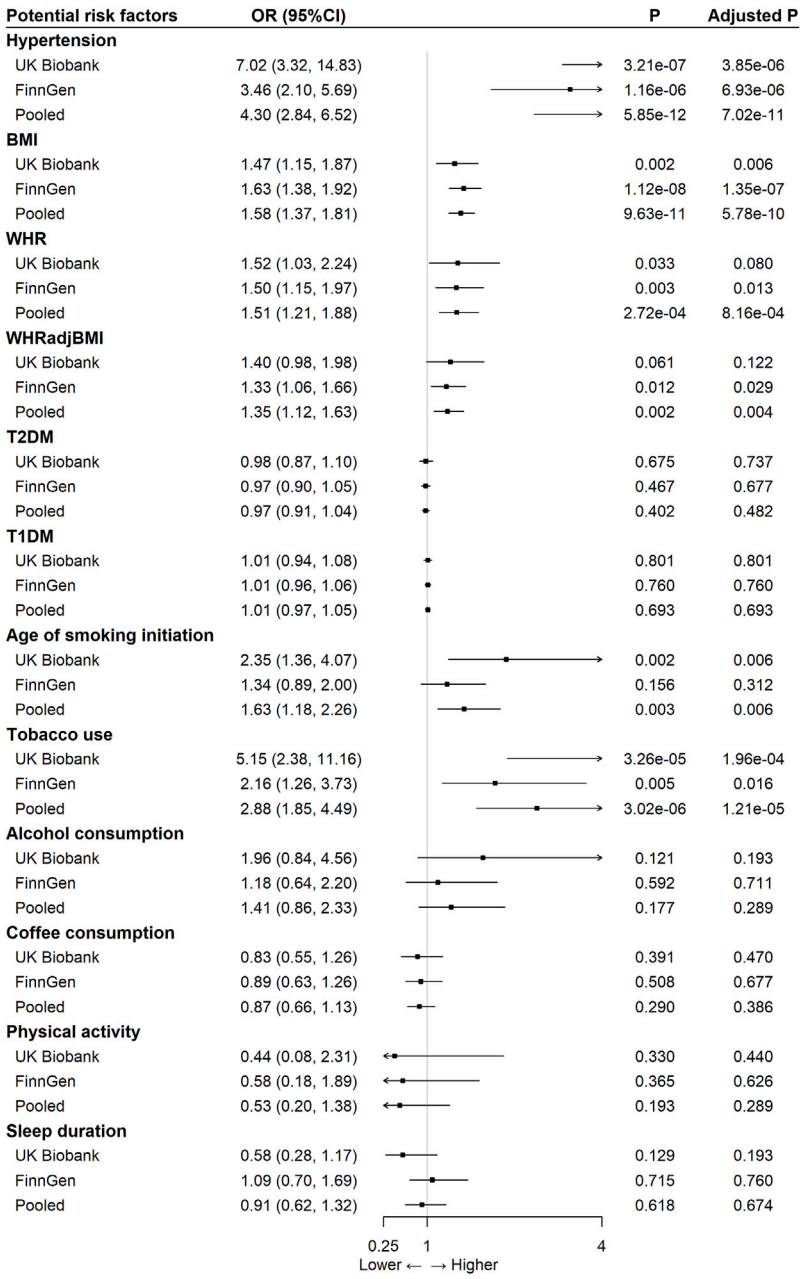

FIGURE 1
The association between 12 modifiable risk factors and aortic aneurysm using the inverse-variance weighted method.

Robustness of the results

Several alternative MR algorithms were further performed, including MR-Egger, WM MR, and MR-PRESSO. MR-Egger method attenuated the causal effects of the following risk factors on aortic aneurysms: hypertension, WHR, and age of smoking initiation (Supplementary Figures S1, S2). The results of the WM approach revealed significant causal relationships between hypertension, BMI, WHRadjBMI, and tobacco use (initiation, cessation, and heaviness) with aortic aneurysm but not for WHR and age of smoking initiation as MR IVW (Supplementary Figures S1, S3). The MR-PRESSO revealed 0/2 (for UK Biobank/FinnGen, respectively) outlier SNPs for BMI, 1/2 outlier SNPs for WHR, 1/2 outlier SNPs for WHRadjBMI, and 0/1 outlier SNP for physical activity (Table 1). Outlier correction did not substantially change the OR estimates for

BMI, WHR, WHRadjBMI, and physical activity (Supplementary Figures S1, S4).

We also used ***phenoscanner*** to identify SNPs associated with confounders for the causal effects of exposure on outcome. We reported 55/54 (for UK Biobank/FinnGen, respectively) SNPs for hypertension, 177/173 SNPs for BMI, 129/127 SNPs for WHR, 64/65 SNPs for WHRadjBMI, 50/49 SNPs for T2DM, 4/4 SNPs for T1DM, 45/44 SNPs for age of smoking initiation, 39/39 SNPs for tobacco use (initiation, cessation, and heaviness), 25/25 SNPs for alcohol consumption, 3/3 SNPs for coffee consumption, 4/4 SNPs for physical activity, and 3/3 SNPs for sleep duration, in order to have pleiotropic effects on confounders, including lipid metabolism (Table 1; Supplementary Table S1). After excluding the pleiotropic SNPs, MR IVW analysis indicated significant causal effects on aortic aneurysm of hypertension, BMI, and tobacco use (initiation, cessation, and heaviness) as shown in Supplementary Figures S1, S5.

The MR-Egger regression findings suggested potential pleiotropy for hypertension and alcohol consumption in UK Biobank-based analysis and potential pleiotropy for T1DM in the FinnGen cohort, as the intercept were all significantly not equal to 0 (p = 0.025, 0.040, and 0.041, respectively). The Cochran's Q statistic with the IVW method revealed significant heterogeneity across SNPs used in the analysis for WHR and WHRadjBMI based on UK Biobank GWAS and in the analysis using FinnGen cohort data for BMI, WHR, WHRadjBMI, age of smoking initiation, and physical activity (Supplementary Table S3).

Reverse causality was explored by the bidirectional MR and MR Steiger tests. Bidirectional MR suggested a noncausal relationship between the aortic aneurysm on any of the 12 risk factors after multiple comparisons (Supplementary Table S4). As for the MR Steiger test to orient the causal direction between the exposure and the risk for aortic aneurysm, we found that there were strong pieces of evidence for hypertension, BMI, WHR, WHRadjBMI, age of smoking initiation, tobacco use (initiation, cessation, and heaviness), and physical activity as the causal risk factors for aortic aneurysm when using UK Biobank data. However, when focused on the FinnGen cohort, only age of smoking initiation and physical activity did not pass the MR Steiger test (Supplementary Table S5).

At last, MR on each SNP individually and leave-one-out sensitivity analysis in UK Biobank and FinnGen cohorts, respectively, were reported in Supplementary Tables S6–S9.

Discussion

Our MR study confirmed a robust causal relationship between several modifiable risk factors and aortic aneurysm for the first time, including hypertension, BMI, WHRadjBMI, and tobacco use (initiation, cessation, and heaviness). In addition, no reversed causation was detected in all investigated factors. Furthermore, we identified WHR and age of smoking initiation as potential causes for aortic aneurysms, but the result varied across different MR algorithms. However, our study did not reveal the significant causal effects of T1DM, alcohol consumption, coffee consumption, physical activity, and sleep duration on the aortic aneurysm in the population of European ancestry.

The relationship between hypertension, BMI, WHRadjBMI, and tobacco use (initiation, cessation, and heaviness) and aortic aneurysm was likely to be causal. The causal relationships were consistent in the main analysis and with a maximum of one failure among the other four sensitivity tests. We noticed that MR-Egger tended to fail more frequently than the other algorithms. The observed failure might suggest outliers or violations of the inside assumptions in the cases of hypertension and age of smoking initiation (Bowden et al., 2015). Among the four risk factors mentioned above, the nonsignificant result after Phenoscanner search and exclusion was observed in WHRadjBMI. The failure might occur because of pleiotropy of the SNPs since 64/65 SNPs used in the main analysis were identified to have associations with traits beyond WHRadjBMI. We also observed a significant Cochran's Q statistic for WHRadjBMI, suggesting potential pleiotropy. However, for WHR and age of smoking initiation, there were apparent inconsistencies among the main analysis and sensitivity analyses. More than half of the SNPs as genetic instruments for WHR were associated with other phenotypes. Similar to WHRadjBMI, the significant effect of WHR on aortic aneurysm was abolished after Phenoscanner exclusion and Cochran's Q statistic revealed pleiotropy. MR-Egger intercepts and Cochran's Q statistic did not report pleiotropy and heterogeneity regarding age of smoking initiation. However, the proportion of potential invalid SNPs for age of smoking initiation was approximately one-quarter, and no significant results were observed after Phenoscanner exclusion.

Hypertension was a widely recognized risk factor in aortic aneurysm development. Although several epidemiological studies revealed a positive correlation between hypertension and aortic aneurysm, no causal relationships have been reported in previous MR studies (Vardulaki et al., 2000; Kobeissi et al., 2019; Mori et al., 2020). Our study identified the forward causation between hypertension and aortic aneurysm (p-adjusted < 0.001) for the first time. In a meta-analysis enrolling 14 cohort studies with a total of 26,943 cases and 5,317,552 participants, the overall RR to develop AAA in hypertensive patients was 1.66 (95% CI: 1.49–1.85) (p < 0.001) compared to patients without hypertension (Kobeissi et al., 2019). In addition to AAA, a cross-sectional study on computed tomographic scans of 21,295 patients investigated the association between atherosclerotic risk factors and ascending TAA (ATAA). The multivariate analysis results indicated that hypertension was positively associated with ATAA ≥ 4.5 cm (OR: 2.08; 95% CI: 1.44–3.03; p < 0.001). On

this basis, we further suggested that hypertension could mediate the susceptibility to aortic aneurysms.

As a critical metabolic factor, several conventional observational studies have investigated the role of BMI in aortic aneurysm development (Cronin et al., 2013; Stackelberg et al., 2013). Our MR study revealed forward causation between indicators for the relative weight and body fat distribution (BMI, WHR, and WHRadjBMI) and aortic aneurysm (p-adjusted < 0.005). The results coincided with a previous meta-analysis in which three studies, involving 305,726 participants, found a positive association between BMI and AAA. Moreover, a cross-sectional study of 12,203 men in Western Australia identified a greater waist–hip ratio as an independent and positive risk factor associated with AAA prevalence. However, considering the association between WHRadjBMI and aortic aneurysm, opposite conclusions were acquired in a previous MR study in the Dutch cohort, which might be related to the study population (van 't Hof et al., 2017). Given the lack of relevant cohort studies, the WHRadjBMI causal effects on aortic aneurysms still needed more investigations.

The causal effect of smoking on aortic aneurysms has been long recognized. The aortic aneurysm was the cardiovascular disease most strongly associated with smoking (Pujades-Rodriguez et al., 2015). Current smoking was the only modifiable risk factor for AAA growth in a meta-analysis involving 15,475 patients (Ulug et al., 2016). Previous MR analysis also suggested a causal relationship between tobacco use (initiation, cessation, and heaviness) and AAA, which is consistent with our findings. However, the same study also reported a significant role in age of smoking initiation (Larsson et al., 2020b). The discrepancy, as suggested by Phenoscanner searching and exclusion, might be because nearly one-quarter of the genetic instruments used for age of smoking initiation were invalid.

Last but not least, we performed an MR study on the causal relationship between diabetes and aortic aneurysm. In a cohort study involving 1.9 million participants to explore the association between T2DM and AAA, T2DM was negatively associated with AAA with a median follow-up of 5.5 years (OR = 0.46, 95% CI: 0.35–0.59, p < 0.0001) (Shah et al., 2015). A Spanish national retrospective study involving 1,15,020 patients admitted with AAA suggested that the incidences of AAA were significantly higher among nondiabetic elders (age > 70 years old) (p < 0.05) (Lopez-de-Andrés et al., 2015). Another population-based prospective study also reported the protective role of T2DM in AAA development (OR = 0.57, 95% CI: 0.40–0.82) (Larsson et al., 2018). However, our analysis did not reveal statistically significant causal effects for both T1DM and T2DM. The failure might be because of the lacking of statistical power of our study as suggested using power analysis. Nonetheless, we cannot exclude the possibility that previous observational studies were confounded. Further well-designed aortic aneurysm GWASs might facilitate the MR analysis on the effects of diabetes on the risk of aortic aneurysm.

Strengths and limitations

A key strength of our MR study was that we utilized the two independent aortic aneurysm GWASs with large sample sizes to investigate the causal effects of various relevant risk factors on aortic aneurysms. We also conducted multiple sensitivity analyses, pleiotropy detection, and reverse causality tests to carefully draw a conclusion and fulfill MR design's advantages of minimizing residual confounders.

Our study has several limitations. First, we only focused on European ancestry, and analyses were performed with combined data for males and females. The generalizability of the results to non-European populations and certain sex was limited. Second, the overlap between the population of exposure and outcome was inevitable, especially for UK Biobank, which could cause a shift of error toward the observational study. Third, the small variance explained by using the genetic instruments for several risk factors might result in insufficient statistical power to draw a powerful conclusion. Our power analysis also suggested lacking statistical power. It might be because the UK Biobank and FinnGen cohorts utilized in the present study were not case–control designs, which meant the detection of a particular disease might be insufficient. Fourth, horizontal pleiotropy was present and might introduce bias from the unexpected association between SNPs and potential confounders through relevant tests that were already conducted. Last but not least, some practical differences do exist between AAA and TAA. Further relevant studies might be needed to explore the difference..

Conclusion

In conclusion, we identified a positive causal relationship between hypertension, BMI, WHRadjBMI, and tobacco use (initiation, cessation, and heaviness) and aortic aneurysm. The potential harmful effect of WHR and age of smoking initiation could be further explored. Our study might contribute to the understanding of aortic aneurysm etiology and management of population health. In addition, the negative result of T2DM on the risk of aortic aneurysm indicated that some confounding factors contributed to the protective role of T2DM in observational studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

YZ is responsible for substantial contributions to the conception and substantively revision. JZ is responsible for study design, data acquisition, manuscript writing, and substantively revision. JL is responsible for study design, data analysis, data interpretation, manuscript writing, and substantively revision. All the authors approved the submitted version and agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.925874/full#supplementary-material

SUPPLEMENTARY TABLE S1
Single nucleotide polymorphisms (SNPs) used as genetic instruments for Mendelian randomization.

SUPPLEMENTARY TABLE S2
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SUPPLEMENTARY TABLE S3
Pleiotropy test for SNPs used for 12 risk factors for aortic aneurysm.

SUPPLEMENTARY TABLE S4
Bidirectional MR tests for the role of aortic aneurysm role 12 risk factors.

SUPPLEMENTARY TABLE S5
Mendelian randomization (MR) Steiger tests for orientation of the association between 12 risk factors and aortic aneurysm.

SUPPLEMENTARY TABLE S6
Individual SNP MR on 12 risk factors and aortic aneurysm in the UK Biobank.

SUPPLEMENTARY TABLE S7
Individual SNP MR on 12 risk factors and aortic aneurysm in FinnGen cohort.

SUPPLEMENTARY TABLE S8
Leave-one-out MR analysis on 12 risk factors and aortic aneurysm in UK Biobank.

SUPPLEMENTARY TABLE S9
Leave-one-out MR analysis on 12 risk factors and aortic aneurysm in FinnGen cohort.

SUPPLEMENTARY FIGURE S1
xxxx

SUPPLEMENTARY FIGURE S2
Association between 12 modifiable risk factors and aortic aneurysm using the MR-Egger regression method.

SUPPLEMENTARY FIGURE S3
Association between 12 modifiable risk factors and aortic aneurysm using the weighted median method.

SUPPLEMENTARY FIGURE S4
Association between 12 modifiable risk factors and aortic aneurysm using the MR-PRESSO method.

SUPPLEMENTARY FIGURE S5
Association between 12 modifiable risk factors and aortic aneurysm using the IVW method after phenoscanner exclusion of pleiotropic SNPs.

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Association of insomnia and daytime sleepiness with low back pain: A bidirectional mendelian randomization analysis

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Purpose: Observational research has indicated the presence of a causal relationship between sleep disturbances and low back pain (LBP). However, the link may have been biased by confounding factors. The purpose of this study was to examine the potential causal association of insomnia and daytime sleepiness with LBP by using mendelian randomization (MR).

Methods: Genome-wide association study (GWAS) summary statistics of insomnia were obtained from a large-scale GWAS meta-analysis (n = 1,331,010; individuals from UK Biobank and 23andMe) or UK Biobank alone (n = 453,379). The summary statistics of daytime sleepiness were from UK Biobank (n = 452,071) and LBP were provided by the FinnGen Release 6 (210,645 individuals with 16,356 LBP cases and 194,289 controls) or UK Biobank (5,423 cases versus 355,771 controls). Linkage disequilibrium score (LDSC) regression and bidirectional MR analysis was employed to estimate genetic correlation and causal relationship. In the MR analysis, the inverse variance weighted method (IVW) was utilized as the main analysis procedure, while MR-Egger, Weighted median and Robust adjusted profile score (RAPS) were utilized for supplementary analyses.

Results: LDSC analysis showed that LBP were significantly genetically correlated with insomnia (rg = 0.57, p = 2.26e-25) and daytime sleepiness (rg = 0.18, p = 0.001). The MR analysis revealed that genetically predicted insomnia was significantly associated with an increased risk of LBP (OR = 1.250, 95% CI: 1.186–1.318; p = 1.69e-16). However, the reverse causality was not confirmed. No evidence was identified supporting causality of daytime sleepiness and LBP.

Conclusion: This study demonstrates a putative causal link of insomnia on LBP and a null causal effect of LBP on insomnia. Furthermore, a causal link between daytime sleepiness and LBP were not reported. This finding may stimulate new strategies for patient management in clinical practice, benefiting public health.

KEYWORDS

insomnia, daytime sleepiness, low back pain, single nucleotide polymorphism, mendelian, randomization, causal association

Introduction

Low back pain (LBP) is one of the most common patient complaints in orthopedic outpatient departments and imposes a high burden on individuals and society. According to The Global Burden of Disease study 2013, chronic LBP is the most frequent cause of disability worldwide (Global Burden of Disease Study 2013 Collaborators, 2015). The prevalence of LBP has been estimated between 60% and 80% (Hagen et al., 1997). Chronic LBP is associated with many risk factors and diseases, such as overweight and obesity, heavy physical effort and depression (Nieminen et al., 2021). Nonetheless, its etiology is multifarious, and in 90% of back pain patients, the underlying reason cannot be identified. Therefore, better identification of LBP risk factors could help advance public health and reduce the cost of treatment.

Insomnia, a frequently occurring sleep disorder characterized by trouble falling and/or staying asleep, is commonly reported by LBP patients, with a prevalence of at least 50% (Alsaadi et al., 2013). A cohort study by Agmon and coworkers documented that after adjusting for several potential confounding variables, such as socioeconomic status, self-rated health, lifestyle behavior, and anthropometrics, healthy employed adults are almost 1.5 times more likely to suffer from LBP after developing insomnia (Agmon and Armon, 2014). Daytime sleepiness, defined by having trouble staying awake during the day, is also associated with the increasing frequency of back pain (Gustafsson et al., 2018). Although many observational studies have revealed a bidirectional relationship between sleep disturbances and LBP, the causal association are unclear due to the confounders (Kelly et al., 2011; Goforth et al., 2014; Uhlig et al., 2018; Ho et al., 2019; Bilterys et al., 2021; Oliveira et al., 2022). A recent review focused on the association between sleep and spinal pain (including LBP) showed weak to moderate evidence of causality (Van Looveren et al., 2021). Thus, further research with more advanced methodology is necessary to establish a cause-and-effect link exists between LBP and sleep disturbances.

Traditional observational studies are inevitably biased since they are subject to many confounding factors. On the other hand, large randomized trials, which can provide the best evidence for causation, are expensive and time-consuming. Mendelian randomization (MR) provides an ingenious method for inferring the causal nature of the exposure-outcome relationship by using instrumental variables (IVs), such as single nucleotide polymorphisms (SNPs) (Gao et al., 2021; Zhou et al., 2022). Due to the random segregation of alleles at conception, genetic variants are fixed and not affected by environmental risk factors, which minimizes residual confounding and reverse causality. Therefore, MR is also considered as a "natural" randomized controlled trial.

Previous MR studies have successfully confirmed that sleep traits contributed to the risk of several diseases including psychiatric disorder (Gao et al., 2019), cardiovascular diseases (Yuan et al., 2021), amyotrophic lateral sclerosis (Zhang et al., 2021) and even osteoarthritis (Ni et al., 2022). Thus, the present study aimed to corroborate the putative causal link of insomnia and daytime sleepiness with LBP using bidirectional MR.

Materials and methods

Study design

The overall design of the bidirectional MR study is shown in Figure 1. We selected SNPs as the genetic instrument and performed the MR analysis by the three key assumptions of MR design: 1) Relevance, i.e., genetic IVs are strongly associated with the exposure (Assumptions I); 2) Independence, i.e., IVs are independent of any confounders (Assumptions II); and 3) Exclusiveness, i.e., IVs do not affect the outcome directly, only possibly indirectly *via* the exposure (Assumptions III) (Burgess et al., 2013). Robust MR methods with different model assumptions were utilized, such as inverse variance weighted (IVW), MR-Egger, Weighted median and Robust adjusted profile score (RAPS). Subsequently, we performed a series of analyses to identify potential pleiotropy and heterogeneity. Since all analyses in our study were based on publicly available GWAS datasets, ethics approval was not required.

Genome-wide association study data sources

All IVs were retrieved from publicly available GWAS summary data. Our studies were restricted to subjects of European ancestry to minimize confounding caused by population stratification. The insomnia GWAS was reported by Jansen and collaborators and represents a large-scale (N = 1,331,010 individuals from UK Biobank and 23andMe) GWAS meta-analysis of insomnia from individuals of European ancestry (Jansen et al., 2019). The sample size of this data was 109,402 cases and 277,131 controls in the UK Biobank and 288,557 cases and 655,920 controls in the 23andMe database. Summary genetic statistic data for self-reported daytime sleepiness were available from the UK Biobank

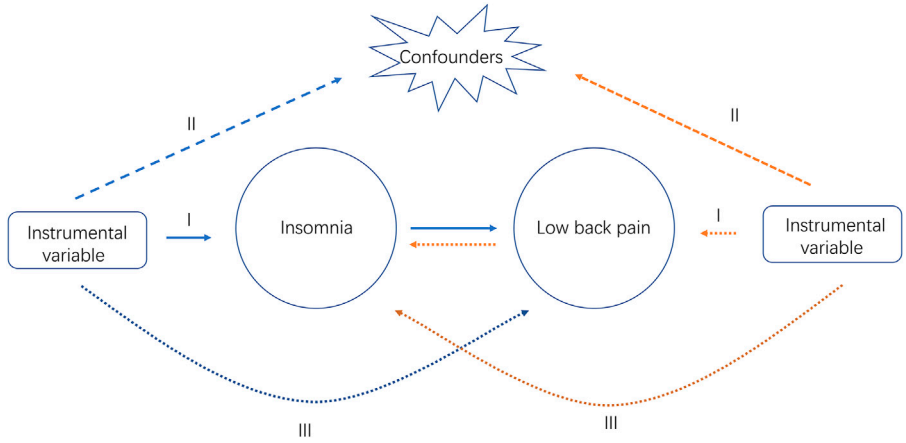

FIGURE 1
Study design overview. I, II, III, represent the three key assumptions of MR design. The blue arrows represent the forward study whereas the yellow arrows represent the reverse study.

(452,071 individuals) (Wang et al., 2019). The summary statistics from GWAS for LBP were retrieved from the FinnGen Release 6 (R6), which includes 16,356 cases and 194,289 non-cases of European ancestry.

Importantly, the (Lane et al., 2019) summary statistics for insomnia were used for outcome data in our reverse research, because of it is higher powered than the Jansen data without 23andMe data. In the validation analysis, genetic association data for LBP from UK Biobank data were also used on the web https://gwas.mrcieu.ac.uk/datasets/ukb-d-M13_LOWBACKPAIN. Finally, the exposure and outcome data were rearranged according to effect alleles to obtain harmonized datasets for the next step in the analysis. The characteristics of participants and additional information about the GWAS datasets are listed in Supplementary Table S1.

Genetic correlation analysis

Genetic correlations (rg) of insomnia, daytime sleepiness with LBP were estimated using Linkage disequilibrium score regression (LDSC) software package (https://github.com/bulik/ldsc) (Bulik-Sullivan et al., 2015).

Mendelian randomization analysis

First, we evaluated the potential issue of weak IVs in MR analysis by performing the F-statistics. A mean F-statistics markedly greater than 10 usually indicates a small instrument bias (Bowden et al., 2016). Next, the conventional fixed-effect IVW method, which has the highest precision and assumes any horizontal pleiotropy is balanced, was implemented for the main analyses. The methods which included MR-Egger, Weighted median and RAPS were also employed for MR sensitivity analyses. Weighted median method can provide consistent estimates when at least half of the IVs are valid (Burgess et al., 2019). When there is pleiotropy in IVs, the MR-Egger method can obtain an effective estimation and the intercept in the MR-Egger regression can also detect pleiotropy (Bowden et al., 2015). RAPS method is robust to weak genetic IVs and can produce approximately unbiased results (Zhao et al., 2020). Cochran's Q-test was used to detect potential heterogeneity. We also performed the MR-PRESSO analysis (SignifThreshold = 1) to detect outliers. If the MR-PRESSO outlier test calculated a p value less than the threshold, the outlier SNPs were removed. We performed the leave-one-out analysis to further identify potentially influential SNPs. Scatter plot and forest plot of the MR analyses were used to visualize the results.

All data analyses were conducted using the "Two-Sample MR" and "MR-PRESSO" packages in R version 4.0.2. Two-tailed p values < 0.05 were considered statistically significant putative causal link. A power calculation was performed using the online website (https://sb452.shinyapps.io/power/) developed by Burgess and coworkers (Burgess, 2014).

TABLE 1 Genetic correlation estimates from LDSC regression.

Phenotype 1	Phenotype 2	Rg (SE)	Pval
Insomnia	LBP	0.57 (0.05)	2.26e-25
Daytime sleepiness	LBP	0.18 (0.05)	0.001

Rg, genetic correlation; Pval, p-value for rg; SE, the standard error of Rg.

TABLE 2 Causal correlation estimates from MR analysis.

Method	nSNP	Beta	SE	Pval	OR	OR_lci95	OR_uci95
Insomnia on LBP							
MR Egger	138	0.143	0.106	0.179	1.154	0.937	1.421
Weighted median	138	0.188	0.043	1.47e-05	1.207	1.108	1.313
IVW	138	0.223	0.027	1.69e-16	1.250	1.186	1.318
RAPS	138	0.235	0.030	1.08e-14	1.266	1.192	1.343
Daytime sleepiness on LBP							
MR Egger	36	−2.593	1.637	0.123	0.075	0.003	1.851
Weighted median	36	−0.315	0.468	0.500	0.729	0.292	1.824
IVW	36	−0.151	0.318	0.636	0.860	0.461	1.606
RAPS	36	−0.147	0.399	0.713	0.863	0.395	1.887
LBP on insomnia							
MR Egger	23	−0.011	0.009	0.235	0.989	0.971	1.007
Weighted median	23	−3.09e-05	0.007	0.996	1.000	0.986	1.014
IVW	23	0.003	0.005	0.573	1.003	0.994	1.012
RAPS	23	0.002	0.005	0.663	1.002	0.993	1.012
LBP on daytime sleepiness							
MR Egger	26	−0.002	0.007	0.739	0.998	0.985	1.011
Weighted median	26	0.003	0.005	0.592	1.003	0.993	1.012
IVW	26	0.004	0.003	0.235	1.004	0.998	1.010
RAPS	26	0.004	0.004	0.303	1.004	0.997	1.011

nSNP, number of single-nucleotide polymorphism; Beta, the regression coeficient; SE, the standard error of the effect size; IVW, inverse-variance weighted; RAPS, Robust adjusted profile score.

Results

Linkage disequilibrium score regression

Using LDSC regression, we found that LBP were significantly genetically correlated with insomnia and daytime sleepiness, with the highest correlation seen for insomnia (rg = 0.57, p = 2.26e-25) and the lowest for changes in daytime sleepiness (rg = 0.18, p = 0.001) (Table 1).

IVs selection for mendelian randomization analysis

Firstly, we adopted the Jansen et al. insomnia dataset, in which 248 independent SNPs were identified as IVs of insomnia from combined results of the UK Biobank and 23andMe. Then, to satisfy the assumptions II, a total of 158 independent SNPs was screened out after the LD test based on Europeans (r^2 < 0.001). Unless otherwise specified, significance threshold p of < 5×10^{-8} and the absence of linkage disequilibrium (LD) (cut-off r^2 < 0.001, clump window > 10,000 kb) were selected in our MR analysis. Under the same conditions, 38 SNPs as IVs of daytime sleepiness were obtained. In the reverse direction study, SNPs were identified to associate with LBP at a broadened threshold ($p < 5 \times 10^{-6}$) (Wu et al., 2021; Zhou et al., 2022). We extracted 26 SNPs from the FinnGen study and 20 SNPs from UK Biobank, respectively. The information of these SNPs is detailed in Supplementary Table S2.

The causal effect of insomnia on low back pain

Initially, 4 SNPs of 158 lead SNPs were not found in the summary statistics for LBP (rs1064939, rs11001276, rs2286729, and rs73671843). Subsequently, nine SNPs (rs12991815, rs1731951, rs2030672, rs2221119, rs4858708, rs6119267, rs7044885, rs8180817, and rs830716) were removed for being palindromic with intermediate allele frequencies. For the remaining 145 SNPs, MR-PRESSO test detected seven potential outliers (rs1038093, rs12251016, rs12666306, rs429358, rs66674044, rs671985, and rs77641763). Finally, 138 SNPs as IVs of insomnia were selected to perform the MR analysis.

Our MR analyses showed that genetically predicted insomnia was causally associated with an increased risk of LBP. By using fixed-effect IVW method, the OR of LBP per genetically predicted insomnia was 1.25 [95% confidence interval (CI), 1.186–1.318; p = 1.69e-16] (Table 2). This statistically significant trend was further proven by Weighted median (OR = 1.21, CI: 1.105–1.318; p = 2.98e-05), RAPS (OR = 1.27, CI: 1.192–1.343; p = 1.08e-14) except for MR-Egger method

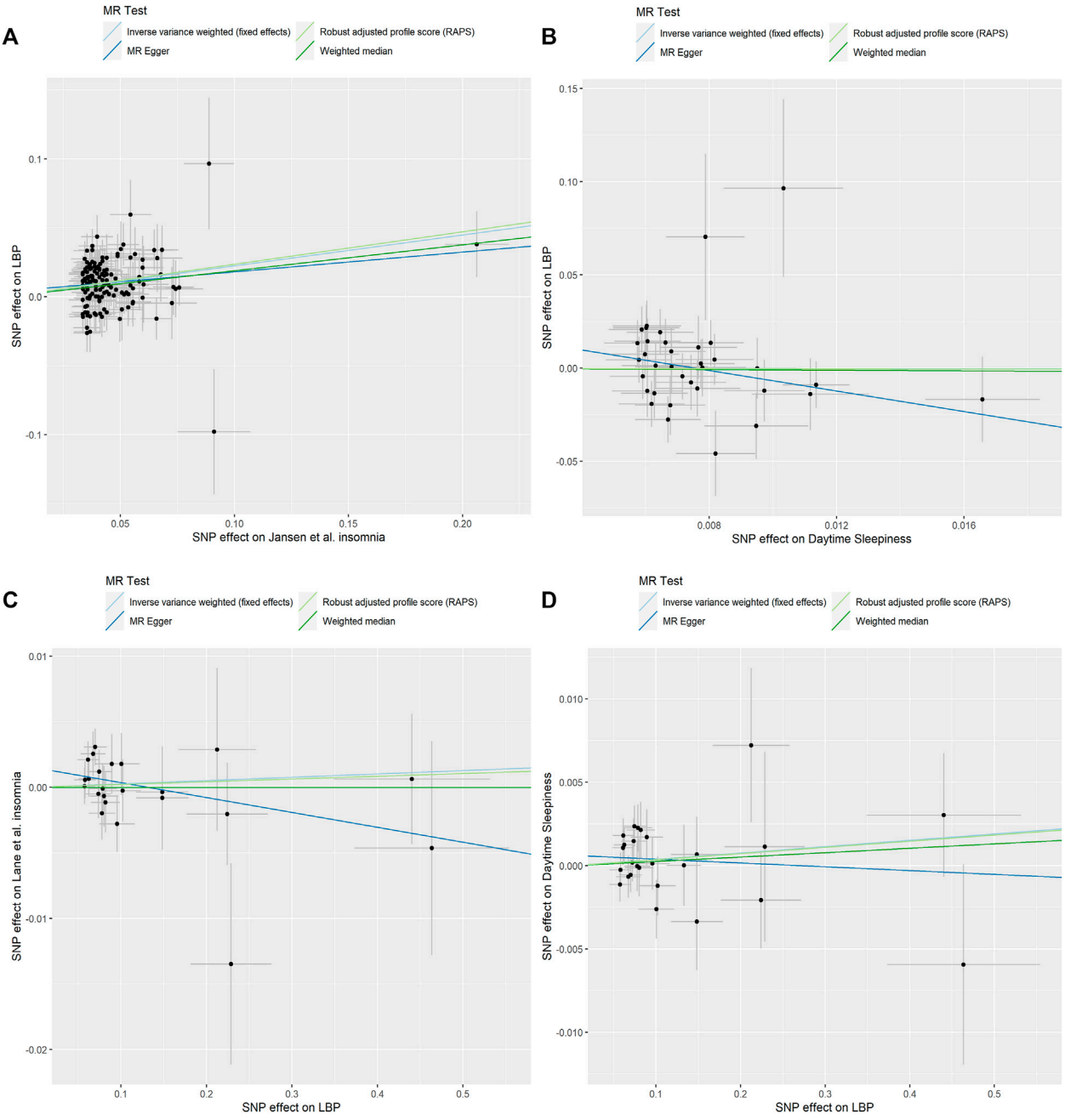

FIGURE 2
Scatter plots of causality. **(A)** Jansen et al. Insomnia on LBP. **(B)** Daytime Sleepiness on LBP. **(C)** LBP on Lane et al. Insomnia. **(D)** LBP on Daytime Sleepiness.

(OR = 1.15, CI: 0.937–1.421; $p = 0.179$) (Table 2). The MR-Egger regression analysis did not indicate the presence of pleiotropy (intercept $p = 0.435$) and Cochran's Q test did not detect significant heterogeneity in both MR-Egger and IVW methods ($p > 0.05$) (Supplementary Table S3). The causal effect of insomnia on LBP is illustrated by scatter plot (Figure 2A). Based on the effect size analysis, each insomnia SNP appeared to have a robust effect on LBP (Supplementary Figure S1). Furthermore, the leave-one-out analysis indicated that the significant result was not driven by any single SNP (Supplementary Figure S2). The mean F-statistics for IVs of insomnia is 43.73 in our study, which had ~100% power to detect a 25% increase in overall LBP risk (OR = 1.25). Altogether, these results indicate that our data are robust and without obvious bias.

The causal effect of daytime sleepiness on low back pain

For 38 SNPs, one SNP (rs11078398) was not found in the summary statistics for LBP and one SNP (rs2048522) was removed for being palindromic. In the MR-PRESSO test, no outliers were detected. Finally, 36 SNPs as IVs of daytime sleepiness remained in this part of the analysis. No genetic effect of daytime sleepiness on the risk of LBP was found by IVW method (OR = 0.86, CI: 0.461–1.606; $p = 0.636$), and the other methods were concordant with this result (Figure 2B; Table 2). MR-Egger regression analysis did not indicate that 36 IVs had horizontal pleiotropy (intercept $p = 0.135$). Cochran's Q test did not report any heterogeneity ($p > 0.05$) (Supplementary Table S3). Supplementary Figures S3, S4 shows the forest plot and the plot of leave-one-out analysis.

The causal effect of low back pain on insomnia

All 26 SNPs were available for insomnia datasets and three SNPs (rs34960666, rs35989721, and rs4024198) were found as a potential outlier by MR-PRESSO analysis. All MR methods did not support significant causal effect of LBP on insomnia (IVW: OR = 1.00, CI: 0.994–1.012, $p = 0.573$; MR-Egger: OR = 0.989, CI: 0.971–1.007, $p = 0.235$; Weighted median: OR = 1.00, CI: 0.986–1.014, $p = 0.996$; RAPS: OR = 1.00, CI: 0.993–1.012, $p = 0.663$) (Table 1). The scatter plot shows the distribution of the effect of single LBP SNP on insomnia (Figure 2C). The forest plot and the plot of the leave-one-out analysis are shown in Supplementary Figures S5, S6. The sensitivity analysis excluded horizontal pleiotropy and heterogeneity ($p > 0.05$, Supplementary Table S3).

The causal effect of low back pain on daytime sleepiness

In the analysis, 26 SNPs were taken as IVs for LBP. The results of IVW model suggested that LBP was not associated with the risk of daytime sleepiness (OR = 1.00, CI: 0.998–1.010, $p = 0.235$). The Weighted median, MR-Egger and RAPS estimations provided supporting evidence (Figure 2D; Table 2). The results did not display obvious evidence of heterogeneity or pleiotropy ($p > 0.05$, Supplementary Table S3). The forest plot and the plot of the leave-one-out analysis are displayed in Supplementary Figures S7, S8.

Robustness check

Summary statistics of LBP from UK biobank were included for replication purposes. In the analysis, genetically determined insomnia was positive associated with the risk of LBP (IVW: OR = 1.00, CI: 1.003–1.005, $p = 9.54e\text{-}12$), while there was no evidence of a reverse causality. No association was found between daytime sleepiness and LBP. No significant heterogeneity and pleiotropy were found in the replication practice. Specific information could be found in Supplementary Material. Obviously, these results were consistent with that in initial practice, indicating our MR analysis results are robust.

Discussion

Our findings reported coheritability of LBP to both insomnia and daytime sleepiness. By performing bidirectional MR analyses, our results provided genetic evidence that insomnia causally affects LBP and there is no reverse causality. Specifically, individuals having insomnia have an increased risk for LBP, and LBP patients do not have a statistically significant trend to develop insomnia. In addition, our study found no evidence of a bidirectional causal link between daytime sleepiness and LBP.

In the past decade, substantial studies have shown an association between sleep problems and the risk of chronic pain in the low back and neck/shoulders, as well as chronic widespread pain (Gupta et al., 2007; Canivet et al., 2008; Mork et al., 2014; Uhlig et al., 2018; Skarpsno et al., 2021). A previous prospective cohort study by Agmon and collaborators documented that healthy employed adults with insomnia have a higher risk of experiencing back pain than subjects without insomnia (Agmon and Armon, 2014). A recent study by Ho and coworkers showed that the adjusted risk ratio of chronic LBP in insomnia participants was 1.20 (Ho et al., 2022). On the other hand, it has been reported daytime sleepiness positively correlated with the symptom and frequency of back pain (Gustafsson et al., 2018; Uchmanowicz et al., 2019). The previous MR study conducted by Broberg and coworkers demonstrated that genetic liability to insomnia symptoms is significantly causal to reporting pain, including LBP (Broberg et al., 2021). Take advantage of the UK Biobank as well as the latest FinnGen cohort of LBP, the present study provided more robust evidence that insomnia, not daytime sleepiness, leads to a higher risk for developing LBP. Note that the reduction of sleep problems is accompanied by an improvement in LBP (Pakpour et al., 2018; Skarpsno et al., 2020). Particularly, the treatment of insomnia significantly improved the pain symptoms of patients with LBP in a randomized, double-blind placebo-controlled trial (Goforth et al., 2014).

Although sleep disturbances are becoming increasingly recognized as one of the most reported comorbidities in patients with LBP, the causal relationship of LBP on sleep traits is controversial. Tang et al. (2007) found that individuals with LBP are 18 times more likely to suffer from insomnia. The study by Bahouq et al. (2013) revealed that 78% of patients with chronic LBP experienced insomnia, and in 64% of them, insomnia was caused by LBP. A retrospective study on the Korea population found that 43% of LBP patients developed mild to severe insomnia (Kim et al., 2015). Similarly, studies also have shown that at least

50% of patients with LBP have insomnia at the same time (O'Donoghue et al., 2009; van de Water et al., 2011). However, the LBP was not identified as the predictor for insomnia in a prospective cohort study (Agmon and Armon, 2014). Our findings were in line with this research and did not provide significant genetic evidence for LBP as a risk factor for insomnia. Besides, the causal link of genetically predicted daytime sleepiness on the odds of reporting LBP has not been found.

There are several potential biological mechanisms underlying the link between sleep deficiency and LBP. Sleep deprivation may lead to upregulation of inflammatory mediators, possibly by affecting the immune system, ultimately leading to hyperalgesia (Mullington et al., 2010; da Costa Souza and Ribeiro, 2015). In animal studies, sleep loss increases pain sensation in healthy mice, which is involved in decreasing dopaminergic activity in the nucleus accumbens or increasing adenosinergic activity in median preoptic nucleus (Alexandre et al., 2017; Sardi et al., 2018). Moreover, individuals with insomnia symptoms have a mild increase in basal cortisol levels and a hyper-reactivity of the Hypothalamus-pituitary-adrenal axis, which are associated with pain sensitivity (Balbo et al., 2010; Goodin et al., 2012). On the other hand, various other systems, such as the opioid system, the monoaminergic system and adenosine signaling, mediate the effect of deficient sleep on pain (Haack et al., 2020). Further studies are necessary to investigate the mechanisms underlying the association between insomnia and LBP.

Our study has several strengths. First, this is the first analysis focused on the bidirectional causal relationship between two sleep traits (insomnia, daytime sleepiness) and LBP by MR methods, extending the relevant studies. Second, potential confounders and other biases were removed by genetic variants. Third, potential horizontal pleiotropy was detected and corrected by the MR-PRESSO method.

Some inevitable limitations should also be mentioned. Firstly, our study cannot evaluate the influence of within-population structures resulting from the between-sex difference in the prevalence of insomnia (Aili et al., 2015; Van Looveren et al., 2021). Secondly, MR does not inherently completely expel unknown pleiotropy that affected our results. Finally, the association between sleep disturbances and LBP needs to be further explored in other populations, as this study was restricted to subjects of European ancestries.

In summary, the results of this study reveal a deleterious effect of genetically predicted insomnia on LBP and a null causal effect between daytime sleepiness and LBP. Nonetheless, large-scale longitudinal studies, as well as in-depth mechanistic studies, need to be done. Importantly, the MR result implies that attention should be paid in clinical practice to comorbid insomnia and chronic LBP. A better understanding of the relationship between these two conditions will contribute to pain prevention and improvement in global public health.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval were waived for this study, all the data from Mendelian randomization is publicly accessible. Informed consent was obtained from all subjects in the original genome-wide association studies. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PS, LJ, and WL conceptualized the study. PS and LJ took part in drafting and reviewing the main manuscript. PS and ZP contributed to acquisition and analysis of data and validation and visualization of results. ZS and WL participated in reviewing and editing the manuscript. All authors contributed to the article and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.938334/full#supplementary-material

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Age at menarche and ischemic heart disease: An update mendelian randomization study

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Background: Although earlier menarche age has been associated with ischemic heart disease in previous observational studies, the relationship's causation has not been shown. Through two-sample Mendelian randomization (MR), we were able to define the causal connection.

Methods: We performed Mendelian Randomization (MR) analysis to explore the associations between genetically predicted AAM and IHD. Summary-level databases for exposure and outcome were selected from the MR-Base database (https://gwas.mrcieu.ac.uk/). Single-nucleotide polymorphisms (SNPs) connected to AAM at genome-wide significance level ($p < 5 \times 10^{-8}$) were considered as instrumental variables (IVs). We used four methods to pool MR estimates, including fixed-effects inverse variance weighting (fe-IVW), multiplicative random-effects inverse variance weighting (mre-IVW), weighted median (WM), and MR-Egger regression methods. Sensitivity analyses were performed to evaluate the robustness of the results. PhenoScanner searches and Multivariable Mendelian randomization (MVMR) analysis was used for assessing confounders.

Results: 117 SNPs significantly correlated with AAM were screened as instruments, the results of three main methods showed that genetically earlier AAM may have a causal effect on the higher risk of IHD (fe-IVW: OR = 0.80, 95% CI: 0.72–0.88, $p < 0.001$; mre-IVW: OR = 0.80, 95% CI: 0.70–0.90, $p < 0.001$; WE: OR = 0.79, 95% CI: 0.66–0.93, $p = 0.006$). These results were consistent across sensitivity analyses. MR analysis revealed that there was still a relationship between AAM and IHD even when pleiotropic SNPs of confounders were removed employing PhenoScanner searches. In MVMR, the significant association remained after adjusting for biological sex, but it was attenuated with adjustment of body mass index including childhood and adult.

Conclusion: Our MR analysis revealed a substantial genetically determined confounder-mediated relationship between an increase in genetically predicted AAM and a lower risk of IHD. By addressing the intervention of body mass index, the risk of IHD may be lowered.

KEYWORDS

age at menarche, ischemic heart disease, mendelian randomization, causality, single nucleotide polymorphisms

Introduction

Menarche has a distinctive function during a girl's youth as a lifetime marker of first menstruation. Because of this, the age at menarche (AAM) is typically well remembered in adulthood, and many epidemiological studies, therefore, prefer to see it as an essential object of study (Parent et al., 2003). In some reports (Day et al., 2015b; Chan et al., 2022), AAM has been used as a proxy for the time of pubertal maturation or the onset of puberty to explore the impact of earlier or later puberty on cardiovascular health in adults, such as hypertension, angina and heart attacks in later life. It has also been shown in several studies that early AAM does increase the likelihood of cardiovascular illnesses (Lakshman et al., 2009; Ibitoye et al., 2017).

Despite advances in disease prevention and diagnosis, an increasing number of women are still diagnosed with or die from cardiovascular disease each year (Mosca et al., 2011; Zheng et al., 2021). Ischemic heart disease (IHD) is the main cause of mortality in women worldwide, yet because of a lack of understanding of gender differences and inadequate management systems, more women than men pass away from IHD every year (Davies and Rier, 2018; Schmidt et al., 2018; Majidi et al., 2021). As cardiovascular risk factors, several physiological issues unique to women, such as early menopause, polycystic ovary syndrome, eclampsia, and early menarche, are currently increasingly being researched (Ahmed et al., 2014; Mehilli and Presbitero, 2020). These studies also include research on the correlation between AAM and IHD (Schmidt et al., 2018).

Mendelian randomization (MR) was proposed by Katan in 1986 to exploit the causal association between phenotypes and diseases through the use of genotypes as instrumental variables (Katan, 1986). As an analytical method using ready-made epidemiological data to identify causal estimates (Davies et al., 2018), MR carries out the genetic instruments that are fixed before birth, so confounding factors or reverse causality has little influence on instrumental genetic predisposition. Genetic predisposition can contribute to the occurrence of a target exposure, and if a significant association exists between the genetic predisposition and outcome, it suggests a causal effect of exposure. MR has been widely described in the medical literature and has identified many significant causal relationships between multiple exposures and outcomes (Park et al., 2021b).

Even though IHD is also referred to as coronary artery disease (CAD) (Khan et al., 2020) and an MR study found little evidence to support a causal effect of AAM on the risk of CAD (Cao and Cui, 2020), women exhibit less physically obstructive CAD and relatively more preserved left ventricular function while having higher rates of myocardial ischemia and mortality than men with the corresponding age adjustment (Shaw et al., 2009; Smilowitz et al., 2011). Sex-specific pathophysiology of myocardial ischemia, including coronary microvascular dysfunction, a feature of the "Yentl Syndrome," appears to be connected to this paradoxical sex difference. In light of this, the term IHD, as opposed to CAD, or coronary heart disease (CHD), is more appropriate for a topic that is special to women. Furthermore, even though Zheng et al. similarly recognized that women were more susceptible to the ischemia symptoms caused by myocardial infarction (MI) and discovered a genetic connection between earlier AAM and higher risk of MI by two-sample MR analysis (Zheng et al., 2022), studies of body mass index (BMI) and gender were neglected when evaluating the impacts of confounding or mediating factors. In our two-sample MR, we changed the outcome (CAD or MI) to the broader one (IHD) and used the external data source or multivariable MR analysis to further explore the effects of confounders, especially BMI and sex. Consequently, we explored the causal relationship between AAM and IHD *via* two-sample MR analysis to update the aforementioned studies, despite having a similar theoretical basis or starting point.

Materials and method

Study overview

The fundamental study concept for the two-sample MR analysis is shown in Figure 1. In brief, when single nucleotide polymorphisms (SNPs) are used as instrumental variables (IVs) to probe the causal relationship between exposure (AAM) and outcome (IHD), there are three assumptions needed to be satisfied in this study: (1) IVs are strongly associated with age at menarche; (2) IVs should not be associated with confounders in the exposure-outcome association; (3) IVs should influence the outcome only *via* exposure instead of other pathways.

Data source

The GWAS summary databases of exposure (AAM) and outcome (IHD) were obtained from the MR-base database (https://gwas.mrcieu.ac.uk/) (Hemani et al., 2018b). We filtered the MR-base database for the European population up to December 2021 using "menarche", and "age when periods started" as keywords for the exposure database, and then did the same with "ischemic heart disease ", "cardiac ischemia" as keywords for the outcome database. If there are multiple

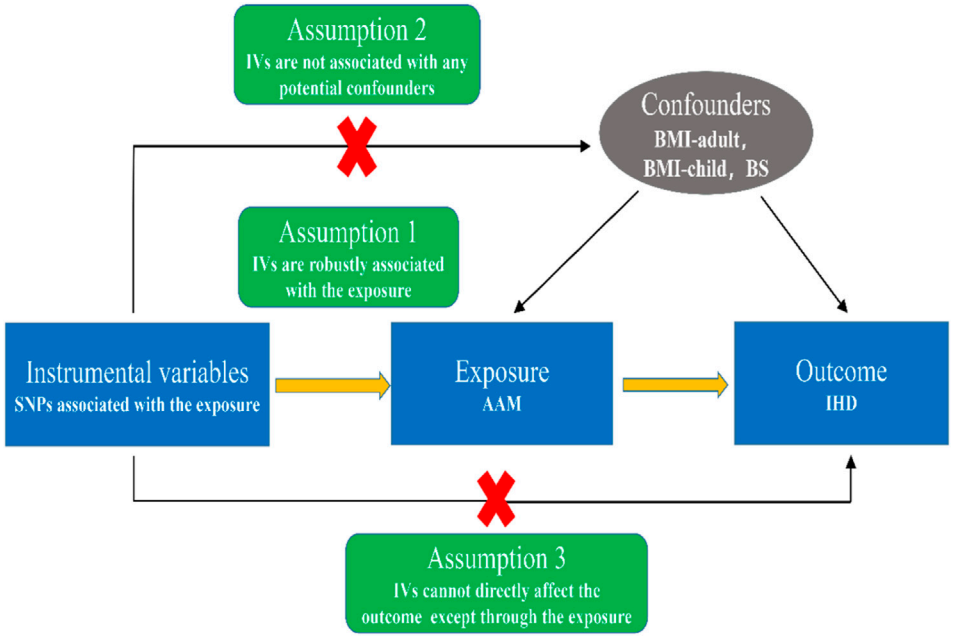

FIGURE 1
Schematic diagram showing the assumptions of Mendelian randomization analysis. Abbreviations: SNPs, single nucleotide polymorphisms; IVs, instrumental variables; AAM, age at menarche; IHD, ischemic heart disease; BMI, body mass index; BS, biological sex.

GWAS databases, we prioritized GWASs with the maximum number of SNPs, largest sample sizes, and the year closer to now. (Supplementary Table S1; databases were accessed in January 2022).

Selection of instrumental variables

The genetic IVs linked with exposure (AAM) were collected from the GWAS summary database with a sample size of 218,796 Europeans, and we underwent a variety of quality control procedures in our analysis to choose suitable instrumental SNPs that showed significant relationships with AAM. Firstly, we identified the SNPs based on the genome-wide significance threshold ($p < 5 \times 10^{-8}$), and then we removed highly correlated variants with $r^2 >$ 0.001 to avoid linkage disequilibrium (LD) in the range of 10000 KB (Savage et al., 2018; Park et al., 2021a). All of these SNPs have minor allele frequencies (MAF) over 0.01, indicating a small statistical bias caused by poor confidence (Chen et al., 2022). Secondly, the F-statistic was calculated for each IV using the following formula to determine its strength: $F = R^2\frac{N-2}{1-R^2}$ (Burgess et al., 2011), where R^2 is the proportion of the variability of the AAM explained by each IV and N the sample size of the GWAS for the SNP-AAM association. F-statistic greater than 10 is recommended to avoid using the weakly genetic tool (Burgess and Thompson, 2012). To calculate R^2 for each IV, we used $R^2 = 2 \times EAF \times (1 - EAF) \times$ beta$\hat{}$2/(2 × EAF × (1 − EAF) × beta$\hat{}$2 + 2 × EAF × (1 − EAF) × se × N × beta$\hat{}$2) to obtain it (Shim et al., 2015; Papadimitriou et al., 2020). Among this formula, EAF, beta, se, and N represent the effect allele frequency, effect size, standard error, and sample size, respectively. Eventually, after extracting SNPs associated with exposure from the outcome, some SNPs not having related information in the outcome were eliminated. These quality control steps were completed to ensure the independence of the IVs and exclude the influence of linkage disequilibrium (LD) on the result (Hemani et al., 2018b).

Statistics process

Four MR approaches were utilized as the primary statistical model, including fixed-effects inverse variance weighting (fe-IVW) (Burgess et al., 2013), multiplicative random-effects inverse variance weighting (mre-IVW) (Bowden et al., 2017), weighted median (WM) (Bowden et al., 2016b), and MR-Egger regression methods (Bowden et al., 2015), to evaluate the potential causal effect of AAM on IHD. Without taking into account the intercept term, IVW considered the weight as the reciprocal of the outcome variance (the square of standard error). Under the premise of IVW, we assume that IVs are not pleiotropic. Therefore, while utilizing the IVW approach, we must make sure that these IVs are not pleiotropic; otherwise, the findings were skewed (Bowden et al., 2015). Through its intercept

test, the MR-Egger analysis may spot horizontal pleiotropy (Bowden et al., 2016a). Even if IVs are pleiotropic, the MR-Egger method can provide a more conservative appraisal of causal effects, and the resulting statistics are not susceptible to exaggeration (Burgess and Thompson, 2017). The WM method allowed for up to 50% of the variables in the SNPs to be non-valid instrumental variables, so it can consistently evaluate the causal effects (Bowden et al., 2016b).

To assess potential violations of the model assumptions in the MR analysis, we conducted a sensitivity analysis. First, we performed a heterogeneity test using Cochran's Q statistic from IVW (along with the I^2 statistic), which may show that the heterogeneity is caused by pleiotropy or another factor (Greco et al., 2015). Cochran's Q statistic roughly follows a x^2 distribution with k-1 degrees of freedom (k is the number of genetic variations) (Liu et al., 2013). $I^2 = \frac{(Q-(k-1))}{Q} \times 100\%$, the results can be divided into four intervals (0–25%, 25–50%, 50–75%, and 75–100%), and each interval corresponds to low, moderate, large and extreme heterogeneity (Liu et al., 2013). It would be difficult to directly combine IVs if there is heterogeneity (P_Q<0.05). Second, since IVs do not follow MR's presumptions when they may directly affect the outcome without exposure, the amount of pleiotropy in the test findings will cause significant bias in MR. (Hemani et al., 2018a; Ong and MacGregor, 2019), thus we employed MR pleiotropy residual sum and outlier (MR-PRESSO) to locate outliers and check the level of pleiotropy (Ong and MacGregor, 2019). To ensure adequate verification, we still used the MR-Egger intercept to test the pleiotropy. To further identify whether a single SNP drove causality, we used leave-one-out (LOO) analysis to reassess causal effects by sequentially excluding one SNP. The result we want indicates that the causal effect we found is dependable and robust because there is no discernible variation (Noyce et al., 2017). Power calculations were performed with the online tool mRnd (https://shiny.cnsgenomics.com/mRnd/) based on the outcome sample size, proportion of cases, R^2 sum, and a type I error rate of 0.05 (Freeman et al., 2013).

For the treatment of confounders, we took two different approaches. In the first one, after searching for pleiotropic SNPs of confounders in PhenoScanner V2 (Kamat et al., 2019), we used the remaining IVs for MR analysis after excluding certain IVs that were significantly associated ($p < 5 \times 10^{-8}$) with potential confounders (i.e., risk factors for IHD). In the second one, multivariable MR (MVMR) is carried out for certain important confounders (e.g. BMI). According to a recent paper with a similar starting point to ours (Zheng et al., 2022), smoking habits, blood pressure, lipids, blood glucose and so on, play mediating roles in the relationship between AAM and myocardial infarction (MI), but they did not specifically examine the mediation of BMI, and it has been observed that AAM causes adult obesity with childhood BMI adding to the pleiotropy (Gill et al., 2018), so we selected BMI included both adult body mass index (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST006368/) and childhood body mass index (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90002409/) to conduct MVMR analysis for providing estimates separate from the effects of potential confounders. Additionally, though the GWAS data of the age at menarche is just finished in the females, other databases within our study contain both genders, we attempted to add biological sex (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90013474/) as a confounder in our MVMR analysis. The screening method for the GWAS summary databases of confounders was the same as for the database of exposure and outcome.

Our analyses are conducted by R software (version 4.1.3). Specially, we used the "TwoSampleMR" R package (version 0.5.6) and the "MRPRESSO" R package to perform MR analysis. The level of statistical significance was set at 0.05 in our study.

Result

Instrumental variables

After identifying at the genome-wide significance threshold ($p < 5 \times 10^{-8}$) and clumping ($r^2 \leq 0.001$), there are 125 SNPs remaining (Supplementary Table S2) with each SNP corresponding to an F-statistic >10 (range from 84.06 to 404.26). However, three SNPs (rs6548237, rs12607903, rs10063744) were not available in the summary data for IHD, and one SNP (rs56283944) extracted two kinds of information from the exposure data (Supplementary Table S3). After the harmonizing process, one SNP (rs56283944, β.IHD = 0.185, *P*.IHD = 0.218) was eliminated for incompatible alleles and 5 SNPs (rs1874984, rs4818008, rs61779780, rs6451675, rs9956387) were removed for being palindromic with intermediate allele frequencies. Finally, 117 SNPs were selected for subsequent analyses.

Causal effect of age at menarche on ischemic heart disease

There is a negative correlation between AAM and the risk of IHD, increase in genetically predicted AAM was associated with a lower risk of genetically predicted IHD, with an odds ratio (OR) of 0.80 [95% confidence interval (CI) 0.72–0.88, $p < 0.001$] in the fixed IVW MR analysis, mre-IVW or WM MR analysis has similar result (OR = 0.80,95% CI:0.70–0.90, $p < 0.001$; OR = 0.79, 95% CI: 0.66–0.93, $p = 0.006$). Meanwhile, we have high power to identify the effect of AAM on IHD (100% power to identify an OR of 0.80, minimum and maximum detectable OR with 80% power: 0.9656/1.0348).

Although the MR-Egger regression did not show a significant correlation between AAM and the risk of IHD ($p > 0.05$), the power of the test of the MR-Egger regression analysis was

considered to be relatively low compared to the other methods (Saade et al., 2011), so the above results suggested that later AAM is related with decreased risk of IHD (Supplementary Figures S1, S2; Supplementary Table S4). The funnel plot shows that when individual SNPs are used as IVs, the distribution of causality is roughly symmetrical (Supplementary Figure S3), suggesting that the results obtained by using 117 SNPs were sufficiently consistent and were not expected to be impacted by potential deviations.

Based on IVW, we conducted a heterogeneity analysis by Cochran's Q statistic. The Q-statistic is 160.5(p = 0.004, I^2 = 27.7%), indicating that there might be moderate heterogeneity. Then, to find and exclude the outliers, we chose to conduct the MR-PRESSO analysis. We found two outliers, rs10832013 (RSSobs = 0.002, p = 0.047) and rs9361178 (RSSobs = 0.003, p = 0.023), which might influence the causal effect of IVs. After their removal, the p-value increased from 0.004 to 0.089 for the MRPRESSO global test, which indicated that the pleiotropy was removed, and the P_Q was also increased to 0.090, suggesting that the heterogeneity has been removed. However, when we performed MR-Egger to further check the horizontal pleiotropy of our MR analysis, the result displayed that the intercept term was -0.006 (Supplementary Figure S4), which was not statistically significant with zero (p = 0.160 > 0.05), indicating that there was no horizontal pleiotropy between IVs. We used LOO analysis to examine if the causal effect changed in the presence or absence of the outlier, the LOO plots indicated that the causal estimation was robust in MR analysis (Supplementary Figure S5), and suggested that there was no single SNP that drove the causal relationship. Although there might be differences in the results of the above sensitivity tests, it generally suggested that individual SNP heterogeneity was largely balanced.

Using the online tool PhenoScanner V2, we found 50 SNPs from those 117 SNPs that could not be associated with any potential confounders (blood pressure, blood lipids, body mass index, etc.). Although there were no associations between the 50 SNPs and IHD (IVW, p = 0.09), when we selected and excluded one outlier (rs9361178) by performing MR-PRESSO analysis, the relationship between AAM and IHD re-appeared (IVW, OR = 0.80, 95% CI: 0.66—0.97, p = 0.022) (Supplementary Figure S6A), the intercept of MR-Egger analysis was not statistically significant with 0 (p = 0.129 > 0.05) (Supplementary Figure S6C), and the results from LOO method also showed that the association is robust (Supplementary Figure S6D). In the multivariable MR analysis, the relationship between AAM and IHD was attenuated after adjustment of BMI, including both adult and childhood BMI (OR = 0.86, 95% CI: 0.74 1.00, p = 0.045; OR = 0.83, 95% CI: 0.72 0.96, p = 0.01), even though it was remained significant after adjusting for biological sex (p < 0.001), indicating that sex differences barely affect the correlation between AAM with IHD and that BMI from childhood to adult mediates this association (Supplementary Table S5).

Discussion

The MR analyses employed in this study provided strong evidence for the association between genetically predicted AAM and genetically predicted risk of IHD, but the MVMR analysis showed that this causation could be mediated by confounders, such as adult and child BMI.

Many observational studies, as we know, have been designed to explore the association between AAM and IHD. In the cohort research enrolling 867 White women with college degrees, Cooper et al. (1999) found that the risk of IHD reduced with increasing age of menarche onset (age-adjusted RR 0.76 per year, 95% CI: 0.6–0.95). Higher age at menarche was associated with a mean 6.0% (95% CI 1.2–10.6) decreased mortality from IHD (p = 0.01) in the 12-year cohort research conducted in the United States, although the authors did not display or discuss the findings after controlling for significant confounders like BMI (Jacobsen et al., 2009). According to a study through meta-analysis and seven observational studies, the pooled RR for IHD mortality was 0.969 (95% CI: 0.947–0.993) for every 1-year rise in AAM, with considerable heterogeneity (I^2 = 44.9%, $P_{heterogeneity}$ = 0.092), but in studies with excellent quality, lengthy follow-up (>12 years), and body mass index adjustment, heterogeneity appeared to decline (Chen et al., 2019).

In line with our results about confounders, some findings indicated that the associations between early menarche and cardiovascular health might be mainly driven by its associations with BMI (Elks et al., 2013; Bubach et al., 2018; Zheng et al., 2021). A study using both observational methods and MR analysis has concluded that AAM had an influence on obesity and cardiometabolic traits, and suggested that preventive interventions should instead focus on reducing childhood obesity (Bell et al., 2018). Additional MR study also found that for each 4 kg/m^2 increase in BMI, observational estimates suggest a 26% increase in the odds of IHD, while causal estimates indicate a 52% increase (Nordestgaard et al., 2012). Therefore, further work is needed to detect the underlying mechanisms and the main targets of interventions.

There are several notable strengths of our MR analysis. Firstly, the outcome group is using the most recent broadly-defined IHD with a large sample size for the first time; Secondly, we used F-statistic to ensure that the IV used are strongly genetic tools, which was not previously used in a similar MR study (Cao and Cui, 2020). Thirdly, compared to the previous similar MR analysis (Cao and Cui, 2020; Zheng et al., 2022), we switched the outcome (CAD or MI) to the wider one (IHD), removed confounders by employing an external database (PhenoScanner V2) and utilized MVMR analysis to further investigate the mediating effects of BMI and sex. Finally, our work adds to the body of well-founded information supporting the need for greater investigation into the processes underpinning the early and late effects of AAM in IHD.

Also, our study has some limitations. Firstly, since the age at menarche estimations were provided voluntarily, they are subject to recall bias (Perry et al., 2014). Secondly, we were unable to obtain all sex-specific databases from the public datasets. Although the age at menarche is a sex-specific variable, we used summary-level genetic data from both sexes for IHD and confounders, assuming that the same genetic variations govern age at puberty in both men and women. Results from an LD score regression to determine the genome-wide genetic link between the age at menarche in girls and the age at voice broke in boys revealed a significant positive correlation, suggesting that comparable variations are responsible for controlling the timing of puberty in both sexes (Day et al., 2015a). We included biological sex (BS) as a confounder in our MVMR analysis, which showed a strong negative association between AAM and IHD even after adjusting for BS ($p < 0.001$) (Supplementary Table S4). Hence, the effect of sex is likely to be minimal and polygenic for the wide range of IHD, and further MR studies may yield more accurate and reliable results if gender-specific data are available to validate our results in women only. Thirdly, all participating participants were from Europe, so follow-up work is needed to explore whether the findings of this study can be generalized to other ethnic groups. For example, the index/proxy AAM SNPs at NUCKS1 and TMEM38B were most strongly associated in the Hispanic/Latina subsample (Fernandez-Rhodes et al., 2018), and there are significant inter-ethnic differences exist in allele frequencies of certain genes, which lead to differences in the age of menarche among different ethnic groups (Dvornyk and Waqar-ul-Haq, 2012). Finally, the MR approaches presupposed linearity of the modelled correlations, however, several observational investigations revealed a nonlinear relationship between AAM and cardiometabolic illnesses (Day et al., 2015b). Our MR estimations of the impact of AAM on the IHD may be seen as a causal effect that is representative of the entire population. To handle nonlinearity in MR, both parametric and nonparametric approaches have been proposed (Burgess et al., 2014; Silverwood et al., 2014).

Conclusion

Our MR studies show an association between increased levels of genetically predicted AAM and a decreased risk of genetically predicted IHD, and this relationship was discovered to be mediated by both adult and childhood BMI. These findings may assist public health policymakers and physicians in developing more scalable and effective strategies to reduce the incidence of IHD due to early AAM without enacting political and social reforms, given the growing number of adolescent females worldwide who are presently threatened by earlier AAM as a result of social-economic progress. Additional interventional studies should be carried out to determine whether restricting BMI in females with a history of earlier AAM reduces their risk of IHD, and to gain a better understanding of other relevant mediators as well as how they interact.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: MR-base database (https://gwas.mrcieu.ac.uk/), age at menarche (https://gwas.mrcieu.ac.uk/datasets/ukb-a-315/), ischemic heart disease (https://gwas.mrcieu.ac.uk/datasets/finn-b-I9_IHD/), PhenoScanner V2 (http://www.phenoscanner.medschl.cam.ac.uk/), adult body mass index (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST006368/), childhood body mass index (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90002409/), biological sex (https://gwas.mrcieu. ac.uk/datasets/ebi-a-GCST90013474/).

Ethics statement

Written informed consent was obtained from the individual(s), and minor(s), legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

LL, YW designed the study. JC, HC performed the datasets quality control and the data analysis. YZ, QZ interpreted the analysis results. JC and QL wrote the draft manuscript. JC, HC and YW revised the article. All the authors accepted the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.942861/full#supplementary-material

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Causal association between inflammatory bowel disease and IgA nephropathy: A bidirectional two-sample Mendelian randomization study

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Background: An association between inflammatory bowel disease (IBD) [which includes ulcerative colitis (UC) and Crohn's disease (CD)] and IgA nephropathy (IgAN) has been discovered in observational studies, but the causal relationship is still unknown. The aim of this study was to clarify the causal link between IBD (which includes UC and CD) and IgAN *via* a two-sample Mendelian randomization (MR) analysis.

Methods: Eligible single-nucleotide polymorphisms (SNPs) were selected as instrumental variables (IVs) for analyses and were obtained from the publicly available genome-wide association study (GWAS) summary statistics. Inverse-variance weighting (IVW), Mendelian randomization–Egger (MR-Egger) regression, the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test, and the weighted median were utilized to obtain the results. The MR-PRESSO test and MR-Egger regression were also performed to detect and correct horizontal pleiotropy. The Cochran's Q test and "leave-one-out" analysis were also conducted to assess the stability and reliability of the MR results.

Results: This study found that IBD, UC, and CD all had significant positive causal effects on IgAN risk (IBD: OR = 1.58, 95% CI 1.15–2.16, $p = 4.53 \times 10^{-3}$; UC: OR = 1.55, 95% CI 1.14–2.11, $p = 4.88 \times 10^{-3}$; CD: OR = 1.57, 95% CI 1.21–2.03, $p = 5.97 \times 10^{-4}$). No significant horizontal pleiotropic effect was found for the causal association between IBD, UC, CD, and the risk of IgAN. Cochran's Q test identified no evidence of heterogeneity for the IV estimates. The "leave-one-out" sensitivity analysis also revealed that the MR results were robust.

Abbreviations: CD, Crohn's disease; CI, confidence intervals; ESKD, end-stage kidney disease; Gd-IgA1, galactose-deficient IgA1; GWAS, genome-wide association study; IBD, inflammatory bowel disease; IgAN, immunoglobulin A nephropathy; IV, instrumental variable; IVW, inverse-variance weighting; MR, Mendelian randomization; MR-Egger, Mendelian randomization–Egger; SNP, single-nucleotide polymorphism; UC, ulcerative colitis.

Conclusion: The results of this two-sample MR analysis supported that IBD, UC, and CD were causally associated with the risk of IgAN, while there was no sufficient evidence for the causal effect of IgAN on IBD, UC, or CD. Our findings provide theoretical support and a new perspective for the diagnosis and treatment of these two diseases.

KEYWORDS

inflammatory bowel disease, ulcerative colitis, Crohn's disease, IgA nephropathy, Mendelian randomization, causality

1 Introduction

Immunoglobulin A nephropathy (IgAN) is the most common primary glomerulonephritis worldwide and can progress to chronic kidney disease and renal failure (Pattrapornpisut et al., 2021). In the early stage, it is characterized by asymptomatic hematuria with minimal proteinuria, which can be detected by screening programs. As the disease progresses, a proportion will develop hypertension, significant proteinuria, progressive glomerulonephritis, and even end-stage kidney disease (ESKD). Another phenotype presents recurrent macroscopic hematuria and is associated with a favorable prognosis in the short term, commonly in patients aged under 40 years. The incidence of IgAN varies from 0.54 to 10.5 per 100,000 population per year, with a high prevalence in young people aged between 20 and 40 years (Schena and Nistor, 2018). IgAN is considered an immune-mediated disease influenced by a combination of genetic and environmental factors. The typical pathological feature of IgAN is the deposition of polymeric and galactose-deficient IgA1 (Gd-IgA1) in the glomerular mesangium. Kiryluk et al. (2011) indicated that circulating levels of Gd-IgA1 were a heritable trait and were increased in patients with IgAN and their first-degree relatives. Genome-wide association study (GWAS) data sets also identified multiple susceptibility loci for IgAN and several risk alleles associated with intestinal epithelial barrier maintenance and mucosal immunity (Yu et al., 2011; Kiryluk et al., 2014; Li et al., 2020).

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is a chronic and idiopathic inflammatory disease of the gastrointestinal tract (Torres et al., 2017; Ungaro et al., 2017). Mucosal inflammation in UC mainly affects the rectum to the proximal segments of the colon, whereas in CD, all segments of the gastrointestinal tract can be affected, with the terminal ileum being the most common site. The common features of IBD are abdominal pain, chronic diarrhea, weight loss, and varying degrees of systemic symptoms (Flynn and Eisenstein, 2019). Some patients with IBD might exhibit extraintestinal manifestations, such as arthritis, spondyloarthropathy, episcleritis, uveitis, erythema nodosum, and primary sclerosing cholangitis. With societies in newly industrialized countries becoming more westernized, the incidence of IBD is increasing worldwide. It has been reported that the incidence of UC ranges from 0.15 to 57.9 per 100,000 person-years, while the incidence of CD ranges from 0.09 to 23.82 per 100,000 person-years (Ng et al., 2017). As a multifactorial disease, the pathogenic etiology of IBD is still incompletely understood. At present, it is generally believed that chronic intestinal inflammation is caused by an interaction of genetic susceptibility, excessive immune response, gut microbiota, and various environmental triggers. As shown by existing evidence, the first-degree relatives of patients with IBD have a five-fold increased risk of developing IBD (Flynn and Eisenstein, 2019). Hundreds of genetic loci have also been shown to be associated with IBD *via* GWAS (Liu et al., 2015). Moreover, a nationwide cohort study in Sweden has revealed an association between IgAN and IBD (Rehnberg et al., 2021).

Mendelian randomization (MR) can be used to explore the causal effect of exposure on outcomes by taking the differences in genotype as instrumental variables (IVs) (Emdin et al., 2017). Due to the natural random assortment of genetic variation during meiosis, Mendelian randomization has been proposed as a method analogous to classic randomized controlled trials (Emdin et al., 2017; Wu et al., 2020). Because genotypes appear before diseases and are largely independent of the postnatal lifestyle and environmental factors, MR is free from confounding factors and can avoid the biasing effect of reverse causality. As the large-scale GWAS reliably identifies genetic variants, MR has been successfully adopted for investigating causal links.

In this two-sample MR study, significant and independent single-nucleotide polymorphisms (SNPs) were chosen as IVs to clarify the causal association at the genetic level using the GWAS data. The aims of this study are to clarify if IBD, UC, and CD have the potential causal effect on IgAN and whether the reverse causal effect exists.

2 Material and methods

2.1 Data source

The genetic data of IBD, UC, CD, and IgAN originated from the large published GWAS. All participants were of European ancestry. The summary statistics of exposures included IBD (N =

31,665 cases and 33,977 controls), UC (N = 6,968 cases and 20,464 controls), and CD (N = 5,956 cases and 14,927 controls). Liu et al. (2015) elucidated the details of the data used for IBD, UC, and CD. For IgAN, the GWAS summary statistics included 977 subjects with IgAN and 4,980 disease-free controls (Feehally et al., 2010).

2.2 Selection of instrumental variables

In MR, there are three key assumptions that are required to be fulfilled: first, the SNPs must be associated with the exposure; second, the SNPs should be randomly distributed and independent of any confounders; and third, the SNPs affect the outcome only through the exposure (Sekula et al., 2016). When SNPs meet these three stringent assumptions, they are treated as IVs. In this study, we extracted SNPs that were significantly associated with exposures with genome-wide significance ($p < 5 \times 10^{-8}$) as IVs. Then, to ensure that all the instrumental SNPs had independent inheritance, we used a clumping algorithm with a cut-off of $r^2 = 0.1$. The SNPs in linkage disequilibrium ($r^2 > 0.1$) were excluded. Furthermore, the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test was used to identify and remove pleiotropic variants that might cause significant pleiotropy. The F-statistic was used to measure the strength of each instrument, and SNPs with an F value < 10 labeled "weak instruments" were excluded (Burgess et al., 2017). Additionally, R^2 was calculated to explain the proportion of the variation of IBD, UC, and CD by each IV (Burgess and Thompson, 2011). Finally, a calculation of statistical power was performed using mRnd (https://sb452.shinyapps.io/power/), developed by Brion et al. (2013).

2.3 Effect size estimate

The causal associations between exposures (IBD, UC, and CD) and outcomes (IgAN) were mainly estimated with inverse-variance weighting (IVW), Mendelian randomization–Egger (MR-Egger) regression, and the weighted median. The MR-PRESSO test was also used to estimate the causal relationships between IBD, UC, and CD and IgAN to acquire robust causal effects. The IVW method that combines the Wald ratios of each SNP provides the most precise estimates of the causal effect when each genetic variation satisfies the assumption of the IVs (Burgess et al., 2013). MR-Egger regression is used to perform a weighted linear regression of the outcome coefficients on the exposure coefficients. The intercept of MR-Egger regression can estimate the presence of directional pleiotropy. The statistical significance of MR-Egger regression might be inaccurate and strongly influenced by unrelated genetic variations (Bowden et al., 2016b). The weighted median provides valid MR estimates, with more than 50% weights coming from effective IVs in the analysis. Compared with the MR-Egger regression, the weighted median has distinct superiorities for its improved power of causal effect detection (Bowden et al., 2016a). However, when the percentage of horizontal pleiotropic variants is high (≥50%), the opposite conclusion is reached. Hence, MR-Egger regression provides a more robust estimate of potential violations of the MR assumptions (Verbanck et al., 2018).

2.4 Sensitivity analyses

To meet MR assumptions, we conducted multiple sensitivity analyses to assess heterogeneity and pleiotropy within the genetic instruments. Pleiotropy refers to a locus affecting multiple phenotypes, and a genetic variant is associated with more than one phenotype, which is a violation of MR assumption 3. MR-PRESSO and MR-Egger regression were performed to assess the potential pleiotropic effect of the SNPs used as IVs after removing the outliers. The "leave-one-out" sensitivity analysis is an algorithm to ensure the reliability of the association of the SNPs with exposure. The algorithm could reanalyze the results and draw a forest map by leaving out each SNP in turn.

For the heterogeneity analysis, Cochran's Q test and I^2 statistics were performed. The Cochran Q statistic was calculated as the weighted sum of the squared differences between each SNP effect and the summed effect across all SNPs. The I^2 statistic values indicated the expected relative bias of the MR-Egger causal estimate (Bowden et al., 2016b).

The results are presented as odds ratios (ORs) and 95% confidence intervals (CIs). A p-value less than 0.017 (0.05/3 adjusted with the Bonferroni method) was considered statistically significant. All statistical analyses were conducted using the R (version 4.1.2) software with the "Two-Sample MR" package. This analysis used the published study data or publicly available GWAS data and therefore did not require an ethics committee approval. In all original studies, ethical approvals were obtained.

3 Results

3.1 Selected single-nucleotide polymorphisms of the study

A total of 56, 33, and 55 significant ($p < 5 \times 10^{-8}$) and independent ($r^2 < 0.1$) SNPs were selected as IVs for IBD, UC, and CD, respectively (Table 1). The F-statistics for every instrument exposure association was much greater than 10 in this study, with average F values of 53.04, 64.64, and 62.58 for IBD, UC, and CD, respectively. The F-statistics ranged from 30 to 213 for IBD, 29 to 342 for UC, and 30 to 348 for CD, which indicated that the strength of the variables satisfied the relevance assumption of MR. Furthermore, the instrument bias was weak,

TABLE 1 Causal effects of IBD, UC, and CD on IgA nephropathy based on MR.

Exposure	MR method	IgA nephropathy				
		SNPs	OR	95% CI	*p*-value	Statistical power (%)
IBD	IVW	56	1.58	1.15–2.16	4.53×10^{-3}	100
IBD	MR-Egger	56	1.33	0.43–4.13	0.623	
IBD	Weighted median	56	1.52	0.98–2.35	0.063	
UC	IVW	33	1.55	1.14–2.11	4.88×10^{-3}	99
UC	MR-Egger	33	1.37	0.54–3.48	0.507	
UC	Weighted median	33	1.54	1.02–2.33	0.039	
CD	IVW	55	1.57	1.21–2.03	5.97×10^{-4}	100
CD	MR-Egger	55	0.90	0.42–1.96	0.794	
CD	Weighted median	55	1.42	1.03–1.97	0.034	

IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; MR, Mendelian randomization; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; IVW, inverse-variance weighting; MR-Egger, Mendelian randomization–Egger.

TABLE 2 Heterogeneity and pleiotropy analyses of IBD, UC, and CD with IgA nephropathy.

Exposure	MR-Egger				IVW		I^2
	Intercept	Pleiotropy *p*-value	Cochran's Q statistic	Heterogeneity *p*-value	Cochran's Q statistic	Heterogeneity *p*-value	
IBD	0.014	0.760	65.052	0.144	65.166	0.164	15.60%
UC	0.015	0.785	41.618	0.096	41.719	0.117	23.30%
CD	0.065	0.144	70.103	0.058	73.008	0.043	26.04%

IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; MR, Mendelian randomization; MR-Egger, Mendelian randomization–Egger; IVW, inverse-variance weighting.

which could not substantially influence the estimations of the causal effect. Detailed information about the genetic variants is listed in Supplementary Tables S1–S3.

3.2 Causal effect of inflammatory bowel disease on immunoglobulin A nephropathy

The MR analysis assessing the causal effect of IBD on IgAN is shown in Tables 1, 2, Figure 1, and Supplementary Figures S1–S4. The IVW method indicated that IBD had a positive causal effect on IgAN (OR = 1.58, 95% CI 1.15–2.16, $p = 4.53 \times 10^{-3}$) with 100% statistical power (Table 1; Figure 1). The MR-PRESSO analysis also supported the positive causal effect of IBD on IgAN ($p = 0.006$). Scatter plots, forest plots, and funnel plots of SNPs associated with IBD and IgAN are shown in Supplementary Figures S1–S3. Pleiotropy, heterogeneity, and sensitivity analyses were conducted for quality control. For pleiotropy, MR-Egger regression (intercept = 0.014, $p = 0.760$) and the MR-PRESSO global test ($p = 0.159$) revealed that horizontal pleiotropy was unlikely to bias the causality of IBD (Table 2). The Cochran Q-value and the I^2-value indicated that there was no heterogeneity between the IV estimates (MR-Egger Q = 65.052, $p = 0.144$; IVW Q = 65.166, $p = 0.164$; $I^2 = 15.60\%$) (Table 2). The "leave-one-out" sensitivity analysis evaluated the influence of outlying or pleiotropic SNPs by leaving out each SNP in turn. The results revealed that the causal link between IBD and IgAN was not significantly affected by any one SNP (Supplementary Figure S4).

3.3 Causal effect of ulcerative colitis on immunoglobulin A nephropathy

The MR results regarding the causal relationship between UC and IgAN with great statistical power are shown in Tables 1-2, Figure 1, and Supplementary Figures S4–S6. After removing the SNPs (rs434841) that might cause pleiotropy with the help of the outlier test in MR-PRESSO analyses, no horizontal pleiotropy was identified according to the MR-Egger regression (intercept = 0.015, $p = 0.785$) (Table 2). Then, the IVW method results

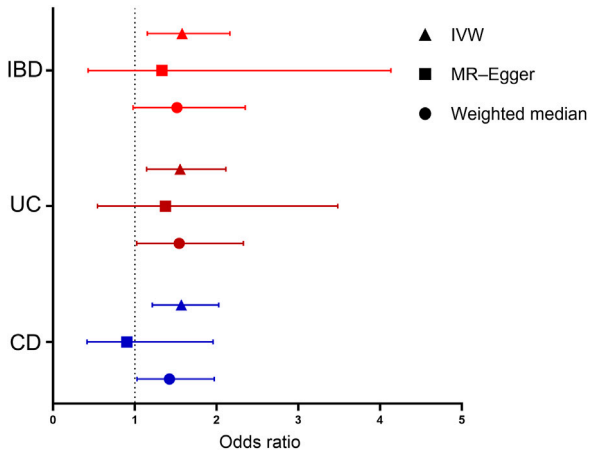

FIGURE 1
Forest plot for the causal effects of inflammatory bowel disease (IBD), ulcerative colitis (UC), and Crohn's disease (CD) on IgA nephropathy (IgAN). Each point represents the combined causal estimate using all SNPs together in a single instrument with the IVW (triangle), MR-Egger (square), and weighted median (circle) methods. Horizontal lines represent the 95% confidence interval (red: IBD, brown: UC, and blue: CD). (IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; SNP, single-nucleotide polymorphism; IVW, inverse-variance weighting; MR-Egger, Mendelian randomization–Egger.)

suggested that the per unit increase in the log-odds of having UC increase the risk of having IgAN was 0.55-fold (OR = 1.55, 95% CI 1.14–2.11, $p = 4.88 \times 10^{-3}$) (Table 1; Figure 1). The outlier-corrected MR-PRESSO analysis ($p = 0.008$) also found an analogous result (Table 1; Figure 1). Scatter plots, forest plots, and funnel plots of SNPs related to the causal effect of UC on IgAN are presented in Supplementary Figures S5–S7. The Cochran Q-value and the I^2-value indicated that there was no heterogeneity between the IV estimates (MR-Egger Q = 41.618, $p = 0.096$; IVW Q = 41.719, $p = 0.117$; $I^2 = 23.30\%$) (Table 2). Moreover, the causal effect of UC on IgAN did not significantly fluctuate with any single SNP in the "leave-one-out" sensitivity analysis (Supplementary Figure S8).

3.4 Causal effect of Crohn's disease on immunoglobulin A nephropathy

As shown in Table 1 and Figure 1, the IVW results found that CD was positively associated with IgAN (IVW: OR = 1.57, 95% CI 1.21–2.03, $p = 5.97 \times 10^{-4}$) with 100% statistical power. The positive causal relationship of CD with IgAN was also supported by the causal estimate in MR-PRESSO analysis ($p = 0.001$). Supplementary Figures S9–S11 show the scatter plots, forest plots, and funnel plots of SNPs for the causal effect of CD on IgAN. The MR-PRESSO analysis ($p = 0.057$) and MR-Egger regression analysis (intercept = 0.065, $p = 0.144$) suggested no evidence of horizontal pleiotropy. In addition, there was no heterogeneity (MR-Egger Q = 70.103, $p = 0.058$; IVW Q = 73.008, $p = 0.043$; $I^2 = 26.04\%$) across the IV estimates in this part (Table 2). Moreover, the results were also confirmed by the "leave-one-out" sensitivity test, suggesting that they were stable and reliable (Supplementary Figure S12).

3.5 Causal effects of immunoglobulin A nephropathy on inflammatory bowel disease, ulcerative colitis, and Crohn's disease

Furthermore, this study also conducted MR analyses of the causal effects of IgAN on IBD, UC, and CD. Only one SNP (rs3115573) was selected to access the causal effects of IgAN on IBD, UC, and CD, respectively. According to the results of the Wald ratio method, IgAN was positively associated with IBD (OR = 1.06, 95% CI = 1.02–1.11, $p = 0.008$), UC (OR = 1.08, 95% CI = 1.02–1.14, $p = 0.008$), and CD (OR = 1.07, 95% CI = 1.01–1.12, $p = 0.013$). However, there were not enough SNPs available for heterogeneity or pleiotropy analyses, leading to unreliable results. Hence, these causal effects of IgAN on IBD, UC, and CD should be treated cautiously, and more studies are required to determine their causal relationships.

4 Discussion

To the best of our knowledge, this study has been considered the first to illustrate the causal effect of IBD (which includes UC and CD) on IgAN using MR analysis and summary statistics from GWAS. As the data from this study revealed, IBD, UC, and CD causally increased the risk of IgAN in individuals of European descent. However, there was no sufficient evidence supporting the inverse causal relationship between these two diseases.

IgAN is the most common type of primary glomerulonephritis worldwide, with an increasing incidence. An analysis of cohorts from Europe and North America found that the 5- and 10-year risks of a 50% decrease in eGFR or ESKD in IgAN patients were 11.2% and 26.8%, respectively (Barbour et al., 2016). The pathological mechanism of IgAN is complex and is yet to be fully elucidated. The "multi hit hypothesis" has been widely accepted to explain IgAN pathogenesis. In this hypothesis, aberrant Gd-IgA1 increased and was targeted by anti-glycan IgG autoantibodies, causing the formation of immune complexes. These immune complexes cannot be adequately cleared from circulation and deposit in the mesangium, which might activate the complement system and trigger glomerular inflammation (Pattrapornpisut et al., 2021).

Previous case reports and clinicopathologic series have shown the relationship between IBD and IgAN and discussed the pathophysiologic links. In a case study from Korea, it was reported that a patient with rapidly progressive IgAN, concurrently presented with CD exacerbation, demonstrating a direct link between the aggravation of renal function and intestinal symptoms. Moreover, both renal and inflamed colonic tissues were positive for IL-17 and responded to immunosuppressive therapy. This might indicate a potential common pathogenesis of these two diseases (Choi et al., 2012). Ambruzs et al. (2014) concluded that the frequency of IgAN in IBD was significantly higher than that in other native kidney biopsy specimens during the same period [20 of 83 (24%) *versus* 2,734 of 33,630 (8%)]. A cross-sectional study from China evaluated 33 renal biopsy specimens from patients with IBD, showing that more than half (66%) of the pathological lesions were IgAN (Zhao et al., 2021). Elaziz and Fayed (2018) included 896 patients with IBD in a study and found that 218 (24.3%) of them had developed renal manifestations, of whom 35 patients were diagnosed with IgAN. Furthermore, Rehnberg et al. (2021) launched a nationwide cohort study and found that IgAN patients had an increased risk of IBD both before and after a confirmed IgAN diagnosis. It has been reported that patients with IBD show increased serum IgA levels and an increased incidence of abnormal hematuria, while patients with IgAN present significantly higher intestinal permeability, which is related to increased hematuria, proteinuria, and serum levels of IgA (Wang et al., 2004; Papista et al., 2011).

Although the exact mechanisms linking IBD and IgAN are not fully understood, the gut–kidney axis hypothesis might potentially support the pathophysiological relationship. It was assumed that dysregulation of the interplay among intestinal immunity, microbiota, and diet could lead to the production of Gd-IgA1, which plays an important role in IgAN (Joher et al., 2022). Together with mucus and antimicrobial peptides, IgA forms the first line of defense against environmental and microbial antigenic exposures occurring in the intestinal mucosa (He et al., 2020). A disruption of the epithelial barrier is more likely to lead to exposure to immunogenic alimentary antigens and increased IgA production. Kovács et al. (1996) found that significantly higher intestinal epithelial permeability was associated with increased serum levels of IgA against food antigens, such as soy protein. Chronic intestinal inflammation contributes to persistent and excessive activation of toll-like receptors, which might eventually result in the overproduction of IgA1/Gd-IgA1 (He et al., 2020). Then, in patients with IBD, the overstimulation of mucosal B cells significantly shifts immunoglobulin production from IgA2 to IgA1 (Brandtzaeg et al., 2006). Moreover, the number of N-acetylgalactosamines in the O-linked oligosaccharides of IgA significantly decreased in CD patients and strongly correlated with clinical activity (Inoue et al., 2012). Additionally, Wang et al. (2004) demonstrated that abnormally activated T cells initiated severe intestinal inflammation that could result in the dysregulation of serum IgA levels, indicating that T-cell-mediated intestinal mucosal immunity was critical in the pathogenesis of IgAN. Hence, a complex interplay of mucosal inflammation, chronic immune stimulation, microbiota, abnormal IgA glycosylation, and dysregulated IgA production and transport might potentially influence the pathogenesis of IBD and IgAN. Furthermore, genetic studies have found that GWAS of patients with IgAN identifies risk loci involved in intestinal mucosal integrity and the immune network (Li et al., 2020).

In addition, patients with IgAN comorbid with IBD have accelerated disease progression, while the remission of bowel disease could also improve renal function. Rehnberg et al. (2021) confirmed that patients with IgAN and IBD had an increased risk of progression to ESKD when compared with patients with IgAN without IBD. The identification of IBD might be useful for predicting the risk of ESKD in patients with IgAN. In 1984, a French physician first reported IgAN associated with IBD (one patient with UC and one patient with CD). With symptomatic treatment of the intestinal disease, not only did hematuria subside but also did mesangial hyperplasia and IgA deposition disappear (Hubert et al., 1984). Since then, a growing number of studies have suggested that intestinal disease therapy is associated with improvements in renal manifestations (Onime et al., 2006; Filiopoulos et al., 2010). Smerud et al. (2011) suggested that enteric budesonide targeted to the ileocecal region could represent a new treatment of IgAN for reducing urine albumin excretion.

There are several limitations in this present research. First, a bidirectional two-sample MR analysis to identify the causal relationships between IgAN and IBD was attempted, but sufficient numbers of SNPs were not available to determine the causal effect of IgAN on IBD, UC, and CD, which led to biased results. Second, this analysis was based on GWAS data sets, making it difficult to stratify the causal effect by race, age, sex, or other risk factors. Although the evaluation of other IgAN cohorts would be ideal to confirm our findings, no other IgAN cohorts could be found in the GWAS data sets. As a result, we performed direct experiments on the causal relationships between IgAN and IBD, UC, and CD in a follow-up study. Lastly, although the SNPs that might cause pleiotropy were identified and removed with the outlier test in the MR-PRESSO analysis, there was still no evidence for the causal effect of IBD, UC, and CD on IgAN from the MR-Egger analysis. Hence, more advanced MR methods have to be developed for the causal estimate after suppressing the interference of pleiotropy.

In conclusion, we found a positive causal relationship of IBD, UC, and CD with IgAN at the genetic level by using two-sample MR analysis. However, there was not sufficient evidence for the causal effect of IgAN on IBD, UC, and CD. The findings of this study should remind clinicians that regular monitoring of renal function in patients with IBD should not be ignored and that optimized treatment for IBD could improve prognosis in patients with IgAN. However, additional studies are required to investigate and elucidate the potential mechanistic links between IBD and IgAN.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of the International IBD Genetics Consortium. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MX and YR conceptualized and designed the study. JS, ZL, and YC analyzed the data in the study. MX drafted the manuscript. YL gave constructive suggestions when writing the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.1002928/full#supplementary-material

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Genetically determined serum bilirubin level and the risk of heart failure: A mendelian randomization study

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Background: The association between serum bilirubin level and heart failure (HF) was controversial in previous observational studies and the causal effects of bilirubin on HF have not been investigated. Here, we conducted a Mendelian randomization (MR) study to investigate the associations between genetically determined bilirubin level and HF.

Methods: Summary data on the association of single nucleotide polymorphisms (SNPs) with serum bilirubin levels were obtained from genome-wide association study (GWAS) for individuals of European descent and East Asian descent separately. Statistical data for gene-HF associations were extracted from three databases: the HERMES Consortium (47,309 cases and 930,014 controls), FinnGen study (30,098 cases and 229,612 controls) for European population and Biobank Japan (2,820 HF cases and 192,383 controls) for East Asian population. We applied a two-sample Mendelian randomization framework to investigate the causal association between serum bilirubin and HF.

Results: Findings from our MR analyses showed that genetically determined serum bilirubin levels were not causally associated with HF risk in either European or East Asian population (odds ratio [OR] = 1.01 and 95% confidence interval [CI] = .97–1.05 for HERMES Consortium; OR = 1.01 and 95% CI = .98–1.04 for FinnGen Study; OR = .82, 95% CI: .61–1.10 for Biobank Japan). These results remained unchanged using different Mendelian randomization methods and in sensitivity analyses.

Conclusion: Our study did not find any evidence to support a causal association between serum bilirubin and HF.

KEYWORDS

bilirubin, heart failure, mendelian randomization, causal effect, Antioxidants

Introduction

Heart failure (HF), as the end-stage of all sorts of cardiac disorders, has become a serious public health concern afflicting more than eight million of people in China and 64 million of patients worldwide (Guo et al., 2016; McDonagh et al., 2021). Due to the progress made in alleviating ischemic cardiomyopathy, growing prevalence in

comorbidities and aging, the prevalence of HF has been constantly climbing (Ponikowski et al., 2016; Castiglione et al., 2022). Overwhelmed oxidative stress has been recognized as the common pathological mechanism underlying the development of HF (Lambeth, 2004; Luo et al., 2021; Pigazzani et al., 2022). Increased oxidative stress could result in myocardial growth abnormity, extracellular matrix remodeling and cardiac energy metabolism disturbance (Senoner and Dichtl, 2019; Rotariu et al., 2022; Singh et al., 2022). Currently, strategies aiming at alleviating the oxidative stress have become heated topics in treating HF (Wang et al., 2021; Pigazzani et al., 2022).

Serum bilirubin is derived from heme catabolism within aging erythrocyte and is regarded as an important endogenous antioxidant (Schwertner and Vítek, 2008; Vítek, 2012; Kunutsor et al., 2017). It was reported that moderately elevated serum bilirubin concentration could exert cellular protective effects on oxidative stress-related cardiovascular disorders (Sedlak and Snyder, 2004; Gazzin et al., 2016). An inverse association was found between the circulating bilirubin concentration and the incidence of coronary heart disease, hypertension, and stroke (Kunutsor et al., 2015; Choi et al., 2020). In lieu of the unignorable involvement of oxidative stress in various cardiovascular diseases, bilirubin may play an important role in HF (Singh et al., 2020; Lamina and Kronenberg, 2021). Previous observational studies reported significant association between bilirubin level and HF risk (Adamson et al., 2022). However, the circulating bilirubin level undergoes constant fluctuation and is easily affected by venous pressure level and hepatic function, especially in HF patients (Moller and Bernardi, 2013). Therefore, whether bilirubin level simply mirrors the cardiogenic hepatic implications or has causal effects on HF has not been determined.

Mendelian randomization (MR) is a form of analysis that makes use of genetic variants as instrumental variables (IVs) to estimate effects of risk factors on outcomes (Davey Smith and Hemani, 2014; Davies et al., 2018). MR analysis takes advantage of the naturally occurring random allocation of alleles at conception and is recognized to overcome the limitations of residual confounding and reverse causation in conventional observational studies (Sekula et al., 2016). With the development of genome-wide association study (GWAS), MR becomes highly suited to investigate the etiological roles of conventional risk factors in HF. Here, we conduct a two-sample MR analysis to investigate the association between genetically bilirubin level and HF in both European and East Asian population.

Methods

Study design and data source

In current study, a 2-sample MR analysis was applied to assess the association between genetically determined serum bilirubin and HF in European population and East Asian population separately (Table 1). MR should abide by three principal assumptions (Burgess et al., 2015). First, the selected genetic instruments should be robustly associated with the exposure. Second, the association between these genetic instruments and the outcome should be exclusively through the exposure. Third, these genetic instruments should be independent of other potential cofounders (Figure 1).

Selection of genetic instruments

Candidate genetic instruments for total serum bilirubin levels were selected from United Kingdom (UK) Biobank and Korean Genome and Epidemiology Study (KoGES) for European and East Asian population respectively (Choi et al., 2020; Seyed Khoei et al., 2020). UK Biobank is a prospective cohort with genetic data collected on more than 500,000 individuals from 2006 to 2010 (Sinnott-Armstrong et al., 2021). Details concerning UK Biobank cohort can be obtained from online (https://www.ukbio bank. ac.uk). KoGES is a community-based cohort study recruited about 210,000 participants from 2005 to 2014 (Kim and Han, 2017). Among these participants, 25,406 were selected with both genetic data and serum bilirubin levels. Further detail information concerning KoGES can be found in previously published study (Kim and Han, 2017).

The single nucleotide polymorphisms (SNPs) reached genome-wide significance ($p < 5*10^{-8}$ for serum bilirubin level were selected as instrumental variables. Independent SNPs were identified by linkage disequilibrium clumped using an R^2 threshold <.001. Palindromic SNPs were further removed to ensure that the effects of the SNPs on the exposure corresponded to the same allele as their effects on HF.

The strength of associations between the genetic instruments and bilirubin levels was reflected by F-statistic, which was calculated by formula previously described (Burgess and Thompson, 2011). To minimize potential weak instrument bias, SNPs with F-statistic more than 10 were considered sufficient to perform MR analysis and were included in current study. To ensure that the genetic variants used to proxy for bilirubin levels are valid genetic proxies, we performed a MR analysis on stroke as a positive control (Supplementary Table S1).

TABLE 1 Detailed information for studies and datasets used for MR analysis.

Population	Contribution	Data source	Sample size	Number of SNPs
European	Exposure (Bilirubin)	United Kingdom Biobank	317,639	9,444,561
	Outcome (HF)	HERMES	977,323	7,773,021
		FinnGen	218,208	16,380,447
East Asian	Exposure (Bilirubin)	KoGES	25,406	~830,000
	Outcome (HF)	Biobank Japan	212,453	8,885,805

MR, mendelian randomization; SNP, single nucleotide polymorphism; CI, confidence interval; HF, heart failure.

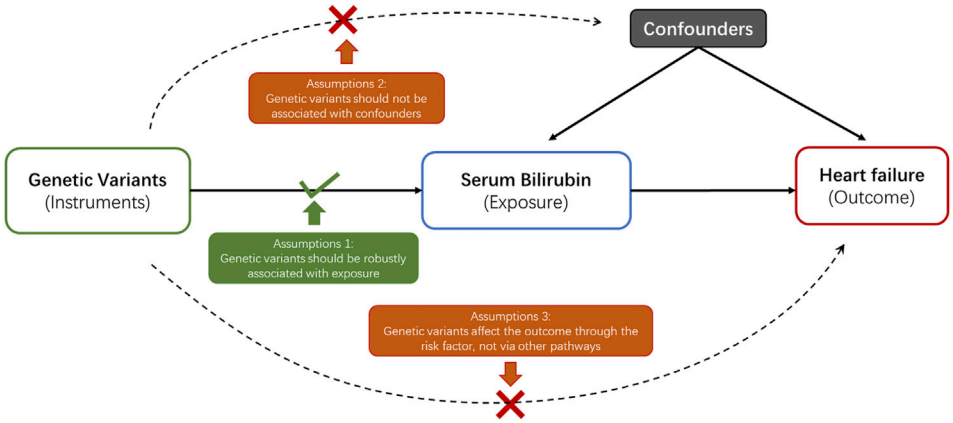

FIGURE 1
Three key assumptions underlying the Mendelian Randomization study design.

Outcome data sets

Summary statistics for outcome data extraction in European population were from the published GWAS performed by the Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES) Consortium and the FinnGen study. The HERMES consortium enrolled 47,309 cases and 930,014 controls from 26 cohorts (Shah et al., 2020). HF cases were extracted based on the clinical diagnostic criteria regardless cause of disease with no specific inclusion criteria for left ventricular (LV) ejection fraction. Details of participants selection can be found elsewhere (Shah et al., 2020).

The FinnGen study is an ongoing nationwide GWAS launched in 2017. This study included genetic data from Finnish biobanks and health record data from Finnish health registries. Details on it including participating biobanks, genotyping, and data analysis can be found on official website (https://www.finng en. fi/en). The latest data, which included 30,098 reported heart failure and 229,612 controls of the FinnGen study (Release 6) were used in current analysis.

For East Asian population, GWAS of BioBank Japan (BBJ) was used for outcome data extraction. BBJ collected genetic and clinical information from more than 201,800 participants from April 2003 to February 2008 and follow-up for 47 target diseases (Ishigaki et al., 2020). Detailed information can be acquired from official website (https://biobankjp.org/en/index.htmlcv). Outcome information of our current analysis were from 2,820 HF cases and 192,383 controls.

The summary genetic association data for HERMES consortium and FinnGen Study are presented in Supplementary Table S2 and Supplementary Table S3 respectively, and the data for BioBank Japan are reported in Supplementary Table S4. The sample overlap between data sources of exposures and outcomes is shown in Supplementary Table S5.

Statistical analysis

For our primary MR analysis, we applied the inverse-variance-weighted (IVW) regression analysis. Specially, the effect on an exposure on an outcome is estimated as the ratio (Wald estimate) of the SNP-outcome association and the SNP-exposure association (Helte et al., 2019). The IVW method assumes the absence of invalid genetic instruments. Cochran's Q statistic was used to test the heterogeneity among the estimated Wald ratios from different genetic variants. To examine if there was violation of MR assumptions due to directional pleiotropy, MR-Egger regression analysis was performed, with the intercept of MR-Egger to estimate the average pleiotropic effect across the genetic variants (Burgess and Thompson, 2017). The weighted median method was used to provide a reliable effect estimate if at least one-half of the instrumental variables were valid (Burgess et al., 2017). Furthermore, Mendelian Randomization Pleiotropy Residual Sum and Outlier (MR-PRESSO) was performed to detect and correct for horizontal pleiotropy through removing outliers (Verbanck et al., 2018). In addition, a leave-one-out sensitivity analysis was conducted to determine whether the results were affected by a single SNP.

All results are presented as odds ratios (ORs) and corresponding 95% confidence intervals (CIs) of the outcomes with per predicted increase in serum bilirubin level. Statistically significant is identified only a two-sided p-value was less than .05. All the analyses were carried out with the TwoSampleMR and MR-PRESSO packages with R version 4.0.2.

Results

Causal association of bilirubin with HF in european population

The results of association between genetically determined bilirubin level and the risk of HF in HERMES Consortium and FinnGen Study were presented in Table 2 and Figure 2. Genetic predisposition to elevated bilirubin level was not associated with HF risk in both cohort by performing IVW method (OR = 1.01 and 95% CI: .98–1.04 in HERMES Consortium; OR = 1.01, 95% CI:0.98–1.05 in FinnGen Study). Sensitivity analyses including weighted-median, MR-Egger, and MR-PRESSO did not find substantial change with IVW analysis (all $p > .05$).

TABLE 2 Association between genetically determined bilirubin and heart failure in European Population.

Methods	Or (95% CI)	IVs (SNPs)	*p*-value	Q-statistics	*P*h
For HERMES consortium					
IVW	1.01 (.97–1.05)	85	.569	173.24	<.001
Weighted median	1.01 (.98–1.04)	85	.399		
MR-Egger	1.02 (.97–1.06)	85	.472		
Intercept[a]	−.001 (.002)		.624		
MR-PRESSO	1.01 (.98–1.04)	83	.582		
For FinnGen study					
IVW	1.01 (.98–1.05)	83	.536	104.48	.048
Weighted median	1.01 (.98–1.05)	83	.509		
MR-Egger	1.01 (.98–1.05)	83	.458		
Intercept[a]	−.001 (.002)		.655		
MR-PRESSO	1.01 (.98–1.05)	83	.537		

IVW, inverse-variance weighted; OR: odds ratio; CI: confidence interval; *P*h, *P* for heterogeneity.
[a]Intercept is presented as β coefficients with SEs.

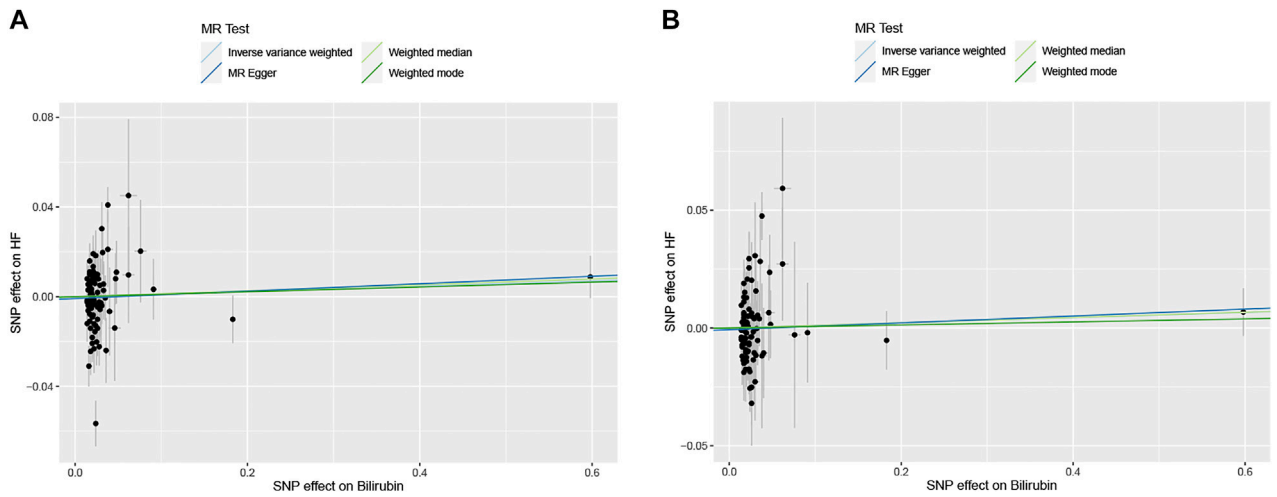

FIGURE 2
The scatter plots of serum bilirubin-associated single nucleotide polymorphisms (SNPs) effects on heart failure (HF) in HERMES Consortium **(A)** and FinnGen Study **(B)**. The 95% CI for the effect size on HF is shown as vertical lines, while the 95% CI for the effect size on bilirubin level is shown as horizontal lines. The slope of fitted lines represents the estimated Mendelian randomization (MR) effect per method.

TABLE 3 Association between genetically determined bilirubin and heart failure in East Asian Population.

Methods	Or (95% CI)	IVs (SNPs)	*p*-value	Q-statistics	*P*h
IVW	.82 (.61–1.10)	9	.190	3.84	.871
Weighted median	.89 (.61–1.31)	9	.559		
MR-Egger	.98 (.53–1.81)	9	.954		
Intercept[a]	−.010 (.010)		.380		
MR-PRESSO	.82 (.65–1.04)	9	.095		

[a]Intercept is presented as β coefficients with SEs.

The MR-Egger intercept estimate did not detect significant directional pleiotropy. MR-PRESSO analysis found two outliers (rs2519093 and rs3184504) and the results remained unchanged after excluding the outliers. Significant heterogeneity was observed among individual SNPs for both cohorts. The result of leave-one-out sensitivity analysis showed the association between bilirubin and HF was not substantially driven by any individual SNP (Supplementary Figure S1).

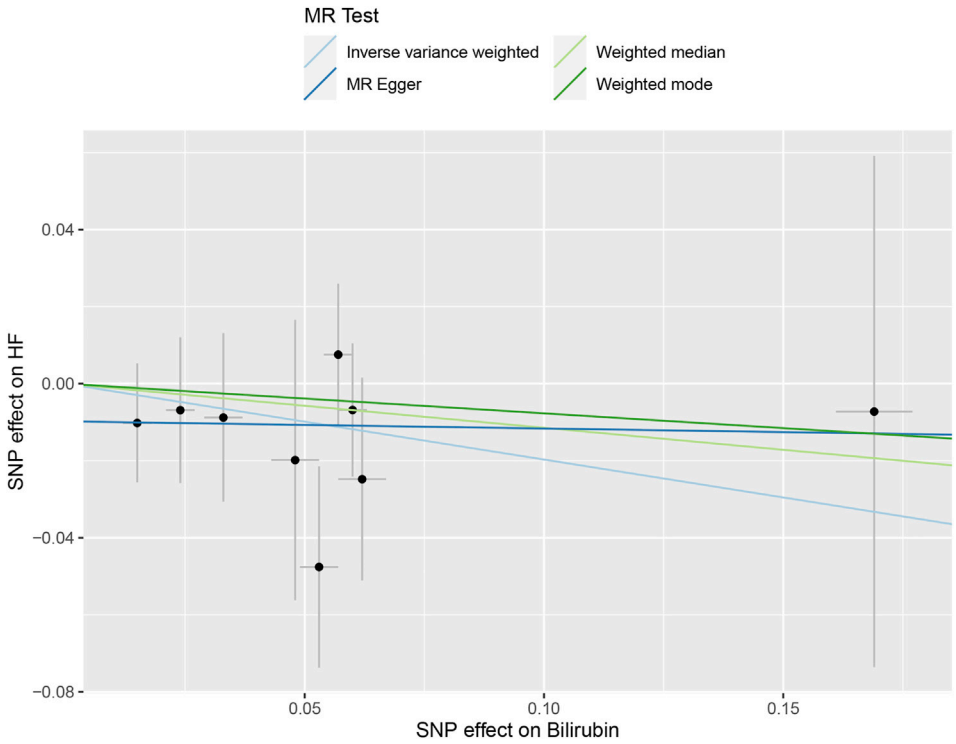

FIGURE 3
The scatter plot of serum bilirubin-associated single nucleotide polymorphisms (SNPs) effects on heart failure (HF) in the BioBank Japan cohort. The 95% CI for the effect size on HF is shown as vertical lines, while the 95% CI for the effect size on bilirubin level is shown as horizontal lines. The slope of fitted lines represents the estimated Mendelian randomization (MR) effect per method.

Causal relationship between bilirubin and HF in East Asian population

Table 3 and Figure 3 depicted the association between genetically determined serum bilirubin and HF risk in BioBank Japan Project. No significant causal effects of genetically determined bilirubin level on HF was observed in East Asian population by IVW method (OR = .82, 95% CI: .61–1.10). The results remained consistent in weighted-median, MR-Egger, and MR-PRESSO methods.

The intercept estimates of MR-Egger method indicated no significant directional pleiotropy and MR-PRESSO method also did not find any potential outliers. Likewise, no heterogeneity was observed among individual SNPs of bilirubin for HF. In addition, leaving out each SNP did not cause substantial change to original results (Supplementary Figure S2).

The associations between direct and indirect bilirubin level with HF were also tested. There was only one SNP for direct bilirubin and one for indirect bilirubin. No significant association was observed either for direct bilirubin or indirect bilirubin with HF (Supplementary Table S6).

Discussion

In the current study, we investigated the etiological role of bilirubin in HF by conducting a two-sample MR analysis. Our results did not find any significant association between genetically determined bilirubin level and HF risk in either European or East Asian population, indicating no causal effects of bilirubin on HF.

Bilirubin, as one kind of important antioxidants in plasma, was reported to exert about one-quarter of the total integral radical scavenging activities (Gopinathan et al., 1994). Evidence from conventional observational studies found that elevated bilirubin level was associated with decreased risk of several cardiovascular diseases. Wang et al., reported that serum bilirubin level was inversely correlated with hypertension risk (Wang and Bautista, 2015). Similarly, Lin et al., found that patients with gene prone to elevated bilirubin level showed a lower chance of coronary heart disease (Lin et al., 2006). Furthermore, Kimm et al., also found that low bilirubin level could serve as an independent risk factor of stroke (Kimm et al., 2009). However, the association between bilirubin and HF was much more complicated. In CHARM and PARADIGM-HF trial, total bilirubin levels were reported to elevate at baseline in 13% of all HF patients and 11.6% of patients with HF with reduced ejection fraction (HFrEF). Similarly, the EVREST and CHARM study both confirmed elevated bilirubin as a strong predictor of cardiovascular death in HF (Allen et al., 2009; Suzuki et al., 2020). However, some studies indicated that high bilirubin level was not associated with long-term cardiac remodeling and dysfunction as well as sudden cardiac death among HF patients (Wu et al., 2013). It should be also noted that serum bilirubin level is easily influenced by liver function and venous pressure, both of which were altered in most HF patients. Therefore, whether the change of serum bilirubin is a reflection of

their involvement in disease pathogenesis or an epiphenomenon of HF induced hepatic injury remains ambiguous.

In recent years, MR research that uses genetic variants inherited randomly from parents has been acknowledged as a reliable method to infer a causal relationship between exposures and outcomes. With the aid of large-scale GWAS datasets, two-sample MR analysis has been widely used to examine the etiological roles of risk factors that reported in conventional observational studies in the development of various cardiovascular diseases (Lamina and Kronenberg, 2021). Similar to our study, previous MR analysis reported non-significant causal associations between cystatin C, galectin-3 and C-reactive protein with HF (van der Laan et al., 2016; Li et al., 2021; Wang et al., 2022), suggesting that these factors may function as bystanders instead of contributors to HF. While most of the previous MR analyses were conducted in European population, our study conducted a separate analysis by utilizing datasets in East Asian to limit the confounding effect of genetic background, which may improve the generalizability of our results.

Previous MR studies have suggested the association between genetically-determined bilirubin level and life-long atherosclerotic cardiovascular diseases (ASCVD), including coronary heart diseases and stroke (Lin et al., 2006; Kunutsor et al., 2017; Lee et al., 2017; Choi et al., 2020). However, the non-significant causal effects of bilirubin were detected on several cardiovascular diseases as well as HF in our current study (Hou et al., 2021). The divergent pathophysiological mechanisms underlying ASCVD and HF may partially explain the discrepancy. Evidence from preclinical studies revealed that bilirubin mainly exerted cardiovascular protection through protecting the endothelium from oxidative damage, maintaining normal flow-mediated vasodilation and inhibiting cholesterol oxidation (Erdogan et al., 2006; Yoshino et al., 2011; Maruhashi et al., 2019), which are major pathophysiological changes related to vascular dysfunction and damage, while no clear evidence showed that bilirubin has direct effects on pathological cardiac hypertrophy, matrix reorganization and cardiomyocyte apoptosis, which are cardinal pathophysiological changes in HF.

Since HF is a condition with heterogenous pathogenesis, the finding from our MR analysis should be treated with caution when extrapolated into the specific subtype of HF. Currently, HF can be classified into three subtypes based on LVEF level, including HF with reduced ejection fraction (HFrEF), HF with mid-range ejection fraction (HFmrEF), and HF with preserved ejection fraction (HFpEF) according to the ESC guidelines, and previous observational studies identified divergent risk factor profiles for these HF subtypes (Dunlay et al., 2017; McDonagh et al., 2021). Moreover, recent genetic studies found pronounced differences in the genetic architectures of HFrEF and HFpEF (Bielecka-Dabrowa et al., 2016; Joseph et al., 2022). For example, a recent large GWAS by Joseph et al. found 13 loci associated with HFrEF, but only one associated with HFpEF at genome-wide significance despite a robust sample size, indicating that HFpEF is likely to be a collective syndrome representing several different pathophysiological entities (Joseph et al., 2022). Therefore, the causal factors identified from MR analysis by combing all kinds of HF as an outcome may not play etiological roles in HFpEF. Future studies to address the genetic variants of type-specified HF and their influences on disease pathogenesis are warranted.

It should also be noted that the causal association between bilirubin and HF may vary in different populations. Previous epidemiological data reported that the population in Asia had higher prevalence of HF compared to that in Europe, both in adults and in the elderly (Lam, 2015; McDonagh et al., 2021), which may be resulted from the discrepancies in lifestyle pattern, environmental contributor, as well as genetic architecture. Similarly, variance of bilirubin levels was observed in different ethnicities, which could be partially due to the heterogeneity of genetic determinants (Dai et al., 2013). Thus, across-ancestry analysis could provide more useful information on the association between bilirubin and HF. Considering this, our current MR analysis incorporated genetic information from the largest GWAS of different population, including European and East Asian, and acquired consistent null causal effects of bilirubin on HF, indicating no etiological role of bilirubin in HF. However, extrapolation of our results to other populations still needs caution and future large GWAS in non-European and non-Asian population are needed.

Though the causal association between bilirubin and HF was not supported from MR analysis, it would not devalue the role of bilirubin acting as a prognostic factor for HF, as it goes for the role of C-reactive protein in coronary heart disease and interleukin 1β in Alzheimer's disease. Bilirubin was reported as an extremely dynamic variable with the potential to change collaterally with disease status, and Adamson et al., proposed that bilirubin level might be more reflective on atrial pressure comparing with BNP (Adamson et al., 2022). Therefore, considering the spurious involvement of bilirubin in HF formation, using bilirubin level in predicting HF prognosis or risk stratification might be more instructive.

A major strength of the present study is the two-sample MR design to reduce bias from confounding factors and reverse causality. In addition, our MR analysis was conducted in both European and East Asian population, which can avoid the chance of results due to genetic divergency. However, some limitations must be noted. First, it is hard to avoid influence of potential directional pleiotropy completely, though no evidence of pleiotropic effect was observed in MR-Egger intercept test. However, SNPs associated with known confounding traits were excluded in our sensitivity analyses. Second, the sample overlapping between exposure dataset for bilirubin and outcome dataset for HF from HERMES consortium may cause some bias to our conclusion. However, our additional analysis using outcome data from FinnGen study to avoid sample overlapping did not find any significant change. Third, due to the unavailability of individual data, we could not conduct analyses grouped by sub-phenotypes and clinical courses of HF. Given the disparate pathophysiological underpinnings of different types of HF, further research in this regard is warranted. Fourth, generalizability of our findings to other ethnicities may be limited. Future large GWAS in other population are warranted.

Conclusion

Our MR analysis did not identify convincing evidence to support the causal relationship between serum bilirubin and HF in either European or East Asian population. Additional human and animal studies are needed to confirm our MR results.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

BG, YW and MY designed this study and conducted main analysis. BG, XS, RL, and YL composed the article. SL and JC reviewed and edited the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1067146/full#supplementary-material

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Causal association between tea intake and risk for gout: a Mendelian randomization study

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Background: Gout, an increasingly prevalent form of inflammatory arthritis, is caused by the accumulation of uric acid crystals in joints, resulting in severe pain, swelling and stiffness that adversely affect physical, mental and emotional wellbeing. The management of gout requires a combination of medication and lifestyle modifications. Recent studies suggest that tea intake may reduce the risk of developing gout; however, further research is needed to establish a causal relationship.

Methods: In this study, we employed a bidirectional two-sample Mendelian randomization (MR) approach, utilizing genome-wide association study (GWAS) summary statistics, to investigate the causal association between increased tea intake and gout. We meticulously selected instrumental variables (IVs) based on rigorous criteria and employed five different MR methods. Heterogeneity was assessed using Cochran's Q statistic, and pleiotropy was evaluated using the MR Egger intercept and MR-PRESSO tests. Weak IVs were identified using F values. The Phenoscanner database was consulted to exclude single nucleotide polymorphisms associated with confounding factors or outcomes.

Results: The study included one dataset related to tea intake (ukb-b-6066) and three datasets related to gout (ukb-b-12765, finn-b-M13_GOUT, and finn-b-GOUT_STRICT). Our forward MR analysis suggest a causal relationship between increased tea intake and reduced risk of gout in all three gout-related datasets [OR (95% CI): 0.9966 (0.9938–0.9993), p = 0.0167; 0.4842 (0.2683–0.8737), p-value = 0.0160; and 0.4554 (0.2155–0.9623), p = 0.0393, respectively]. The reveres MR showed increased risk of gout (ukb-b-12765) was significantly associated with low tea intake according to the IVW analysis [OR (95% CI): 0.0062 (0.0002–0.154), p = 0.0020]. However, this association was not observed in the Finn-b-M13_GOUT and Finn-b-GOUT_STRICT [OR (95% CI): 0.9992 (0.9909–1.0075), p = 0.8453 and OR (95% CI): 0.9996 (0.9932–1.0059), p = 0.8896, respectively]. No significant heterogeneity or potential pleiotropy was detected, and the possibility of weak IVs was also excluded.

Conclusion: Our MR analysis suggest a causal relationship between genetically predicted tea intake and a decreased risk of gout. These findings underscore the potential advantages of increasing tea intake for preventing gout. However, further research is needed to validate these results and elucidate the underlying mechanisms.

KEYWORDS

gout, tea, mendelian randomization, genome-wide association study, causal association

Introduction

Gout is a common inflammatory arthritis resulting from an imbalance between uric acid production and excretion, leading to uric acid crystal buildup in the joints (Dalbeth et al., 2021). In 2015 and 2016, the gout affected around 3.9% United States adults (2.7% in women and 5.2% in men) (Chen-Xu et al., 2019), with a higher prevalence among men, the elderly (Singh and Gaffo, 2020), and those with obesity, hypertension, and diabetes, as well as those with high consumption of alcohol, red meat, and sugary drinks (Dalbeth et al., 2021). The prevalence of gout has exhibited a persistent upward trend throughout the 20th century, in concurrence with alterations in the age composition of the populace and a concomitant surge in the incidence of the metabolic syndrome and its related pathologies (Kuo et al., 2015; Chen-Xu et al., 2019).

The condition can cause severe pain, swelling, and stiffness in the affected joints, with sharp and intense pain that can hinder even slight joint movement or touch (Taylor et al., 2015). While the first metatarsophalangeal joint is the most commonly affected joint, gout can also impact other joints such as the ankle, knee, wrist, and fingers (Bursill et al., 2019). During an attack, the joint becomes hot, red, and swollen, impeding simple activities, and is accompanied by fever and chills, leading to discomfort. Gout attacks, lasting days or weeks, can recur frequently if left untreated. Moreover, its chronic nature and unpredictable attacks can have a significant impact on mental and emotional health, leading to anxiety, depression, social isolation, and reduced quality of life (Zhu et al., 2012; Rai et al., 2017a). Gout can also interfere with daily activities such as work, exercise, and hobbies, affecting overall wellbeing (Zhu et al., 2012). Additionally, gout increases the risk of other severe health problems such as kidney stones, chronic kidney disease, and cardiovascular disease (Bevis et al., 2018), further exacerbating the suffering and increasing the burden on healthcare and society (Kuo et al., 2015).

Gout can cause severe pain and negatively impact an individual's physical, mental, and emotional wellbeing. To prevent and manage gout, a combination of lifestyle modifications and medication is necessary (Juraschek et al., 2016; Nielsen et al., 2017), including reducing the intake of purine-rich foods, sugary drinks, weight loss, and exercise, all of which can help reduce uric acid levels in the body (Richette et al., 2017; FitzGerald et al., 2020). Recent evidence suggests that tea may also be beneficial in reducing the risk of gout (Guo et al., 2023).

Tea is a rich source of flavonoids, which possess anti-inflammatory and antioxidant properties (Trevisanato and Kim, 2000), and may help reduce uric acid levels and inflammation in the body (Chen et al., 2022; Liao et al., 2022). Although the precise mechanism underlying the potential association between tea intake and gout is not fully understood, several hypotheses have been proposed. One theory is that tea flavonoids may inhibit xanthine oxidase, an enzyme involved in uric acid production (Jatuworapruk et al., 2014), while another is that tea flavonoids may improve uric acid excretion by the kidneys (Chen et al., 2015; Feng et al., 2022). However, since observational studies may have numerous confounding factors, large randomized controlled trials are required to determine the effects of tea intake on gout (Zhang et al., 2017).

Mendelian randomization (MR) is a method that utilizes genetic variations as instrumental variables to investigate the causal relationship between a modifiable risk factor and an outcome of interest (Davey Smith and Hemani, 2014). One of the key advantages of MR is its ability to overcome confounding and reverse causation, which are common issues in observational studies (Skrivankova et al., 2021). MR studies also offer several other benefits, including the ability to assess the effects of lifelong exposure to a risk factor and the ability to examine the effects of modifiable risk factors on multiple outcomes (Sanderson, 2021). To explore the relationship between increased tea intake and gout risk, we conducted a two-sample MR study.

Methods

Study design

This investigation employed a bidirectional two-sample MR approach utilizing genome-wide association study (GWAS) summary statistics to examine the association between increased tea intake and gout (Figure 1). In the forward MR analysis, tea intake was considered as the exposure and risk for gout as the outcome. Conversely, in reverse MR analysis, risk for gout was regarded as the exposure and tea intake as the outcome.

The objective of the study was to evaluate the causal association between tea intake and gout incidence as exposure and outcome, respectively, without necessitating ethical approval as publicly available data was utilized. The genetic variation was utilized to assess the causal effect of exposure on outcome. The fundamental requirements for genetic variation to fulfill the instrumental variable assumptions in this research are as follows: 1) The genetic variant must be associated with the exposure; 2) the genetic variant must not be associated with any confounders of the exposure-outcome association; and 3) the genetic variant should not influence the outcome, except possibly via its association with the exposure.

Data source

This investigation utilized genetic associations from independent GWAS datasets of the same ancestral population to circumvent confounding factors. The estimation method for assessing the degree of population overlap between the exposure and outcome datasets is based on the consideration of the maximum potential proportion of overlap. Specifically, this method assumes that all cases in the outcome dataset are derived from the exposure dataset (i.e., maximal overlap). An overlap rate below 1% is considered to have minimal impact on the outcome and can be negligibly ignored (Pierce and Burgess, 2013).

The forward MR

Exposure data source

In the forward MR tea intake was utilized as an exposure. The GWAS dataset for tea intake (ukb-b-6066) was obtained from

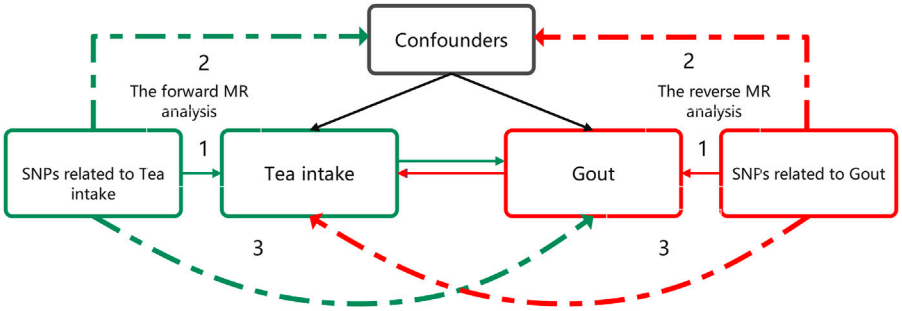

FIGURE 1
Bidirectional MR study design examining the causal effect of tea intake on gout. The green analysis investigates the causal effect of tea intake levels increasing as the exposure on gout risk as the outcome, while the red analysis examines the reverse association. The genetic variant should meet three criteria: 1) The genetic variant is associated with the exposure. 2) The genetic variant is not associated with any confounders of the exposure-outcome association. 3) The genetic variant does not affect outcome, except possibly through association with exposure. MR, Mendelian randomization.

United Kingdom Biobank which encompassed 9,851,867 SNPs and a sample size of 447,485 individuals of European descent. The category of it is continuous and the value type is integer, cups/day. The Mean and Std. dev of the dataset are 3.49631 and 2.84255. The data pertaining to tea intake were sourced from two specific questionnaires: "Screenshot from touchscreen questionnaire used to capture field 1488, Res ID 100318"and "Touchscreen questionnaire ordering, validation and dependencies, Res ID 113241". The questionnaires consisted of the following inquiry: "How many cups of tea do you drink each day? (Include black and green tea)" with a range of 0–99 cups. Participants were instructed to provide an average considering their intake over the past year.

Outcome data source

In the forward MR risk for gout was utilized as the outcome. To enhance the reliability of our findings, we incorporated three datasets related to risk for gout in this study. In our study, cases of Risk for gout identified from the United Kingdom Biobank (ukb-b-12765) and Finngen (finn-b-M13_GOUT and finn-b-GOUT_STRICT) datasets were classified using the International Classification of Disease code 10 (ICD-10) M10, which is defined as a condition characterized by painful joint swelling due to urate crystal deposition. The specific codes assigned to ukb-b-12765, finn-b-M13_GOUT, and finn-b-GOUT_STRICT were M10.99, M10, and M10.0, respectively. M10.99 represents gout without a specified site, indicating the presence of gout without providing specific information about the affected site. This code is used when the site of gout occurrence is not explicitly specified. M10 is the code category encompassing various codes related to gout and its associated disorders. Codes within the M10 category are used to describe gout manifestations in different sites. M10.0 specifically refers to primary gout, which signifies gout where the underlying cause is not specified or clearly associated with any evident underlying disease. It is termed "primary" as it lacks specific triggers or causes but is attributed to abnormal uric acid metabolism.

All three datasets consist of individuals of European ancestry. The ukb-b-12765 dataset comprised 1,042 cases and a control group of 461,968 individuals, with a total of 9,851,867 SNPs. The finn-b-M13_GOUT dataset consisted of 3,576 cases and a control group of 147,221 individuals, with a total of 16,380,152 SNPs. Similarly, the finn-b-GOUT_STRICT dataset included 1,699 cases and a control group of 216,239 individuals, with a total of 16,380,466 SNPs. A detailed summary of the datasets used in this study can be found in Table 1. Due to potential genetic differences between European populations and the Finnish population (Liu and Fu, 2015), the allele frequencies of significant SNPs in the three datasets were compared using Pearson chi-square test to account for the potential impact of genetic background differences on the results. The p-value <0.05 indicates a significant disparity in allele frequencies of the significant SNPs between the two groups. The R software was utilized for performing Pearson chi-square test.

The reverse MR

In reverse MR analysis, the exposed data were derived from the gout dataset above, while the outcome data were derived from the tea intake dataset above.

Selection of instrumental variables

In this study, instrumental variables (IVs) were carefully selected based on rigorous criteria. Specifically, SNPs were considered as valid IVs if they showed a significant genome-wide association with the exposure (at P < 5e-8). Additionally, SNPs with a minor allele frequency (MAF) in the outcome greater than 0.01 and a linkage disequilibrium (LD) r2 of less than 0.001 within a 10,000 kb distance were chosen as IVs. We excluded SNPs that were associated with confounders or outcomes according to the Phenoscanner database (http://www.phenoscanner.medschl.cam.ac.uk/).

The proportion of variance explained by each SNP was calculated, and the F-statistic was computed to evaluate the strength of the IVs (Burgess et al., 2011). A value less than 10 indicated that the selected genetic variants were weak instrumental variables, which may lead to biased results. Therefore, we exercised caution when interpreting the results. The F-statistic was calculated using the formula

TABLE 1 The detailed summary of GWAS datasets in MR analysis.

Trait	GWAS ID	Year	Cases	Controls	Simple size	SNPs	Population	Sex	Category
Tea intake	ukb-b-6066	2018	-	-	447,485	9,851,867	European	Males and Females	Continuous
Diagnoses - secondary ICD10: M10.99 Gout, unspecified (Site unspecified)	ukb-b-12765	2018	1,042	461,968	463,010	9,851,867	European	Males and Females	Binary
Gout	finn-b-M13_GOUT	2021	3,576	147,221	150,797	16,380,152	European	Males and Females	Binary
Gout, strict definition	finn-b-GOUT_STRICT	2021	1,699	216,239	217.938	16,380,466	European	Males and Females	Binary

GWAS, genome-wide association study; MR, mendelian randomization.

$F = \frac{(N-k-1)}{k} \times \frac{R^2}{(1-R^2)}$, where k represents the number of IVs employed in the analysis. The coefficient of determination (R2) was utilized as a metric to quantify the proportion of variance accounted for by individual SNPs. The R^2 was calculated using the formula $R^2 = \frac{2\times \beta^2 \times EAF \times (1-EAF)}{2\times \beta^2 \times EAF \times (1-EAF)+2\times SE^2 \times N \times EAF \times (1-EAF)}$ (Teslovich et al., 2010; Papadimitriou et al., 2020), where β represents the effect size of the SNP, EAF signifies the minor allele frequency, SE2 denotes the standard error of the effect size, and N denotes the total number of individuals in the GWAS study.

Sensitivity analyses

To evaluate heterogeneity, we used the mr_egger and IVW methods to calculate Cochran's Q statistic (Bowden et al., 2015; Burgess and Thompson, 2017). A *p*-value greater than 0.05 indicated no heterogeneity. "leave-one-out" analysis was conducted to assess the influence of individual SNPs on the causal effect of exposure on the outcome. In situations where heterogeneity existed, we employed a random-effects IVW method to estimate the causal association. Otherwise, we used a fixed-effects model. We tested the pleiotropy using the intercept *p*-value obtained from the MR Egger regression and global test *p*-value of MR-PRESSO, with $p > 0.05$ indicating no potential pleiotropy of IVs (Verbanck et al., 2018).

Two-sample MR analysis

We conducted five different MR methods to investigate the causal association between tea intake and risk for gout. The Inverse Variance Weighting (IVW) method was our primary MR analysis, while MR Egger, weighted median, simple mode, and weighted mode methods were used as supplementary analyses. We considered a causal effect of exposure on the outcome to be significant if the *p*-value was less than 0.05. Effect estimates were reported as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). We considered associations statistically significant if *p* values in IVW and MR-PRESSO methods were smaller than 0.05, and the results of MR-Egger, weighted median, Simple mode, and Weighted mode methods were consistent with IVW. R software (version 4.2.2) and R Package "TwoSampleMR" and "MRPRESSO" were used to conduct all statistical analyses in the MR analysis.

Results

In the forward MR analysis, the Pearson chi-square test revealed significant differences in allele frequencies of the IVs: rs10741694, rs10752269, rs10764990, rs2783129, rs4410790, rs4817505, and rs713598 between the ukb-b-12765 and finn-b-M13_GOUT datasets. Therefore, these IVs were deemed unsuitable for further analysis (Tea intake as exposure on Gout finn-b-M13_GOUT as outcome) and were excluded. No significant differences in allele frequencies of the IVs were observed between the ukb-b-12765 and finn-b-GOUT_STRICT datasets (Supplementary Table S1). In the reverse MR analysis, no Pearson chi-square test was conducted as there was no one-to-one SNP correspondence between the three IVs in the ukb-b-12765 dataset and the other two datasets.

As there is no data overlap between the finn-b-M13_GOUT and finn-b-GOUT_STRICT datasets from the Finnish database and the UKB database, any observed differences in results cannot be attributed to this factor. Therefore, we evaluated the extent of sample overlap between Gout (ukb-b-12765) and Tea intake (ukb-b-6066). The findings revealed an overlap rate of 0.23% based on the maximum potential proportion of overlap, which falls below 1% and can be considered negligible in terms of its impact on the outcomes.

The forward MR

MR results of increased tea intake on risk for gout, unspecified (site unspecified) (ukb-b-12765)

We identified a total of 41 SNPs through the MR analysis process, out of which 22 SNPs were excluded for not meeting the inclusion criteria. One SNP, namely, rs2783129, was removed due to its palindromic nature with intermediate allele frequencies. The remaining 18 SNPs were deemed eligible for the analysis, as they did not demonstrate any association with gout or related confounders in Phenoscanner (Supplementary Table S2). and exhibited F-values greater than 10, indicating the absence of weak instruments (Supplementary Table S3).

The MR results demonstrated that genetically predicted increased tea intake were associated with a decreased risk of

TABLE 2 MR analysis of exposure (Tea intake) with outcomes (Risk of Gout).

Exposure	Outcome	Method	Nsnp	b	SE	*p*-value	OR	Lower 95% CI.	Upper 95% CI
Tea intake (ukb-b-6066)	Gout, unspecified (ukb-b-12765)	MR Egger	18	−0.0038	0.0035	0.2975	0.9962	0.9894	1.0031
		Weighted median	18	−0.0035	0.0020	0.0760	0.9965	0.9927	1.0004
		Inverse variance weighted	18	−0.0034	0.0014	0.0167	0.9966	0.9938	0.9994
		Simple mode	18	−0.0034	0.0031	0.2904	0.9967	0.9907	1.0027
		Weighted mode	18	−0.0035	0.0021	0.1197	0.9965	0.9924	1.0007
	Gout (finn-b-M13_GOUT)	MR Egger	32	−2.0002	0.6308	0.0035	0.1353	0.0393	0.4659
		Weighted median	32	−1.3339	0.4016	0.0009	0.2635	0.1199	0.5788
		Inverse variance weighted	32	−0.7253	0.3012	0.0160	0.4842	0.2683	0.8737
		Simple mode	32	−1.1513	0.7648	0.1423	0.3162	0.0706	1.4158
		Weighted mode	32	−1.3863	0.4722	0.0062	0.2500	0.0991	0.6308
	Gout, strict definition (finn-b-GOUT_STRICT)	MR Egger	37	−2.4174	0.7782	0.0037	0.0892	0.0194	0.4098
		Weighted median	37	−1.2744	0.5154	0.0134	0.2796	0.1018	0.7678
		Inverse variance weighted	37	−0.7867	0.3818	0.0393	0.4554	0.2155	0.9623
		Simple mode	37	−1.0202	1.0143	0.3212	0.3605	0.0494	2.6321
		Weighted mode	37	−1.4144	0.5015	0.0078	0.2431	0.0910	0.6495

MR, mendelian randomization; SE, standard error; OR, odds ratio; CI, confidence intervals.

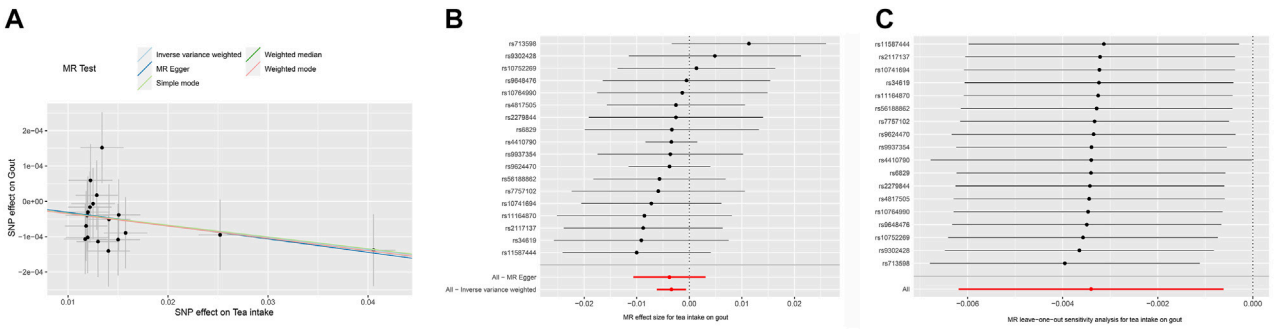

FIGURE 2
The MR analyse using Tea intake as exposure and gout (ukb-b-12765) risk as outcome. **(A)** Scatter plot in the MR analysis of Tea intake increasing on gout risk. The plot shows individual MR estimates, indicating that as the effect of individual SNPs on Tea intake increasing, so does the promotion of gout occurrence by individual SNPs. The *x*-axis indicates the SNP effect and standard error on Tea intake for each of the SNPs, while the *y*-axis shows the SNP effect and standard error on gout. The plot includes the regression line for mr_egger, weighted median, IVW, simple mode, and weighted mode. **(B)** Display of the forest plot for the single SNP analysis of Tea intake increasing on gout risk. The *x*-axis shows the MR effect size for Tea intake increasing on gout, while the *y*-axis illustrates the analysis for each of the SNPs. The dot and bar indicate the causal estimate and 95% CI of the association between Tea intake increasing and gout risk. **(C)** Presentation of the leave-one-out sensitivity analysis for the effect of Tea intake increasing SNPs on gout risk in the context of MR. The dot and bar indicate the estimate and 95% CI when a specific SNP is removed. IV, instrumental variant; IVW, inverse variance weighted; MR, Mendelian randomization; SE, standard error; SNP, single-nucleotide polymorphism.

gout, as evidenced by the results obtained from the IVW models (OR and 95% CI: 0.9966, 0.9938–0.9993; $p = 0.0167$) (Table 2, Figures 2A, B). However, other models failed to demonstrate a significant association, but the direction of their results is consistent with the IVW method. Figure 2A displays the scatter plot illustrating the correlation between genetically predicted increased tea intake

TABLE 3 The Heterogeneity and Pleiotropy test of MR analysis.

Exprosure	Outcome	Heterogeneity test		Pleiotropy test	
		IVW p	MR-egger p	MR-egger *p*	PRESSO *p*
Tea intake	Gout, unspecified (ukb-b-12765)	0.9633	0.9448	0.9084	0.9400
	Gout (finn-b-M13_GOUT)	0.2728	0.4580	0.0512	0.2560
	Gout, strict definition (finn-b-GOUT_STRICT)	0.2041	0.3828	0.1670	0.2300

IVW, inverse variance weighted; MR, Mendelian randomization.

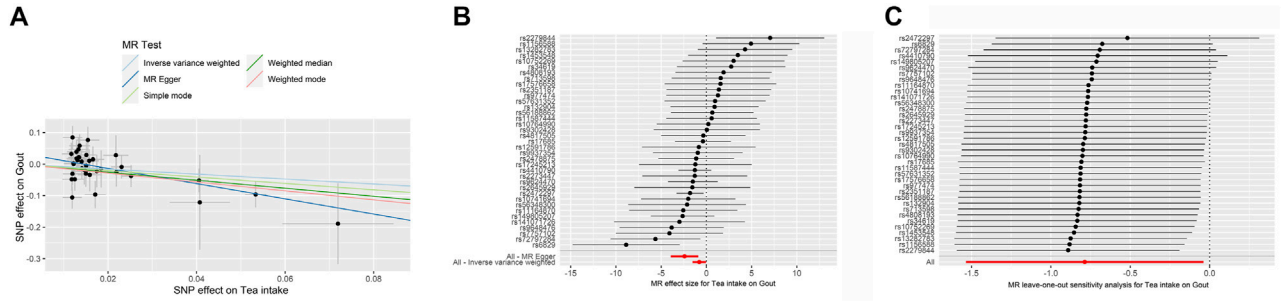

FIGURE 3
The MR analyse using Tea intake increasing as exposure and gout (finn-b-M13_GOUT) risk as outcome. **(A)** Scatter plot in the MR analysis of Tea intake increasing on gout risk. The plot shows individual MR estimates, indicating that as the effect of individual SNPs on Tea intake increasing, so does the promotion of gout occurrence by individual SNPs. The *x*-axis indicates the SNP effect and standard error on Tea intake for each of SNPs, while the *y*-axis shows the SNP effect and standard error on gout. The plot includes the regression line for mr_egger, weighted median, IVW, simple mode, and weighted mode. **(B)** Display of the forest plot for the single SNP analysis of Tea intake increasing on gout risk. The *x*-axis shows the MR effect size for Tea intake increasing on gout, while the *y*-axis illustrates the analysis for each of the SNPs. The dot and bar indicate the causal estimate and 95% CI of the association between Tea intake increasing and gout risk. **(C)** Presentation of the leave-one-out sensitivity analysis for the effect of Tea intake increasing SNPs on gout risk in the context of MR. The dot and bar indicate the estimate and 95% CI when a specific SNP is removed. IV, instrumental variant; IVW, inverse variance weighted; MR, Mendelian randomization; SE, standard error; SNP, single-nucleotide polymorphism.

(ukb-b-12765) and gout risk, with each fitted line slope representing the combined effect obtained through various MR analysis methods. The forest plot depicting the magnitude of the MR effect for increased tea intake (ukb-b-12765) on gout risk is illustrated in Figure 2B. MR Egger and IVW in Cochran's Q test did not reveal any significant heterogeneity among the 18 IVs in the gout GWAS, as evidenced by the absence of significant *p*-values (IVW $p = 0.9633$, MR Egger $p = 0.9448$) (Table 3). Furthermore, MR-Egger regression analyses and MR PRESSO results indicated no potential pleiotropy, further supporting the absence of confounding factors in these IVs (MR Egger $p = 0.9084$, MR-PRESSO $p = 0.94$) (Table 3). The leave-one-out analysis demonstrated that the causal association estimate was not influenced by the exclusion of any individual SNP (Figure 2C).

MR results of increased tea intake on risk for gout (finn-b-M13_GOUT)

We identified a total of 41 SNPs through the MR analysis process, out of which 2 SNPs were excluded for not meeting the inclusion criteria. One SNP, namely, rs2783129, was removed due to its palindromic nature with intermediate allele frequencies. Due to the significant disparities in allele frequencies of rs10741694, rs10752269, rs10764990, rs2783129, rs4410790, rs4817505, and rs713598 compared to the European population, they are deemed unsuitable for further analysis and have been excluded. The remaining 32 SNPs were deemed eligible for the analysis, as they did not demonstrate any association with gout or related confounders in Phenoscanner and exhibited F-values greater than 10, indicating the absence of weak instruments (Supplementary Table S3).

The MR results demonstrated that genetically predicted increased tea intake were associated with a decreased risk of gout, as evidenced by the results obtained from the IVW models (OR and 95% CI: 0.4842, 0.2683–0.8737; $p = 0.0160$) (Table 2, Figures 3A, B). Additionally, there was a significant correlation observed with MR Egger, weighted median, and weighted mode ($p = 0.0035$, 0.0009, and 0.0062, respectively). Although Simple mode did not exhibit a significant correlation, its results align with the direction of the IVW method (Table 2). Figure 3A displays the scatter plot illustrating the correlation between genetically predicted increased tea intake (finn-b-M13_GOUT) and gout risk, with each fitted line slope representing the combined effect obtained through various MR analysis methods. The forest plot depicting the magnitude of the MR effect for increased tea intake (finn-b-M13_GOUT) on gout risk is illustrated in Figure 3B. MR Egger and IVW in Cochran's Q test did not reveal any significant

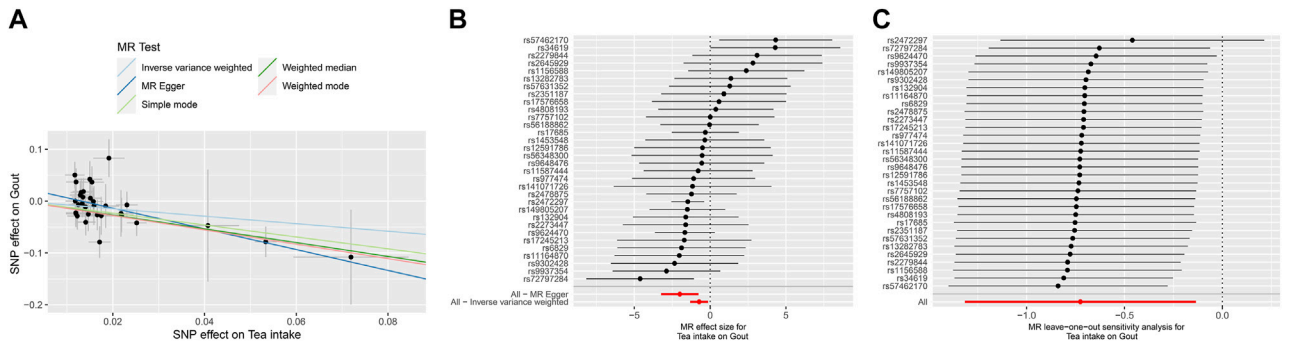

FIGURE 4
The MR analyse using Tea intake increasing as exposure and gout (finn-b-GOUT_STRICT) risk as outcome. **(A)** Scatter plot in the MR analysis of Tea intake increasing on gout risk. The plot shows individual MR estimates, indicating that as the effect of individual SNPs on Tea intake increasing, so does the promotion of gout occurrence by individual SNPs. The *x*-axis indicates the SNP effect and standard error on Tea intake for each of the SNPs, while the *y*-axis shows the SNP effect and standard error on gout. The plot includes the regression line for mr_egger, weighted median, IVW, simple mode, and weighted mode. **(B)** Display of the forest plot for the single SNP analysis of Tea intake increasing on gout risk. The *x*-axis shows the MR effect size for Tea intake increasing on gout, while the *y*-axis illustrates the analysis for each of the SNPs. The dot and bar indicate the causal estimate and 95% CI of the association between Tea intake increasing and gout risk. **(C)** Presentation of the leave-one-out sensitivity analysis for the effect of Tea intake increasing SNPs on gout risk in the context of MR. The dot and bar indicate the estimate and 95% CI when a specific SNP is removed. IV, instrumental variant; IVW, inverse variance weighted; MR, Mendelian randomization; SE, standard error; SNP, single-nucleotide polymorphism.

heterogeneity among the 32 IVs in the gout GWAS, as evidenced by the absence of significant *p*-values (IVW $p = 0.2725$, MR Egger $p = 0.4580$) (Table 3). Furthermore, MR-Egger regression analyses and MR PRESSO results indicated no potential pleiotropy, further supporting the absence of confounding factors in these IVs (MR Egger $p = 0.0512$, MR-PRESSO $p = 0.2560$) (Table 3). The leave-one-out analysis demonstrated that the causal association estimate was not influenced by the exclusion of any individual SNP (Figure 3C).

MR results of increased tea intake on risk for gout, strict definition (finn-b-GOUT_STRICT)

We identified a total of 41 SNPs through the MR analysis process, out of which 3 SNPs were excluded for not meeting the inclusion criteria. One SNP, namely, rs2783129, was removed due to its palindromic nature with intermediate allele frequencies. The remaining 37 SNPs were deemed eligible for the analysis, as they did not demonstrate any association with gout or related confounders in Phenoscanner and exhibited F-values greater than 10, indicating the absence of weak instruments (Supplementary Table S3).

The MR results demonstrated that genetically predicted increased tea intake were associated with a decreased risk of gout, as evidenced by the results obtained from the IVW models (OR and 95% CI: 0.4554, 0.2155–0.9623; $p = 0.0393$) (Table 2, Figures 4A, B). Additionally, there was a significant correlation observed with MR Egger, weighted median, and weighted mode ($p = 0.0037$, 0.0393, and 0.0078, respectively). Although Simple mode did not exhibit a significant correlation, its results align with the direction of the IVW method (Table 2). Figure 4A displays the scatter plot illustrating the correlation between genetically predicted increased tea intake (finn-b-GOUT_STRICT) and gout risk, with each fitted line slope representing the combined effect obtained through various MR analysis methods. The forest plot depicting the magnitude of the MR effect for increased tea intake (finn-b-GOUT_STRICT) on gout risk is illustrated in Figure 4B. MR Egger and IVW in Cochran's Q test did not reveal any significant heterogeneity among the 37 IVs in the gout GWAS, as evidenced by the absence of significant *p*-values (IVW $p = 0.2041$, MR Egger $p = 0.3828$) (Table 3). Furthermore, MR-Egger regression analyses and MR PRESSO results indicated no potential pleiotropy, further supporting the absence of confounding factors in these IVs (MR Egger $p = 0.1670$, MR-PRESSO $p = 0.23$) (Table 3). The leave-one-out analysis demonstrated that the causal association estimate was not influenced by the exclusion of any individual SNP (Figure 4C).

The reverse MR

In the subsequent analysis, we proceeded with a reverse MR investigation to explore the potential causal association between the risk of gout and tea intake levels. We initially harmonized the data with the Tea intake dataset, obtaining three gout-associated genetic variants from the gout, unspecified (ukb-b-12765) dataset and six genetic variants from the Finn-b-M13_GOUT and Finn-b-GOUT_STRICT datasets, which served as IVs (Supplementary Table S3). To ensure the validity of our analysis, we employed MR-PRESSO to identify and address any significant pleiotropy among the independent IVs, resulting in no noteworthy pleiotropic effects. Moreover, the MR-Egger intercept *p*-value indicated the absence of significant pleiotropy. Furthermore, both the MR Egger and IVW methods demonstrated no substantial heterogeneity among the independent IVs based on Cochran's Q statistics (Supplementary Table S4). Consequently, the IVW fixed method, serving as the primary statistical approach in our reverse MR study, was employed to evaluate the potential causal relationship between gout risk and tea intake.

The IVW analysis results provided evidence supporting a causal relationship between a increased risk of unspecified gout (ukb-b-12765) and low tea intake, with an OR of 0.0062 (95% CI:

0.0002–0.1547) and a *p*-value of 0.0020 (Supplementary Table S5; Supplementary Figures S1A, B). However, when examining the Finn-b-M13_GOUT and Finn-b-GOUT_STRICT databases, no significant causal relationship was observed for gout risk and tea intake, as indicated by ORs of 0.9992 (95% CI: 0.9909–1.0075; $p = 0.8453$) and 0.9996 (95% CI: 0.9932–1.0059; $p = 0.8896$), respectively (Supplementary Table S5; Supplementary Figures S2, S3A, B). Furthermore, in the leave-one-out sensitivity analysis, each SNP demonstrated heterogeneity when compared to the other SNPs, as shown in Supplementary Figure S1–S3C.

Discussion

The present study aimed to investigate the causal association between increased tea intake and the risk of gout, utilizing a bidirectional two-sample MR approach. To the best of our knowledge, this study is the first to employ MR methodology to explore the link between tea intake and gout risk. Our results suggest that a correlation between genetically predicted increased tea intake levels and an decreased susceptibility to risk of gout. The MR analysis employed in this study revealed non-significant heterogeneity and pleiotropy for the IVs, while the utilization of PhenoScanner facilitated the identification of SNPs that might be associated with pleiotropy, further confirming the validity of the IVs employed in this study. Notably, all the IVs demonstrated adequate strength, with F values exceeding 10. Furthermore, the utilization of three gout-associated datasets from two distinct biobanks in this investigation, all of which produced consistent outcomes, undoubtedly provides robust validation for our findings. Specifically, the inclusion of patients with a strict diagnosis of GOUT in the finn-b-GOUT_STRICT dataset adds further credibility to our findings that an increased intake of tea is associated with a reduced risk of gout.

We conducted additional reverse MR analyses. The reverse MR analysis revealed that an elevated risk of Gout, unspecified (ukb-b-12765) may lead to lower tea intake. This suggests a potential reverse causal effect between gout risk and tea intake, indicating a bidirectional relationship between gout risk and tea intake. On the one hand, a lifestyle characterized by increased tea intake may potentially reduce the risk of gout. Conversely, individuals with a higher susceptibility to gout may inherently intake less tea, thereby further increasing their risk of developing this condition. However, we did not observe the reverse causal effect in the other two Finnish datasets. There could be several possible reasons for this. Firstly, it is plausible that the reverse causal effect between gout, unspecified (ukb-b-12765) and tea intake may represent a false positive result due to chance or methodological limitations, which prompted us to incorporate multiple datasets related to gout for analysis. Furthermore, the other two datasets are derived from Finnish populations in Europe, which may exhibit dissimilarities compared to the general European population (Liu and Fu, 2015). Moreover, our findings indicate significant differences in allele frequencies of certain validated SNPs between the UKB dataset and the Finn dataset. Given the potential bottleneck encountered by the ancestors of the FIN populace approximately 10,000–20,000 years ago, it is plausible that the occurrence of the founder effect could be observed in these particular SNPs exhibiting substantial allele frequencies disparities (Liu and Fu, 2015). Additionally, these findings imply the existence of possible distinct biological pathways or mechanisms that are specific to gout susceptibility between non-Finnish Europeans and individuals of European descent with Finnish heritage.

Given that the genetic variations associated with Tea intake (ukb-b-6066) and unspecified gout (ukb-b-12765) were obtained from participants in the United Kingdom Biobank, it is important to consider potential bias in the MR estimates due to the likelihood of overlapping samples in the studie (Burgess et al., 2016). Therefore, we employed the maximum proportion overlap method to calculate the sample overlap rate between the two databases. The analysis revealed a sample overlap rate of 0.23%, which is below 1% and thus has minimal impact on the results (Pierce and Burgess, 2013). In light of a substantial overlap between the exposure and outcome data in the United Kingdom Biobank study, the F statistic was computed as a metric to assess the instrument's robustness and efficacy in the analyses (Burgess et al., 2016). The IVs' F-values in our analysis all exceeded 10, providing further reduction in potential bias.

Previous studies investigating the association between tea intake and the risk of gout have reported inconsistent results. Some studies have reported an inverse association (Feng et al., 2022; Guo et al., 2023), while others have found no significant association or even a positive association (Choi and Curhan, 2007; Jatuworapruk et al., 2014). However, the reliability of these studies is questionable due to limitations such as confounding, measurement error, and reverse causality (Sekula et al., 2016). Our findings suggest that increased tea intake may reduce the risk of gout. Specifically, our study found a modest but statistically significant inverse association between genetically predicted increases in tea intake and the risk of gout.

While the biological mechanisms underlying this relationship are not fully understood, it is hypothesized that tea's polyphenols, such as theaflavins, gallic acid, and other relevant compounds, which have antioxidant and anti-inflammatory properties (Chen et al., 2022; Liao et al., 2022), may play a protective role against gout by reducing uric acid production (Chen et al., 2015; Feng et al., 2022) or inhibiting inflammation (Jatuworapruk et al., 2014; Ohishi et al., 2016). Catechins possess antioxidative and anti-inflammatory properties, playing a positive role in regulating the disturbance of uric acid metabolism. In terms of uric acid metabolism regulation, catechins inhibit the activity of xanthine oxidase, reducing the excessive production of uric acid in the liver. They also modulate the expression of uric acid transporters, including URAT1, OAT1, OAT3, ABCG2, and GLUT9, to achieve a balance between uric acid secretion and reabsorption in the kidneys and intestines. Additionally, catechins effectively prevent inflammation caused by urate crystals. Dietary intake of catechins reduces the risk of hyperuricemia (Jatuworapruk et al., 2014; Wu et al., 2020). Moreover, catechins exhibit potent free radical scavenging activity, significantly reducing the secretion of IL-1β and IL-6 in C57BL/6 mice induced by MSU crystals, while inhibiting the activation of the NLRP3 inflammasome, thus effectively reducing the likelihood of developing gout in patients (Jhang et al., 2015). Theaflavins exert their anti-gout effects by downregulating the gene and protein expression of GLUT9 and URAT1 (Chen et al., 2023). On the other hand, gallic acid exhibits anti-inflammatory effects by inhibiting the activation of the NLRP3 inflammasome, suppressing subsequent caspase-1 activation, and reducing the secretion of IL-1β. Further investigations have demonstrated that gallic acid inhibits the generation of ROS, thereby restricting the activation and pyroptotic response of the

NLRP3 inflammasome dependent on the Nrf2 signaling pathway. These findings suggest the potential of gallic acid as a therapeutic agent for the treatment of gouty arthritis (Lin et al., 2020).

Uric acid is a natural metabolic byproduct, that is, typically eliminated from the body via renal excretion. However, excessive uric acid production or reduced elimination can lead to its accumulation in the body, resulting in gout attacks. The excess uric acid forms crystals that deposit in joints and surrounding tissues, triggering an inflammatory response and causing symptoms of gout (Clebak et al., 2020). Tea and its compounds have shown inhibitory effects on xanthine oxidase and adenosine deaminase enzyme activity in animal models (Zhu et al., 2017; 2018). These enzymes play a crucial role in purine metabolism, and their inhibition can effectively reduce the accumulation of serum uric acid, thereby exerting an anti-hyperuricemic effect. Additionally, tea compounds have been shown to modulate the function of uric acid transporters in the kidneys, facilitating renal excretion of uric acid (Chen et al., 2015; Zhu et al., 2018). Moreover, tea and its compounds, particularly polyphenols with potent antioxidant properties, have demonstrated potential in ameliorating uric acid-induced inflammation of endothelial cells, joints, and other tissues. Numerous studies conducted on cells, animals, and humans have provided substantial evidence supporting the notion that tea possesses inhibitory effects on gout inflammation (Bahorun et al., 2010; Jhang et al., 2016; Chen and Xu, 2018; Lee et al., 2019).

There is currently some epidemiological evidence controversy regarding the relationship between tea intake and gout. In a health and nutrition survey conducted among adult participants in the United States, a total of 14,314 individuals without a history of gout or the use of allopurinol or uridine drugs were followed up for 6 years. The study findings showed that increased tea intake did not lead to a decrease in serum uric acid levels in both American males and females (Choi and Curhan, 2007) However, a prospective study involving 9,400 adults (≥40 years old) in Korea revealed a significant association between tea intake and serum uric acid levels in both males and females, although it was unrelated to the risk of hyperuricemia (Bae et al., 2015).

However, a randomized crossover study conducted in Japan indicated that green tea catechins may increase the excretion of uric acid, xanthine, and hypoxanthine (Kawakami et al., 2021). Another cross-sectional study conducted in China suggested a significant association between high levels of tea intake and a reduced risk of hyperuricemia in males, whereas no relationship was found between tea intake and serum uric acid levels in females (Li et al., 2015). Additionally, a prospective cohort study found that consuming at least 2–3 cups of tea per day was associated with a reduced risk of kidney stones, with uric acid stones accounting for 7%–10% of cases (Chen et al., 2019; Barghouthy et al., 2021). Recently, a prospective cohort study based on the United Kingdom Biobank demonstrated a strong nonlinear association between tea intake and the risk of gout, with a significant risk reduction observed by consuming 6 cups of tea per day (Guo et al., 2023). These findings support the notion that promoting tea intake could be a simple and cost-effective approach to reducing the burden of gout, particularly in high-risk individuals (Feng et al., 2022; Guo et al., 2023). However, it is crucial to consider the potential adverse effects of excessive tea intake, such as insomnia, anxiety, and cardiovascular effects (Hayat et al., 2015). Thus, the intake of tea should be balanced and within recommended levels. Future studies should aim to replicate our findings in diverse populations and investigate the specific mechanisms underlying the association between tea intake and gout risk. In addition, it is crucial to determine the optimal dose and duration of tea intake required to reduce the risk of gout and to investigate the potential adverse effects of excessive tea intake.

Despite providing insights into the potential causal relationship between tea intake and gout risk, our study has several limitations that need to be acknowledged. Firstly, like all MR studies, our findings are based on MR assumptions, which may have limitations. Although we conducted multiple sensitivity analyses to assess the robustness of our results, the possibility of unmeasured confounding factors influencing our findings cannot be ruled out (Emdin et al., 2017; Richmond and Davey Smith, 2022). Secondly, the tea intake dataset of our study relied on self-reported data from GWAS studies, which may suffer from recall bias and may not reflect actual tea intake accurately. Meanwhile, tea can be categorized into different types, such as green tea, black tea, and white tea, based on variations in processing methods and procedures. Each type of tea may have distinct or varying concentrations of chemical constituents, resulting in different effects on gout (Chen et al., 2022). Our study solely utilized a dataset related to tea intake, specifically incorporating black and green tea, without analyzing other specific types of tea. Further investigation into the effects of other specific types of tea would contribute to a more comprehensive understanding of the relationship between tea intake and gout. Thirdly, the generalizability of our findings to other populations may be limited as our study was based on data from individuals of European ancestry. Furthermore, it is worth noting that two of the three datasets analyzed in our study, which pertain to gout, were derived from Finnish populations. European populations can be classified into Finnish and non-Finnish Europeans (Liu and Fu, 2015). Furthermore, our study findings also demonstrate differences in allele frequencies of certain significant SNPs between the FINN and UKB datasets, indicating that potential genetic background disparities may still influence our results. Morever, although our findings indicate a reduced risk of gout with increased tea intake across the three associated datasets, caution must be exercised in interpreting these results due to variances in inclusion criteria and potential heterogeneity in the specific mechanisms and sites of gout manifestation within these datasets. Consequently, a prudent approach is necessary when considering the implications of our analysis. While tea intake may be linked to a diminished risk of gout, the precise underlying mechanisms and the influence of tea intake on risk of gout could exhibit individual variations. Therefore, further research is warranted to deepen our understanding of the complex relationship between tea intake and risk of gout, as well as its differential impact on gout occurrence and pathogenesis across diverse populations. Lastly, our study suggests a potential causal relationship between tea intake and gout risk; however, it is important to note that other lifestyle factors such as diet and physical activity may also contribute to the development of gout. Thus, further research is necessary to explore the role of tea intake in the context of other lifestyle factors that may impact gout risk (Rai et al., 2017b; Hui et al., 2017).

Conclusion

In summary, our MR analysis suggest a causal association between genetically predicted increased tea intake and a reduced

risk of gout. Our study contributes to the existing literature on the potential health benefits of tea intake and underscores the value of MR analysis in elucidating causal relationships between exposures and outcomes. Nonetheless, our study has limitations that warrant consideration when interpreting the findings. Further investigation is necessary to validate our results and to investigate the underlying biological mechanisms.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

XL and JC: writing the article, analysis and interpretation. YF: conception and design, data mining, writing the article, critical revision of the article. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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Endoplasmic reticulum stress related genome-wide Mendelian randomization identifies therapeutic genes for ulcerative colitis and Crohn's disease

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Background: Endoplasmic reticulum stress (ERS) is an important pathophysiological mechanism in ulcerative colitis (UC) and Crohn's disease (CD). ERS-related genes may be influenced by genetic factors and intestinal inflammation. However, the role of ERS as a trigger or potential etiological factor for UC and CD is unclear, as the expression of ERS-related genes in UC and CD may be the cause or subsequent changes in intestinal inflammation. Here, we used a three-step summary data-based Mendelian randomization (SMR) approach integrating multi-omics data to identify putative causal effects of ERS-related genes in UC and CD.

Methods: Genome-wide association study (GWAS) summary data for UC (6,968 cases and 20,464 controls) and CD (5,956 cases and 14,927 controls) were extracted as outcome, and DNA methylation quantitative trait loci (mQTL, 1,980 participants) data and expression QTL data (eQTL, 31,684 participants) from the blood were obtained as exposure. The ERS-related genes were extracted from the GeneCards database, and then the GWAS summary data were integrated with the mQTL and eQTL data associated with ERS genes by SMR. Sensitivity analysis included two-sample MR analysis, power calculations, Bayesian co-localization analysis, and phenotype scanning were performed to evaluate the robustness of the results.

Results: A total of 1,193 ERS-related genes were obtained. The three-step SMR analysis showed that cg24011261 CpG site regulating *GPX1* expression was associated with a low risk of UC, whereas *GPX1* expression regulated by a combination of cg05055782, cg24011261, and cg05551922 CpG sites was associated with a low risk of CD. Sensitivity analysis further supports these findings.

Conclusion: This multi-omics integration study identifies a causal relationship between the role of ERS in UC and CD and suggests potential new therapeutic targets for clinical practice.

KEYWORDS

summary data-based Mendelian randomization, endoplasmic reticulum stress, ulcerative colitis, Crohn's disease, integrative omics analysis

1 Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are the two main subtypes of inflammatory bowel disease (IBD) (Hodson, 2016). The main differences between CD and UC are the varying areas of the digestive tract involved. CD is discontinuous and can involve any part of the digestive tract, but mainly the terminal ileum, whereas UC is continuous and involves only the large intestine and mainly the rectum (Abraham and Cho, 2009; Yamamoto and Ma, 2009; Khor et al., 2011). The main clinical symptoms of IBD are abdominal pain, diarrhea, hematochezia, and weight loss. IBD is becoming more prevalent worldwide, particularly in newly industrialized countries such as South Africa and America (Ng et al., 2017). It is worrying that the prevalence of IBD has exceeded 0.3% in many countries in Oceania, Europe and North America (Ng et al., 2017). Despite the growing number of treatment options, the treatment of IBD remains extremely challenging. Many factors are involved in the pathogenesis of IBD, but it is mainly attributed to immunological and genetic factors (Fugger et al., 2020). Unravelling the causality of these interactions may provide important insights into the pathogenesis of IBD and identify potential targets for treating the disease.

Endoplasmic reticulum (ER) is mainly responsible for facilitating protein folding and translocation to the appropriate destinations (Cubillos-Ruiz et al., 2017). The ER is the largest membrane network in the cell and its function is susceptible to extracellular stimuli and intracellular homeostasis (Cubillos-Ruiz et al., 2017). Various injuries, such as hypoxia and inflammation, disrupt ER function and lead to the accumulation of large amounts of unfolded and misfolded proteins, resulting in ER stress (ERS) (Cao, 2015). During this process, unfolded protein response (UPR) is triggered to attenuate ERS-induced cellular damage as well as to enhance cellular resistance to injury (Grootjans et al., 2016). However, UPR fails to compensate for cellular stress to restore ER homeostasis when ERS is persistent and severe, leading to activation of apoptotic signaling pathways and promoting apoptosis.

Disruption of the intestinal mucosal barrier function due to the interaction of multiple factors is a core event in the development and progression of IBD. Therefore, injury to intestinal epithelial cells (IECs), a primary component of the intestinal mucosal barrier, may play a critical role in this event. Research suggests that an imbalance of ERS in IECs promotes the progression of IBD (Grootjans et al., 2016). A study conducted by Xie et al. (2022) also observed a large amount of ERS in IECs of UC. A pilot proteomics study by Vieujean et al. found that ERS was associated with the progression of fibrous strictures in patients with CD (Vieujean et al., 2021). Although many studies have shown that the development of UC and CD is associated with ERS, no study has systematically and comprehensively investigated their potential causal relationship.

Mendelian randomization (MR) is an emerging method of epidemiological investigation that uses genetic variants as instrumental variables to assess whether there is a potential causal association between exposure and outcome (Burgess et al., 2019). In MR, random assignment of effect alleles largely avoids bias from unknown confounders such as lifestyle and environmental factors (Smith and Ebrahim, 2004). Genome-wide association studies (GWAS) investigate genetic associations between traits using single nucleotide variants (SNVs). Integrated multi-omics analysis is a novel approach in the post-GWAS era to investigate disease pathogenesis and identify key therapeutic targets (Do et al., 2017). Summary data-based MR (SMR) is an extension of the MR concept that integrates and analyses GWAS summary data with expression quantitative trait loci (eQTL) or DNA methylation QTL (mQTL), which were developed to prioritize causal variation mediated by gene expression or DNA methylation (DNAm) (Zhu et al., 2016; Wu et al., 2018). In this study, we used three-step SMR to assess the potential causal association between ERS-related genes and the two main subtypes of IBD. We then performed heterogeneity in the dependent instrument (HEIDI) test to investigate whether the causality was due to pleiotropy. Finally, multiple sensitivity analysis was performed to evaluate the robustness of the results.

2 Materials and methods

2.1 Study design

Figure 1 summarizes the workflow of this study. First, we integrated GWAS summary data of the two major subtypes of IBD with the blood eQTL and mQTL summary data for analysis using the SMR method. Second, we identified potential causal ERS-related genes by three-step SMR. Finally, the robustness of the primary findings was further analyzed using two-sample MR analysis, power calculations, Bayesian colocalization analysis, and phenotype scanning.

2.2 Data source

The ERS-related genes were searched in the GeneCards database (https://www.genecards.org/) using the keyword "endoplasmic reticulum stress." Only genes with relevance scores ≥7 were included in the analysis according to previous methods (Zhang et al., 2021; Guo and Liang, 2022; Xu et al., 2023). A total of 1,193 ERS-related genes were obtained (Supplementary Table S1). The blood cis-eQTL data and cis-mQTL data were obtained according to the methods previously reported in the literature (Jacobs et al., 2020; He et al., 2023). The cis-eQTL summary data for ERS-related genes were derived from the eQTLGen consortium, which collects genetic data on gene expression in 31,684 individuals from 37 datasets (Võsa et al., 2021). Every SNV-gene pair for which data was available in more than 1 cohort and with a SNV-gene distance of less than 1 MB was tested. Data were downloaded from the eQTLgen consortium website (https://www.eqtlgen.org/cis-eqtls.html). Notably, the downloaded data included non-significant SNVs. Therefore, a $P < 5E\text{-}8$ was set to extract SNVs of ERS-related genes from these data as instrumental variables. The cis-mQTL summary data were derived from a meta-analysis by Wu et al. (2018). Their study included two European cohorts: the Lothian Birth Cohorts ($n = 1{,}366$) and the Brisbane Systems Genetics Study ($n = 614$). These data are limited to DNAm probes with at least a cis-mQTL at $P < 5E\text{-}8$ and SNVs ≤2 MB from each DNAm probe. BESD format for these data was obtained

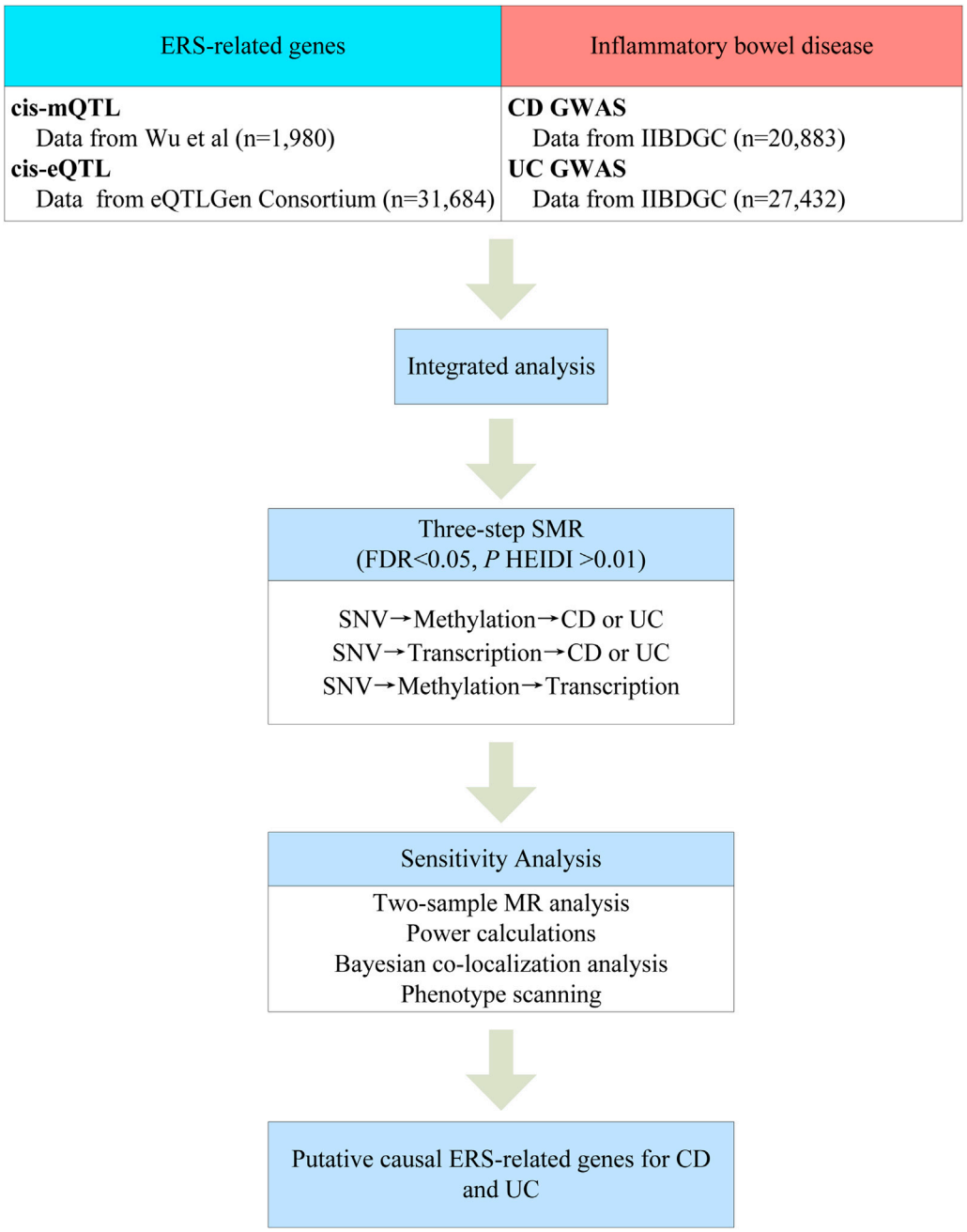

FIGURE 1
Workflow of the study.

from the SMR website (https://yanglab.westlake.edu.cn/software/smr/#mQTLsummarydata). The current study focused only on cis-mQTL summaries of ERS genes. GWAS summary data for UC were obtained from the international inflammatory bowel disease genetics consortium (IIBDGC), including 6,986 cases and 20,464 controls (Liu et al., 2015). Similarly, we also obtained CD GWAS summary data from the IIBDGC, which included 5,956 cases and 14,927 controls. The data used in this study were obtained from public databases that had already been ethically approved for the original study. Therefore, no additional ethical approval was required for this study.

2.3 Statistical analysis

The main analytical process of this study consisted of two stages: primary SMR analysis and sensitivity analysis. The SMR analysis provided the main results, while the sensitivity analysis tested the robustness of the results. We provide more detail on SMR analysis and sensitivity analysis in the Supplementary Methods.

Primary SMR analysis was conducted in three steps (1): SNVs as genetic instrumental variables, DNAm as exposure, and two major subtypes of IBD as outcome; (2) SNVs as genetic instrumental

variables, ERS-related gene expression as exposure, and two major subtypes of IBD as outcome; (3) SNVs as genetic instrumental variables, DNAm as exposure, and ERS-related gene expression as outcome. Only the significance results from the first and second steps were included in the third step of the analysis. The identification of the final putative causal relationships was defined as (1) false discovery rate (FDR) < 0.05 in all three-step SMR; (2) *P* HEIDI >0.01 in all three-step SMR; and (3) the eQTL and mQTL should correspond to the same gene symbol. The results of SMR were estimated using odds ratios (OR), which was calculated as follows: OR = exp (β_{SMR}), where exp represents the base of the natural logarithm.

Sensitivity analysis included two-sample MR analysis, power calculations, Bayesian co-localization analysis, phenotype scanning. Two-sample MR analysis was performed only on the causal results identified by SMR. We also performed power calculations using an online power calculator (https://sb452.shinyapps.io/power/) (Burgess, 2014). A power ≥80% was considered to have high statistical efficacy (Sanderson et al., 2022). Bayesian co-localization analysis was used to test whether GWAS summary data (including UC and CD) and QTL (including eQTL and mQTL) shared the same causal variable. The localization analysis was based on five hypotheses (H0, no association with GWAS or QTL within locus; H1, association with GWAS only; H2, association with QTL only; H3, association with GWAS and QTL but not co-localized; H4, co-localized GWAS and QTL) and the posterior probability of each hypothesis was assessed. Here, all SNVs within 100 kb upstream and downstream of the top SNV of the probe were extracted for co-localization analysis according to previous methods (Li et al., 2023). The posterior probability of H4 (PPH4) ≥ 0.5 was taken as evidence of co-localization of GWAS and QTL (Breen et al., 2019). Although PPH4 ≥ 0.8 is considered strong evidence for Bayesian co-localization, previous research has found that many loci with PPH4 ≥ 0.5 appear to be qualitatively consistent with the co-localization provided by PPH4 ≥ 0.8 (Dobbyn et al., 2018). We also performed phenotype scanning with PhenoScanner database (http://www.phenoscanner.medschl.cam.ac.uk/) to investigate the relationships of identified instrumental variants with other traits. The screening criteria for phenotype scanning were as follows: (1) the GWAS was derived from European ancestry; (2) the effect allele of the instrumental variable was consistent with our results; (3) the association of the instrumental variable with the trait met genome-wide significance (*P* < 5E-8); and (4) the absolute value of the effect size >0.01. In addition, we searched the GWASATLAS database (https://atlas.ctglab.nl/) for associations of putative causal ERS-related genes with traits.

The three-step SMR analysis and HEIDI test were performed using version 1.3.1 of the SMR software (https://yanglab.westlake.edu.cn/software/smr/#Download). Two-sample MR analysis were conducted using the "TwoSampleMR (version 0.5.6)" package of the R software (version 4.2.2). The co-localization analysis was performed using the "coloc (version 5.2.2)" R package.

For the three-step SMR analysis, the Benjamini–Hochberg method was used to adjust for false positives due to multiple testing. FDR <0.05 was defined as significant for SMR analysis. The two-sample MR analysis is a sensitivity analysis based on the SMR results. For two-sample MR analysis, *P* < 0.05 was defined as significant.

3 Results

3.1 SMR analysis for mQTL and GWAS data

The SMR test was used to extract SNVs and DNAm sites for 1,193 ERS-related genes from the blood mQTL data. A total of 4,100 CpG sites of ERS-related genes were obtained, which were associated with 1,321,603 SNVs. We then integrated mQTL for ERS-related genes with GWAS summary data for UC and CD, respectively. In concrete, the integration of mQTL with the UC GWAS summary data identified 34 DNAm probes corresponding to 17 ERS-related genes (SMR FDR <0.05 and *P* HEIDI >0.01). Meanwhile, integration of mQTL with CD GWAS summary data identified 75 DNAm probes corresponding to 37 ERS-related genes. Estimates of causality are expressed as β, and odds ratios of 1 standard deviation (SD) of ERS-associated gene expression level were obtained by calculating the expectation of β, and these results are presented in Supplementary Table S2.

Our results demonstrated that different instrumental variants regulating the same ERS-associated gene have different effects on DNAm levels. For example, one SD increase of *RNF186* methylation by rs3806308 was associated with 34.6% higher risk of UC (OR: 1.346, 95% CI: 1.240–1.461, SMR FDR = 3.97E-10), and conversely, one SD increase of *RNF186* methylation by rs12128452 was associated with 30.5% lower risk of UC (OR: 0.695, 95% CI: 0.626–0.772, SMR FDR = 2.51E-9). However, the effect of instrumental variants of *GPX1* on DNAm levels was synergistic. One SD increase of *GPX1* methylation by rs34293138 and rs111903592 was associated with 20.3% and 48.3% lower risk of UC, respectively. In summary, our analysis of the integration of UC GWAS and mQTL revealed the presence of 31 independent loci regulating methylation levels at 34 different CpG sites within 17 genes associated with ERS. Similarly, our examination of the CD GWAS summary data identified 63 independent loci that regulate methylation levels at 75 different CpG sites within 37 genes associated with ERS.

3.2 SMR analysis for eQTL and GWAS data

A total of 985 eQTL probes of ERS-related genes were obtained, which were associated with 498,776 SNVs. Integration of the eQTL with the UC GWAS and CD GWAS resulted in 11 and 16 ERS-related genes (Supplementary Table S3), respectively. In UC, it was found that two genes (*GPX1* and *CLN3*) exhibited a protective effect, while nine genes (*MAPKAPK2*, *IL24*, *BCL2L11*, *COL7A1*, *NFKB1*, *KDELR2*, *CTSB*, *JAK2*, and *STAT3*) were identified as risk factors. In CD, six genes (*SCARNA5*, *GPX1*, *ATF6B*, *BAD*, *RNFT1*, and *TMED1*) were associated with a low risk of disease and ten genes (*HSPA6*, *COL7A1*, *RFT1*, *ERAP2*, *JAK2*, *TMEM258*, *CCDC88B*, *TMED10*, *ATP2A1*, and *STAT3*) were associated with a high risk of disease.

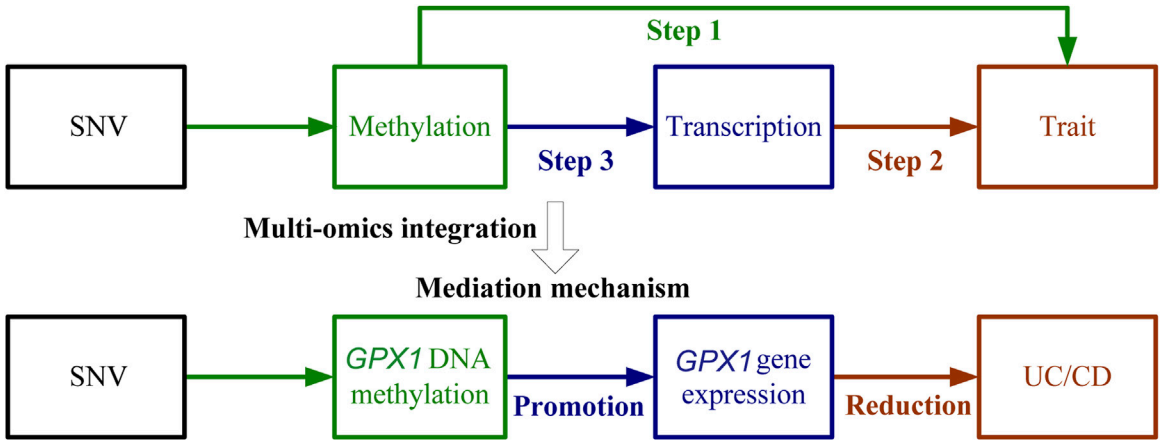

FIGURE 2
Schematic of integrative analysis of multi-omics data. The effects of DNAm on trait, DNAm on gene expression, and gene expression on trait are evaluated using the SMR and HEIDI method and integrated to identify potential mediation mechanisms in which a SNV exerts an effect on the trait by altering the DNAm level, which then regulates the expression levels of a functional gene. The detailed steps were (1) Use SMR and HEIDI to determine associations between DNAm and UC/CD; (2) Use SMR and HEIDI to determine associations between gene expression and UC/CD; (3) Use SMR and HEIDI to determine associations between DNAm and gene expression.

3.3 SMR analysis for mQTL and eQTL data

It is well known that gene methylation affects gene expression. Therefore, we proceeded to explore the possible link between DNAm and gene expression by using DNAm as the exposure and transcripts as the outcome. After screening the results by SMR FDR <0.05 and *P* HEID >0.05, we obtained the regulatory relationships for the expression of five ERS-related genes regulated by nine DNA methylation CpG sites (Supplementary Table S4). For *GPX1*, there were four significantly associated methylation sites (cg07274523, cg05055782, cg24011261, and cg05551922), all of which were positively correlated with *GPX1* expression. For *OS9*, there were two significant methylation sites (cg18799399 and cg15848620) that exerted different regulatory effects on *OS9* expression. For *ATF6B*, *TMED10*, and *TMED1*, they are regulated by cg03317682, cg12149606, and cg01875838, respectively.

3.4 Multi-omics data integration

Three omics results were integrated to identify causal effects of ERS-related genes in UC and CD. Based on the method for integrated analysis of multi-omics data proposed by Wu et al. (2018), we constructed a hypothetical model of the mediation mechanism: a SNV exerts an effect on the trait by altering the DNAm level, which regulates the expression levels of a functional gene (Figure 2). Four CpG sites, cg18799399, cg15848620, cg03317682, and cg12149606, were deleted because these sites did not show significance results in mQTL-GWAS SMR analysis. In addition, the cg01875838 site was discarded because *TMED1* did not show significant results in the eQTL-GWAS SMR analysis. In conclusion, the results of the integration analysis showed that *GPX1* methylation regulated by rs34293138 and rs111903592 influences *GPX1* expression, which is associated with low risk of UC, whereas *GPX1* methylation regulated by rs4855855, rs111903592, and rs4241406 influences *GPX1* expression, which is associated with low risk of CD (Figures 3, 4). For the cg07274523/cg24011261-*GPX1*-UC axis, β values for the causal association between cg07274523 (by the effect of the genetic variant rs34293138) to *GPX1* expression, cg24011261 (by the effect of the genetic variant rs111903592) to GPX1 expression, and *GPX1* expression to UC were 0.160, 0.450, and −1.422, respectively. For the cg05055782/cg24011261/cg05551922-*GPX1*-CD axis, β values for the causal association between cg05055782 (by the effect of the genetic variant rs4855855) to *GPX1* expression, cg24011261 (by the effect of the genetic variant rs111903592) to *GPX1* expression, cg05551922 (by the effect of the genetic variant rs4241406) to *GPX1* expression, and *GPX1* expression to CD were 0.407, 0.450, 0.428, and −1.458, respectively. As identified by the roadmap epigenomics mapping consortium (REMC), the four methylation probes (cg07274523, cg24011261, cg05055782, and cg05551922) that regulate GPX1 gene expression showed no significant differences in chromatin status across multiple tissues and cells (Figure 4), suggesting that there is no apparent tissue specificity to the SMR results. Furthermore, the top SNV in the mQTL-GWAS analysis of the cg07274523, cg05055782, cg24011261, and cg05551922 probes were 4, 15, 148, and 25 kbp distant from the top associated SNV in the eQTL-GWAS analysis of *GPX1*, respectively. Based on this evidence, we hypothesized that specific genetic variants lead to DNAm, which then upregulates *GPX1* expression. Theoretically, high expression of *GPX1* reduces the risk of UC and CD. Sensitivity analysis was performed using two-sample MR methods to verify the robustness of the SMR results. In cases where a single SNV was observed, the Wald ratio method was employed to assess causality. Conversely, when multiple SNVs were present, the inverse variance weighted method was utilized for the evaluation of causality. As expected, two-sample MR analysis supported our findings (Supplementary Table S5). The results of the power calculations also indicated that the data had a high statistical efficiency (Supplementary Table S6).

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Outcome	Probe	Gene	TopSNV	Forest plot	OR (95%CI)	SMR FDR
UC	ENSG00000233276	*GPX1*	rs11130203		0.241(0.154-0.379)	1.90E-07
	cg07274523	*GPX1*	rs34293138		0.797(0.745-0.852)	6.21E-09
	cg24011261	*GPX1*	rs111903592		0.517(0.399-0.670)	9.36E-05
CD	ENSG00000233276	*GPX1*	rs11130203		0.233(0.144-0.376)	5.78E-07
	cg05055782	*GPX1*	rs4855855		0.446(0.320-0.622)	2.86E-04
	cg24011261	*GPX1*	rs111903592		0.506(0.385-0.666)	2.06E-04
	cg05551922	*GPX1*	rs4241406		0.429(0.297-0.618)	6.74E-04
				0.0 0.2 0.4 0.6 0.8 1.0 1.2		

FIGURE 3
Forest plot of three-step SMR analysis results.

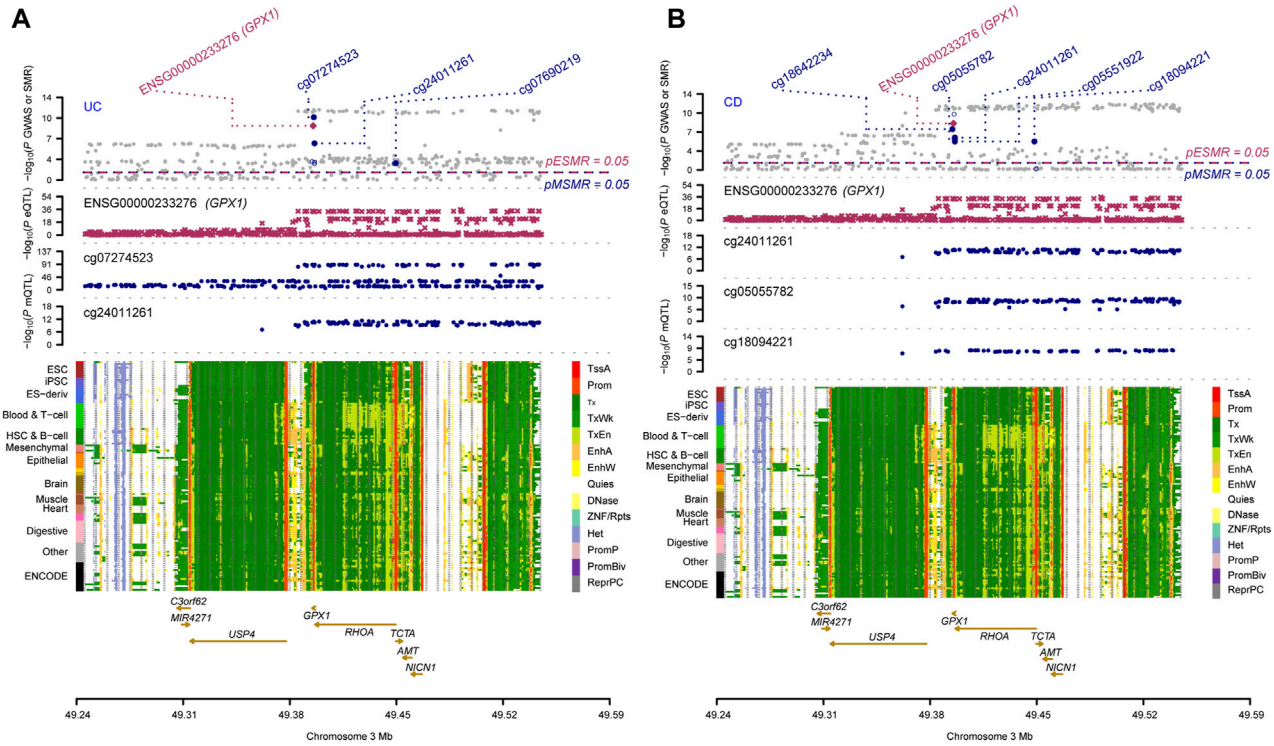

FIGURE 4
Results of SNV and SMR associations across mQTL, eQTL and GWAS at the *GPX1* locus. The top plot shows -$\log_{10}$ (*p*-values) of SNVs from GWAS. The red diamonds and blue circles represent -$\log_{10}$(*p*-values) from SMR tests for associations of gene expression and DNAm probes with trait, respectively. The solid diamonds and circles are the probes not rejected by the HEIDI test. The second plot shows -$\log_{10}$ (*p*-values) of the SNV associations for gene expression probe ENSG00000233276 (*GPX1*). The third plot shows -$\log_{10}$ (*p*-values) of the SNV associations for DNAm probes. The bottom plot shows 14 chromatin state annotations (indicated by colours) of 127 samples from the REMC for different primary cells and tissue types (rows). **(A)** multi-omics data integration for UC; **(B)** multi-omics data integration for CD.

3.5 Bayesian co-localization analysis

Bayesian co-localization analysis was used to rule out confusion due to linkage disequilibrium (LD). Bayesian co-localization results showed that *GPX1* expression (PPH4 = 0.574) and cg24011261 site (PPH4 = 0.961) shared the genetic variant with UC. However, our results did not indicate that cg07274523 site (PPH4 = 0.395) shared the same variant with UC (Figure 5A). Co-localization strongly suggests that *GPX1* expression (PPH4 = 0.834), cg05055782 (PPH4 = 0.937), cg05551922 (PPH4 = 0.909), and cg24011261 sites (PPH4 = 0.957) share genetic effects with CD (Figure 5B).

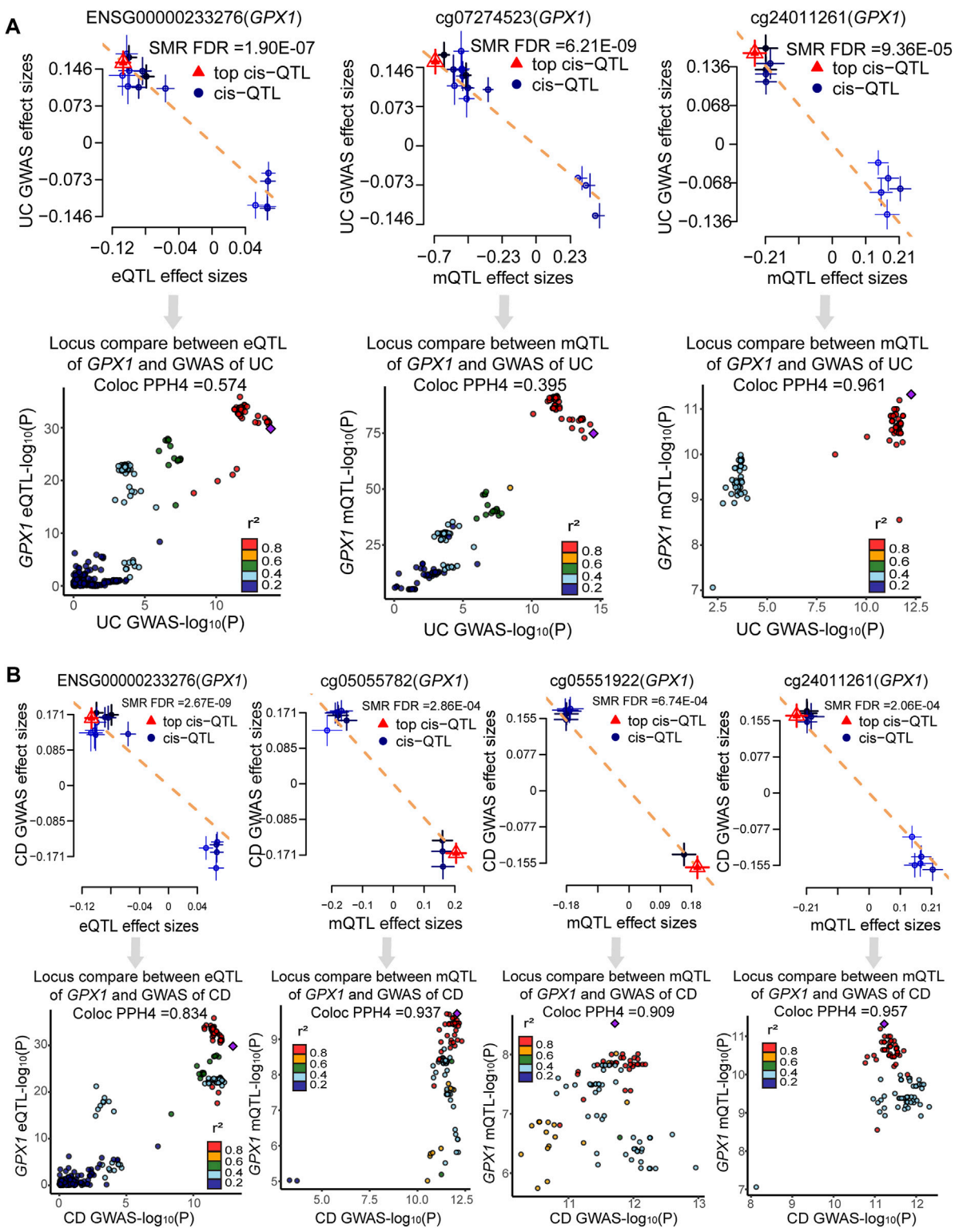

FIGURE 5
Result of SMR and co-localization analysis. **(A)** SMR and co-localization analysis for UC; **(B)** SMR and co-localization analysis for CD.

3.6 Phenotype scanning

To further validate the robustness of the results, we also searched for associations of the identified gene and genetic variants with other traits through the GWASATLAS and PhenoScanner databases, respectively. In the GWASATLAS database, we set $P < 5E\text{-}8$ to search for associations of *GPX1* with other traits (Supplementary Table S7). In addition to the study by Liu et al. (2015), the study by de

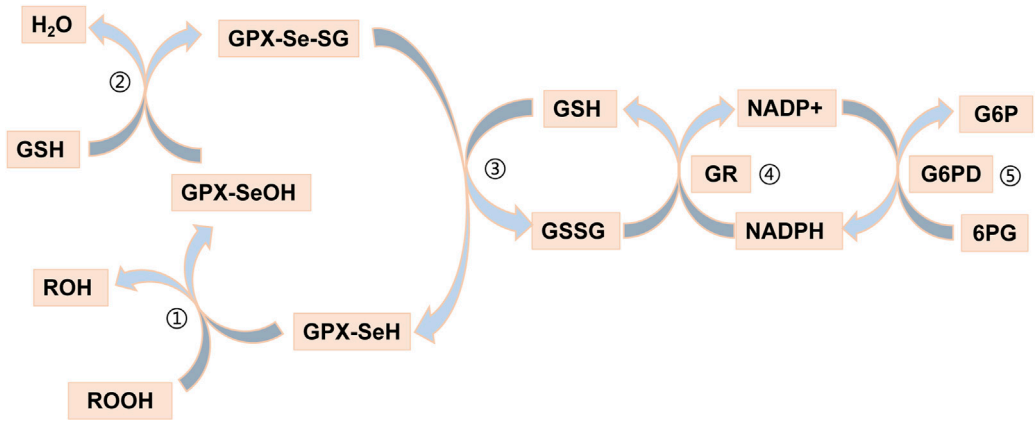

FIGURE 6
Reduction of hydrogen peroxide by *GPX1*. Step ①: The selenol of GPx-SeH (with -SeH representing the Sec active site) is oxidized to selenic acid (-Se-OH) by peroxide (ROOH); Step ②: The first GSH molecule reduces selenic acid (-Se-OH) to form glutathioneated selenol intermediate (-Se-SG) and releases a part of H_2O; Step ③: The second GSH molecule continues to reduce the intermediate (Se-SG) to form oxidized glutathione (GSSG), while the activity of GPX neutrality returns to selenol (-Se-); Step ④: The GSSG is degraded to reduced glutathione (GSH) under the action of NADPH-dependent glutathione reductase (GR), while NADPH loses an electron to NAPD+. Step ⑤: Glucose 6 phosphate (G6P) reduces NAPD + to NADPH when circulating under the action of glucose 6 phosphate dehydrogenase (G6PD).

Lange et al. (2017) and the study by Anderson et al. (2011) also showed that *GPX1* is associated with UC and CD, which is consistent with our findings. We searched the PhenoScanner database and found that rs11130203 and rs111903592 were associated with UC, whereas rs11130203, rs4855855, rs111903592, and rs4241406 were associated with CD (Supplementary Table S8). This also indicates the robustness of our findings.

4 Discussion

To our knowledge, this study is the first to identify putative causal ERS-related genes in UC and CD using a multi-omics integration approach and Bayesian co-localization. Integration of UC GWAS summary data and mQTL and eQTL for ERS-related genes prioritized one gene expression (*GPX1*) and two CpG sites (cg07274523 and cg24011261). However, the cg07274523 site did not pass co-localization analysis (PPH4 = 0.395), suggesting that the observed causality may be due to LD. Integration of CD GWAS and QTL data prioritized one gene expression (*GPX1*) and three CPG sites (cg05055782, cg24011261, and cg05551922). Our findings provide strong evidence for potential mechanisms linking the genetic locus, methylation and expression of *GPX1* to UC and CD.

Recently, an increasing number of blood-based biomarkers have been used to predict the risk of developing UC and CD. We found a negative (protective) effect of *GPX1* expression with susceptibility to UC (OR_{SMR} = 0.241) and CD (OR_{SMR} = 0.233) by SMR analysis. Glutathione peroxidase 1 (*GPX1*) is a redox-active enzyme that mitigates cytotoxicity by catalyzing the reduction of organic hydroperoxides or H_2O_2 to the corresponding alcohols or water (Li et al., 2000). Selenocysteine (Sec) is an important component of the active site of *GPX1* (Flohe et al., 1973; Rotruck et al., 1973). The *GPX1*-catalysed process usually also requires the use of glutathione (GSH) as a reducing agent (Barbosa et al., 2017). Indeed, detoxification of peroxides by mammalian *GPX1* occurs via a bi-substrate ping-pong type enzymatic mechanism (Lubos et al., 2011). During peroxidase reduction, modification of the Sec active site is required to form a stable intermediate (Flohé et al., 1972; Flohe et al., 1973; Kraus et al., 1980). After reaction with peroxides, the active site of sec (-SeH) is oxidized to selenenic acid (Se-OH) (Kraus et al., 1980; Flohé et al., 2022). One of the GSH molecules then binds to selenenic acid and undergoes a reduction reaction to produce a glutathione selenol (Se-SG) intermediate (Kraus et al., 1980; Flohé et al., 2022). Another GSH molecule continues to bind to the intermediate and reduces it to oxidized glutathione (GSSG), a process that will restore the active site of sec (Kraus et al., 1980; Flohé et al., 2022). The GSSG is in turn degraded to GSH by NADPH-dependent glutathione reductase (GR) (Lubos et al., 2011). The process of reduction of hydrogen peroxide by *GPX1* is illustrated in Figure 6. *GPX1* is usually classified as an oxidative stress enzyme because of its ability to reduce hydroperoxides. Superficially, *GPX1* KO mice develop normally, but these mice are susceptible to injury due to increased oxidative stress and ERS when stimulated (Liu et al., 2021). Wild-type mice supplemented with selenium under the same conditions survived normally. A number of studies have been conducted to show that overexpression of reactive oxygen species (ROS) leads to inflammatory bowel disease due to damage to the intestinal mucosal barrier (Sun et al., 2023; Yao et al., 2023). The study by Huang et al. showed that scavenging ROS toxicity, improving cellular antioxidant capacity and alleviating cellular ERS by increasing *GPX1* expression could greatly rescue the cell death situation (Tao et al., 2023). The UPR signaling, which plays a major role in restoring ER homeostasis, is initiated by IRE1, PERK and ATF6 (Cantoni et al., 2022). The evidence suggested that PERK signaling and Nrf2 signaling were in crosstalk with each other (Harding et al., 2003). The activated PERK promotes Nrf2 phosphorylation and transcription (Cullinan et al., 2003). Activation of the Nrf2 pathway is known to help maintain the integrity of the intestinal epithelial barrier, involving mechanisms such as Zonula Occludens-1, claudin and MUC2 (Wen et al., 2019; Yuan et al., 2019; Wang and Wang, 2022). Interestingly, there seems

to be a cycle, as activated Nrf2 increases *GPX1* and GSH expression to improve cellular stress resistance (Yin et al., 2015; Chen et al., 2019; Liang et al., 2021). In conclusion, *GPX1* has a protective effect against UC and CD, which could serve as a potential pharmacological target for the diseases.

This study has four strengths. Firstly, this study integrated multi-omics data to dissect GWAS signals and identify prioritization of gene expression and methylation. SMR was used as the primary analysis, and the results were integrated through a three-step approach, indicating strong evidence for their robustness. We conducted a comprehensive and systematic evaluation of the causal association between 1193 ERS-related genes and the two major subtypes of IBD. Secondly, summary data for UC and CD were extracted from the IIBDGC database, which defines them critically. Thus, the findings are free of bias due to the co-occurrence of the two subtypes. Thirdly, we performed sensitivity analysis using two-sample MR methods, Bayesian co-localization analysis and phenotype scanning, which further demonstrated the reliability of our results. Finally, the samples included only individuals of European ancestry, thereby reducing biases arising from diverse genetic backgrounds.

Our study also has limitations. Despite obtaining considerable GWAS summary data, there was a lack of protein quantitative trait loci data that were associated with ERS. Additionally, the available eQTL and mQTL datasets did not contain information on genetic variables present on the X and Y chromosomes. Secondly, our study exclusively focused on cis-eQTL and cis-mQTL data for ERS-related genes. However, whether trans-eQTL and trans-mQTL data might also influence the regulatory network to a large extent is unknown. Finally, it is still necessary to perform functional experiments to validate our findings. In addition, as ERS gene expression can be affected by a variety of factors, we believe that combining data from different molecular levels (e.g., metabolites and proteins) with GWAS data may lead to new discoveries in the future.

5 Conclusion

In conclusion, our three-step SMR analysis showed that cg24011261 CpG site regulating *GPX1* expression was associated with a low risk of UC, whereas *GPX1* expression regulated by a combination of cg05055782, cg24011261, and cg05551922 CpG sites was associated with a low risk of CD. This study identifies a causal relationship between the role of ERS in UC and CD and suggests potential new therapeutic targets for clinical practice.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

MZ: Writing–original draft, Methodology, Supervision. QL: Data curation, Formal Analysis, Visualization, Writing–review and editing. WZ: Data curation, Formal Analysis, Visualization, Writing–review and editing. YZ: Funding acquisition, Writing–review and editing. YX: Funding acquisition, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1270085/full#supplementary-material

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Common gastrointestinal diseases and chronic obstructive pulmonary disease risk: a bidirectional Mendelian randomization analysis

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Background: Observational studies suggest an association between gastrointestinal diseases and chronic obstructive pulmonary disease (COPD), but the causal relationship remains unclear.

Methods: We conducted bidirectional Mendelian randomization (MR) analysis using summary data from genome-wide association study (GWAS) to explore the causal relationship between common gastrointestinal diseases and COPD. Gastrointestinal diseases included gastroesophageal reflux disease (GERD), peptic ulcer disease (PUD), irritable bowel syndrome (IBS), Crohn's disease (CD), ulcerative colitis (UC), functional dyspepsia (FD), non-infectious gastroenteritis (NGE), and constipation (CP). Significant MR analysis results were replicated in the COPD validation cohort.

Results: Bidirectional MR analysis supported a bidirectional causal relationship between GERD and COPD, and COPD was also found to increase the risk of IBS and CP. Our study also provided evidence for a bidirectional causal relationship between PUD and COPD, although the strength of evidence may be insufficient. Furthermore, we provided evidence that there is no causal association between CD, UC, FD, NGE, and COPD.

Conclusion: This study offers some evidence to clarify the causal relationship between common gastrointestinal diseases and COPD. Further research is needed to understand the underlying mechanisms of these associations.

KEYWORDS

chronic obstructive pulmonary disease, gastrointestinal diseases, gastroesophageal reflux disease, peptic ulcer disease, irritable bowel syndrome, constipation, Mendelian randomization

Abbreviation: COPD, chronic obstructive pulmonary disease; GWAS, Genome-wide association study; MR, Mendelian randomization; GERD, gastroesophageal reflux disease; PUD, peptic ulcer disease; IBS, irritable bowel syndrome; CD, Crohn's disease; UC, ulcerative colitis; FD, functional dyspepsia; NGE, non-infectious gastroenteritis; CP, constipation; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; SNP, single nucleotide polymorphism; OR, odds ratio; IVW, inverse variance weighting; MR-Egger, MR-Egger regression; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test; MR-RAPS, Mendelian Randomization Robust Adjusted Profile Score.

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most common chronic respiratory diseases, with a forced expiratory volume in one second to forced vital capacity ratio (FEV1/FVC ratio) less than 70% after bronchodilator is the gold standard for COPD diagnosis (Halpin et al., 2021). COPD affects 544.9 million people globally and is the third leading cause of death in the past decades, accounting for 7% of all deaths (GBD Chronic Respiratory Disease Collaborators, 2020). Observational studies have indicated an association between gastrointestinal diseases and COPD (Wang et al., 2023), but the precise causal relationship has not been confirmed.

Gastroesophageal reflux disease (GERD) involves the reflux of stomach contents into the esophagus, causing various uncomfortable symptoms. Approximately 20% of adults in the Western world have GERD (Maret-Ouda et al., 2020a). Observational studies have found that GERD is one of the most common complications of COPD (Zou et al., 2022), and the risk of worsening COPD in GERD patients is doubled (Rascon-Aguilar et al., 2006). GERD and COPD appear to have a mutually reinforcing effect, not merely a shared susceptibility to certain environmental factors. Peptic ulcer disease (PUD) primarily includes open ulcers in the gastric mucosa and upper small intestine, and it can also occur in the lower esophagus, small intestine, gastrojejunal anastomosis, etc (Lanas and Chan, 2017). The estimated lifetime prevalence of peptic ulcer disease is approximately 5%–10% (Lanas and Chan, 2017). Observational studies have found that the risk of PUD is increased by 17%–24% in COPD patients (Schneider et al., 2010; Huang et al., 2017). Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by symptoms related to changes in stool form or frequency, and it affects approximately 5%–10% of the global population (Ford et al., 2020a). A cohort study involving 14,021 IBS patients found a significant increase in the risk of COPD in IBS patients ($p < 0.0001$) (Lai et al., 2020), with a hazard ratio (HR) of 1.512. Another study also found an increased risk of IBS in COPD patients (HR = 1.55, $p < 0.001$) (Chiu et al., 2022). Inflammatory bowel disease (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC) and is a chronic, recurrent gastrointestinal disease (Wehkamp et al., 2016). Approximately 1 in 198 individuals has UC, and 1 in 310 individuals has CD (Wehkamp et al., 2016). IBD has numerous extraintestinal manifestations, including pulmonary manifestations (Rogler et al., 2021). A retrospective cohort study in Canada indicated a 55% higher incidence of CD and a 30% higher incidence of UC in COPD patients (Brassard et al., 2015). There is also evidence linking severe IBD progression to pulmonary inflammation and pathology (Vutcovici et al., 2016; Raftery et al., 2023). Functional dyspepsia (FD) encompasses a range of symptoms involving the gastroduodenal region, including upper abdominal pain or burning sensations and postprandial fullness or early satiety (Ford et al., 2020b). FD affects approximately 18% of the general population (Ford et al., 2020b). Observational studies have shown a significant increase in the risk of FD in COPD patients (HR = 1.34, $p < 0.003$) (Chiu et al., 2022). Non-infectious gastroenteritis (NGE) is a group of diseases, including gastritis, duodenitis, and esophagitis, typically not caused by infections (Mathews et al., 2022). Globally, more than half of the population may suffer from chronic gastrointestinal inflammation (Sipponen and Maaroos, 2015), and COPD patients appear to have a higher prevalence of NGE (Fedorova et al., 2003). Constipation (CP) is another common functional gastrointestinal disorder, affecting approximately 16% of adults (Bharucha and Wald, 2019). Previous studies have shown an increased risk of CP in COPD patients (Sun et al., 2013; Gursoy Coskun et al., 2021). The aforementioned observational studies suggest a complex interplay between gastrointestinal diseases and COPD, which is also referred to as the "gut-lung axis" (Gokulan et al., 2022).

There seems to be a delicate relationship between gastrointestinal diseases and COPD. Understanding the potential causal relationships can aid in better disease management, with the aim of improving patient prognosis. However, there are currently no appropriate randomized controlled trials (RCTs) to elucidate these causal associations. Designing RCTs for these gastrointestinal diseases and COPD is impractical due to confounding factors such as smoking, obesity, and asthma. In the absence of feasible RCTs, Mendelian randomization (MR) is a reliable causal inference method (Skrivankova et al., 2021). MR uses genetic variations as instrumental variables (IVs) and provides evidence regarding the causal relationship between modifiable exposure factors and diseases (Skrivankova et al., 2021). MR is less susceptible to confounding and reverse causation and is effective in reducing financial and human resource costs (Davies et al., 2018). To provide more evidence to clarify the causal relationship between gastrointestinal diseases (GERD, PUD, IBS, CD, UC, FD, NGE, and CP) and COPD, this study explored the potential causal effects using bidirectional MR.

Materials and methods

Study design

An overview of the research design is shown in Figure 1. Our research is based on three main assumptions of MR research (Haycock et al., 2016). I: The instrumental variable is related to exposure; II: The instrumental variable is not related to any known or unknown confounding factors that can mediate from exposure to outcome; III: The outcome is associated with the genetic instrument only through the effect of the exposure.

GWAS summary data source

The summary data for GERD is derived from a genome-wide association study (GWAS) meta-analysis conducted by Ong et al. (2022), which includes 129,080 cases of European ancestry and 473,524 European ancestry controls. The summary data for PUD is obtained from a GWAS meta-analysis by Wu et al. (2021), comprising 16,666 European ancestry cases and 439,661 European ancestry controls. The GWAS summary data for IBS is sourced from Eijsbouts et al.'s report (Eijsbouts et al., 2021), encompassing 53,400 cases of European ancestry and 433,201 European ancestry controls. The summary data for CD and UC are obtained from Lange et al.'s report (de Lange et al., 2017). CD includes 12,194 cases of mixed ancestry and 28,072 mixed ancestry controls, while UC includes 12,366 cases of mixed ancestry

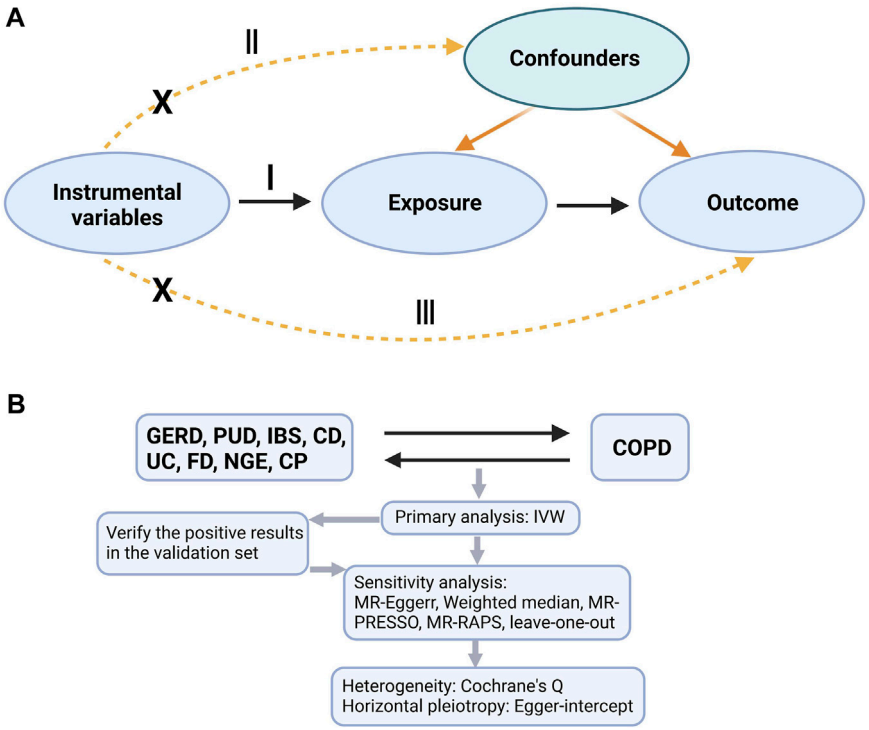

FIGURE 1
A is a bidirectional acyclic graph. B is an overview of our study. I: assumption I, II: assumption II; III: assumption III; GERD, gastroesophageal reflux disease; PUD, peptic ulcer disease; IBS, irritable bowel syndrome; CD, Crohn's disease; UC, ulcerative colitis; FD, functional dyspepsia; NGE, noninfectious gastroenteritis; CP, constipation; COPD, chronic obstructive pulmonary disease; IVW, inverse variance weighting; MR-Egger, MR-Egger regression; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test; MR-RAPS, Mendelian Randomization Robust Adjusted Profile Score.

and 33,609 mixed ancestry controls. The summary data for FD is sourced from FinnGen (https://www.finngen.fi/en) (Kurki et al., 2023), including 8,875 European cases and 320,387 European controls. The data for NGE is obtained from Jiang et al.'s analysis of the UK Biobank data (Jiang et al., 2021), comprising 11,373 European cases and 444,975 European controls. The summary data for CP is sourced from a trans-ethnic GWAS meta-analysis conducted by Sakaue et al. (2021), which includes 16,299 cases of European and East Asian mixed ancestry and 571,953 European and East Asian mixed ancestry controls. The summary data for COPD (discovery) is obtained from Sakaue et al.'s trans-ethnic GWAS meta-analysis (Sakaue et al., 2021), which includes 17,574 cases of European and East Asian mixed ancestry and 617,598 European and East Asian mixed ancestry controls. COPD (validation) data is from FinnGen, comprising 18,266 European ancestry cases and 311,286 European ancestry controls. Ethical approval for each of the summary datasets is available in the original studies, and these data can be used without restrictions. Table 1 provides a brief overview of the summary data.

Instrumental variables

We extracted SNPs strongly associated with GERD, CD, and UC using a threshold of $p < 5.0E-08$. To ensure an adequate number of SNPs, we used a threshold of $p < 1.0E-05$ to extract SNPs for PUD, IBS, FD, NGE, CP, and both the discovery and validation sets of COPD. We calculated the F-statistic for each SNP as well as the overall F-statistic for all SNPs. The F-statistic for an individual SNP was calculated using the following formula (Codd et al., 2013): $F = \frac{beta^2}{se^2}$, where "beta" is the effect size of the SNP on the exposure, and "se" is the standard error corresponding to "beta." The overall F-statistic was calculated using the following formula (Codd et al., 2013): $F = \frac{N-K-1}{K} \times \frac{R^2}{1-R^2}$, $R^2 = 2 \times eaf \times (1 - eaf) \times beta^2$, where N is the sample size of the exposure, K is the number of IVs, R^2 is the proportion of exposure variance explained by the SNPs, eaf is the effect allele frequency of the SNP, and beta is the effect size of the SNP on the exposure. An F-statistic greater than 10 indicates a strong association between the SNP and the phenotype (Lawlor et al., 2008). The R^2 and overall F-statistic for all causal estimates in this study can be found in Table 1, and individual SNP F-statistics are described in Supplementary Table S2.

We applied strict criteria for removing linkage disequilibrium, with a clustering window set at 10,000 kb and an r^2 threshold set at 0.001. We also removed palindromic SNPs with intermediate allele frequencies (minor allele frequency >0.42). Additionally, based on a threshold of $p < 1.0E-05$, we searched all SNPs in the PhenoScanner database, and SNPs associated with the outcome or potential confounders were excluded.

TABLE 1 A brief description of each GWAS summary statistics.

Combination	Ancestry	Sample size of exposure	PMID	Group	NSNP	R2 (%)	F
GERD-COPD	European	129,080 cases, 473,524 controls	34,187,846	Discovery	40	1.66	253.66
				Validation	39	1.61	252.49
PUD-COPD	European	16,666 cases, 439,661 controls	33,608,531	Discovery	36	5.72	768.69
				Validation	34	5.46	774.85
IBS-COPD	European	53,400 cases, 433,201 controls	34,741,163	Discovery	63	3.66	293.80
CD-COPD	Mixed	12,194 cases, 28,072 controls	28,067,908	Discovery	50	55.40	999.21
UC-COPD	Mixed	12,366 cases, 33,609 controls	28,067,908	Discovery	42	39.55	715.50
FD-COPD	European	8,875 cases, 320,387 controls	—	Discovery	14	3.62	884.56
NGE-COPD	European	11,373 cases, 444,975 controls	34,737,426	Discovery	19	3.78	944.44
CP-COPD	Mixed	15,902 European ancestry cases, 395,721 European ancestry controls, 397 East Asian ancestry cases, 176,232 East Asian ancestry controls	34,594,039	Discovery	21	4.04	1180.00
COPD-GERD	Mixed	13,530 European cases, 454,945 European controls, 4,017 East Asian cases, 162,653 East Asian controls	34,594,039	Discovery	31	4.59	986.16
	European	18,266 European cases, 311,286 European controls	—	Validation	31	4.50	501.09
COPD-PUD	Mixed	13,530 European cases, 454,945 European controls, 4,017 East Asian cases, 162,653 East Asian controls	34,594,039	Discovery	53	8.44	1104.63
	European	18,266 European cases, 311,286 European controls	—	Validation	70	10.42	547.53
COPD-IBS	Mixed	13,530 European cases, 454,945 European controls, 4,017 East Asian cases, 162,653 East Asian controls	34,594,039	Discovery	60	12.37	1494.22
COPD-IBS	European	18,266 European cases, 311,286 European controls	—	Validation	75	11.18	552.91
COPD-CD	Mixed	13,530 European cases, 454,945 European controls, 4,017 East Asian cases, 162,653 East Asian controls	34,594,039	Discovery	55	11.25	1464.44
COPD-UC	Mixed	13,530 European cases, 454,945 European controls, 4,017 East Asian cases, 162,653 East Asian controls	34,594,039	Discovery	55	11.25	1464.44
COPD-FD	Mixed	13,530 European cases, 454,945 European controls, 4,017 East Asian cases, 162,653 East Asian controls	34,594,039	Discovery	58	11.80	1464.72
COPD-NGE	Mixed	13,530 European cases, 454,945 European controls, 4,017 East Asian cases, 162,653 East Asian controls	34,594,039	Discovery	57	9.34	1148.39
COPD-CP	Mixed	13,530 European cases, 454,945 European controls, 4,017 East Asian cases, 162,653 East Asian controls	34,594,039	Discovery	61	12.74	1520.40
	European	18,266 European cases, 311,286 European controls	—	Validation	76	10.99	535.02

NSNP, Number of SNPs; R2, exposure variance explained by all IVs; F, F-statistic; GERD, gastroesophageal reflux disease; PUD, peptic ulcer disease; IBS, irritable bowel syndrome; CD, Crohn's disease; UC, ulcerative colitis; FD, functional dyspepsia; NGE, noninfectious gastroenteritis; CP, constipation; COPD, chronic obstructive pulmonary disease.

Previous studies have shown a strong association between asthma and COPD, and both gastrointestinal diseases and asthma are influenced by smoking (Hikichi et al., 2018). Among the instrumental variables (IVs) representing gastrointestinal diseases, SNPs related to smoking, asthma, and lung function will be excluded. Previous research has also indicated an increased risk of gastrointestinal diseases with asthma (Althoff and Sharma, 2023), and both gastrointestinal diseases and COPD are susceptible to the influence of smoking (Berkowitz et al., 2018; Maret-Ouda et al., 2020b). Therefore, when selecting IVs for COPD, SNPs related to smoking and asthma will be excluded. Furthermore, considering the influence of obesity on GERD (Friedenberg et al., 2008), when analyzing the causal effect of COPD on GERD, SNPs related to body mass index will be excluded from the IVs for COPD.

Statistical analysis

We conducted MR analysis on 8 pairs of causal combinations: GERD-COPD, PUD-COPD, IBS-COPD, CD-COPD, UC-COPD, FD-COPD, NGE-COPD, and CP-COPD, where GERD, PUD, IBS, CD, UC, FD, NGE, and CP were treated as exposures and COPD as the outcome. Subsequently, reverse MR analysis was performed with COPD as the exposure to obtain COPD-GERD, COPD-PUD, COPD-IBS, COPD-CD, COPD-UC, COPD-FD, COPD-NGE, and COPD-CP, totaling 8 reverse causal combinations. Finally, validation analysis was carried out on the positive results using the COPD validation dataset.

In the primary analysis, the Inverse Variance Weighting (IVW) random-effects model was used to estimate the combined causal effects (Bowden et al., 2017). Sensitivity analyses included MR-Egger

regression (Bowden et al., 2015), Weighted Median (Bowden et al., 2016a), Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) (Verbanck et al., 2018), and Mendelian Randomization Robust Adjusted Profile Score (MR-RAPS) (Zhao et al., 2020). MR-PRESSO was used to detect potential horizontal pleiotropy and, if significant, provide IVW estimates corrected for outliers (Verbanck et al., 2018). In this study, MR-PRESSO was set with a Distribution of 1000 and SignifThreshold of 0.05. Cochrane's Q and I^2 statistics were calculated to assess heterogeneity (Bowden et al., 2016b; Bowden et al., 2019), with I^2>90% indicating robust and reliable results (Bowden et al., 2016b). Furthermore, MR-Egger regression intercept testing was conducted to assess horizontal pleiotropy, with a non-zero intercept suggesting the presence of horizontal pleiotropy (Bowden et al., 2017). Finally, we performed leave-one-out analyses to ensure that our MR results were not unduly influenced by individual SNPs.

MR results were presented as odds ratios (ORs), with estimates primarily used to determine the direction of causality (Burgess and Labrecque, 2018). In the bidirectional MR analysis of gastrointestinal diseases and COPD, causal estimates were conducted in both directions for all 8 combinations. Considering multiple testing contributions, we considered MR results statistically significant when $p < 0.05/8 = 0.00625$ based on the Bonferroni correction, and suggestive significance when $0.00625 < p < 0.05$. All analyses were performed using the open-source statistical software R (version: 4.2.3). MR analysis was based on "TwoSampleMR (https://github.com/MRCIEU/TwoSampleMR.git)," "mr.raps (https://github.com/qingyuanzhao/mr.raps.git)," and "MR-PRESSO (https://github.com/rondolab/MR-PRESSO.git)." Data visualization was based on "TwoSampleMR" and "forestploter (https://github.com/adayim/forestploter.git)."

Results

IVs for gastrointestinal diseases

In MR analyses with gastrointestinal diseases as exposures, preliminary analyses initially identified 80, 41, 63, 89, 58, 20, 24, and 24 IVs for GERD, PUD, IBS, CD, UC, FD, NGE, and CP, respectively. After screening these IVs in the PhenoScanner database, 29 IVs from GERD, 3 IVs from PUD, 8 IVs from IBS, 30 IVs from CD, 10 IVs from UC, and 1 IV from FD were found to have potential pleiotropy or relevance to the outcomes. Harmonization with discovery data for COPD was performed, which involved the removal of palindromic structure SNPs and missing SNPs in the outcomes. Finally, 40, 36, 63, 50, 42, 14, 14, and 21 SNPs remained as IVs for GERD-COPD, PUD-COPD, IBS-COPD, CD-COPD, UC-COPD, FD-COPD, NGE-COPD, and CP-COPD, respectively. MR analysis for GERD-COPD and PUD-COPD yielded positive results, leading us to perform validation analyses using COPD validation data. Following the same IV selection process, 39 and 34 SNPs were selected as IVs for validation analysis in GERD-COPD and PUD-COPD, respectively.

IVs for COPD

In MR analyses with COPD as the exposure, we initially obtained 81 and 103 SNPs as discovery and validation IVs for COPD, respectively. In the COPD-GERD MR analysis, we excluded 19 SNPs with potential pleiotropy from the COPD discovery IVs and 18 SNPs with potential pleiotropy from the COPD validation IVs based on the PhenoScanner database. In the COPD-PUD, COPD-IBS, COPD-CD, COPD-UC, COPD-FD, COPD-NGE, and COPD-CP MR analyses, 14 SNPs with potential pleiotropy were identified from the COPD discovery IVs, along with 13 SNPs with potential pleiotropy from the COPD validation IVs based on the PhenoScanner database. Harmonization was conducted between COPD discovery data and gastrointestinal disease data, involving the removal of palindromic structure SNPs and missing SNPs in the outcomes. Ultimately, 31, 53, 60, 55, 55, 58, 57, and 61 SNPs remained as IVs for COPD-GERD, COPD-PUD, COPD-IBS, COPD-CD, COPD-UC, COPD-FD, COPD-NGE, and COPD-CP, respectively. Positive MR results were obtained for COPD-GERD, COPD-PUD, COPD-IBS, and COPD-CP, prompting validation analyses using COPD validation data. Following the same IV selection process, 31, 70, 75, and 76 SNPs were chosen as IVs for validation analysis in COPD-GERD, COPD-PUD, COPD-IBS, and COPD-CP, respectively. Supplementary Tables S1, S2 provide basic information on the SNPs that were excluded in this study and those used in the final MR analysis, respectively.

MR Results and Sensitivity Analysis

The IVs for each phenotype investigated in this study consisted of 14–76 SNPs, explaining exposure variance ranging from 1.61% to 55.40%. The F-statistics for individual IVs (see Supplementary Table S2) and the overall F-statistics (Table 1) were all greater than 10, indicating minimal bias due to weak instrument variation. In summary, after excluding potentially pleiotropic SNPs, MR results suggested a potential bidirectional causal relationship between GERD and COPD, with COPD also increasing the risk of IBS and CP. These findings were consistent in the validation analyses. Additionally, limited evidence of a bidirectional causal relationship between PUD and COPD was found, as only positive results were present in the discovery phase. The evidence provided also indicated no causal associations between CD, UC, FD, NGE, and COPD. Figures 2, 3 provide an overview of the causal estimates and sensitivity analyses. Table 2 presents the results of heterogeneity and pleiotropy analyses for all MR analyses in this study. Supplementary Table S3 provides a more detailed breakdown of the results for all MR analyses in this study. Supplementary Figures S1–S3 present leave-one-out results for MR analyses with gastrointestinal diseases as exposures, COPD as an exposure, and validation MR analyses, respectively.

In the discovery phase, IVW results showed that GERD increases the risk of COPD (OR = 2.005, 95% CI: 1.691–2.377, p = 1.21E-15), and COPD also increases the risk of GERD (OR = 1.124, 95% CI: 1.063–1.189, p = 4.31E-05). These results were supported by the Weighted Median method, MR-PRESSO method, and MR-RAPS method. The MR-Egger method provided causal estimates in the same direction as the IVW method, although the results were not statistically significant. In bidirectional analyses, some heterogeneity was detected by the Q-statistic and MR-PRESSO, but an I^2 value greater than 90% suggests robust results. Additionally, the Egger intercept did not

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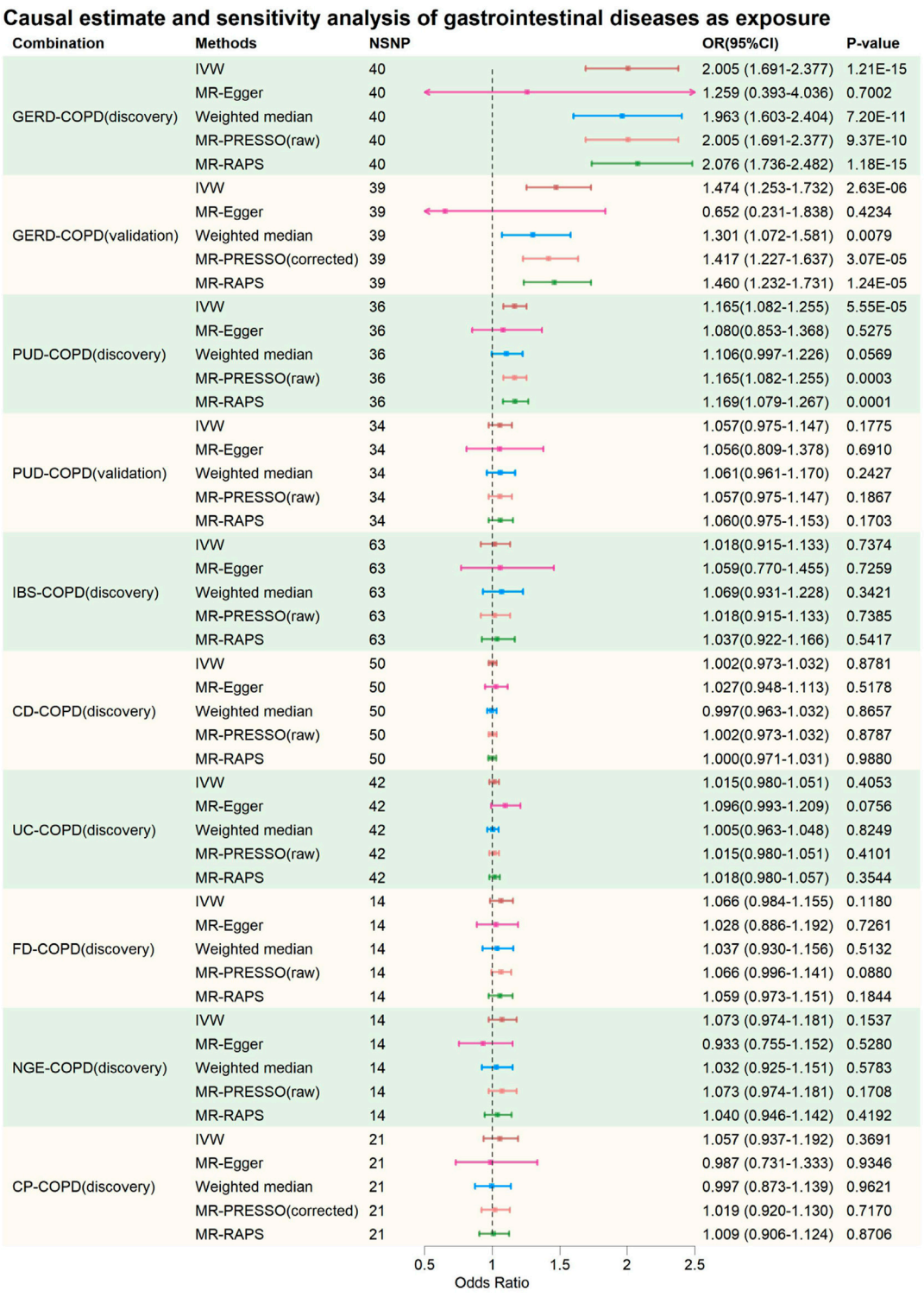

FIGURE 2
Causal estimate and sensitivity analysis of gastrointestinal diseases as exposure. NSNP, Number of SNPs; OR, odds ratio; 95%LCI, lower limit of 95% confidence interval of OR; 95%UCI, upper limit of 95% confidence interval of OR; *p*-value, *p*-value of OR; IVW, inverse variance weighting; MR-Egger, MR-Egger regression; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test; MR-RAPS, Mendelian Randomization Robust Adjusted Profile Score; GERD, gastroesophageal reflux disease; PUD, peptic ulcer disease; IBS, irritable bowel syndrome; CD, Crohn's disease; UC, ulcerative colitis; FD, functional dyspepsia; NGE, noninfectious gastroenteritis; CP, constipation; COPD, chronic obstructive pulmonary disease.

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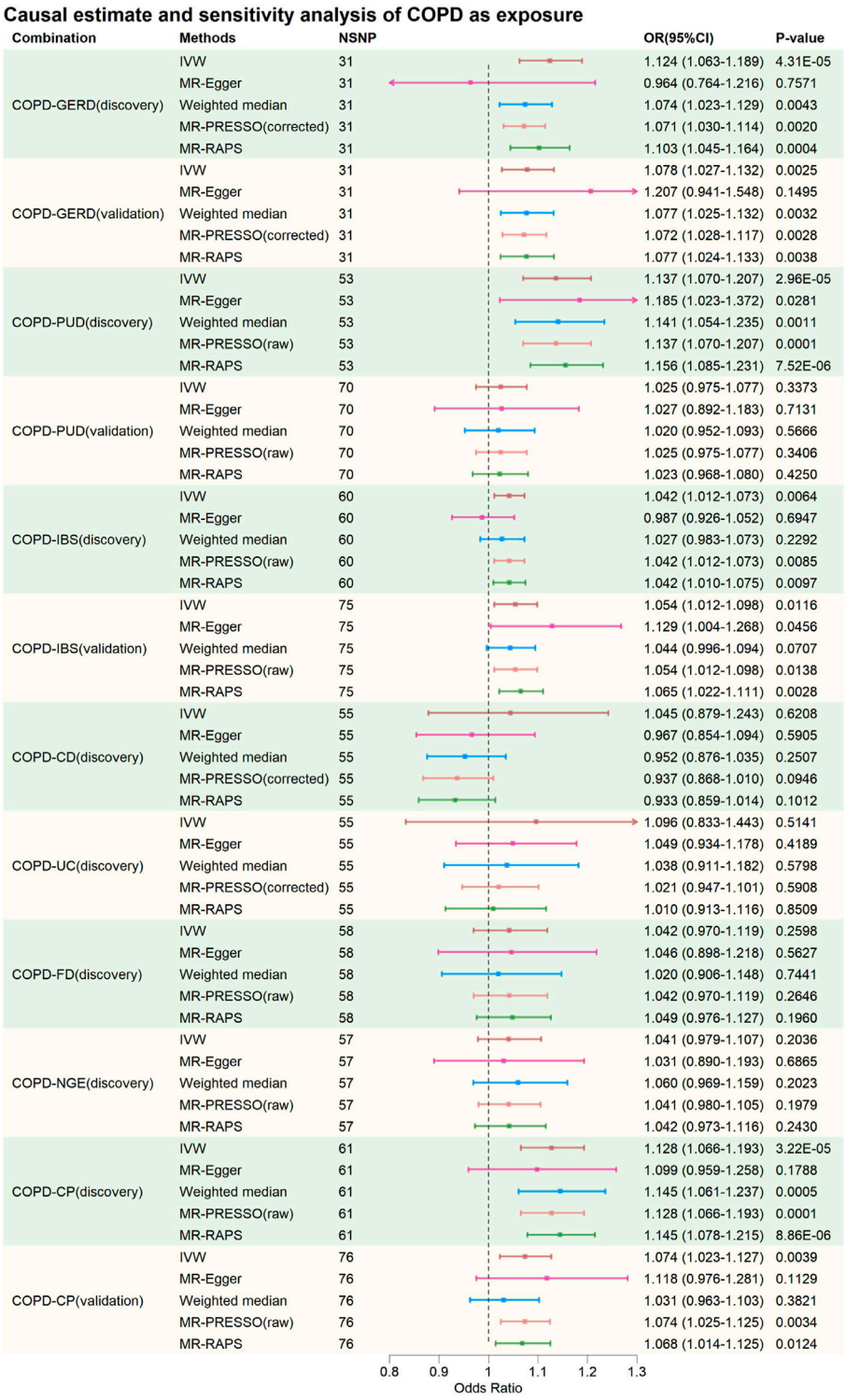

FIGURE 3
Causal estimate and sensitivity analysis of COPD as exposure. NSNP, Number of SNPs; OR, odds ratio; 95%LCI, lower limit of 95% confidence interval of OR; 95%UCI, upper limit of 95% confidence interval of OR; *p*-value, *p*-value of OR; IVW, inverse variance weighting; MR-Egger, MR-Egger regression; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test; MR-RAPS, Mendelian Randomization Robust Adjusted Profile Score; GERD, gastroesophageal reflux disease; PUD, peptic ulcer disease; IBS, irritable bowel syndrome; CD, Crohn's disease; UC, ulcerative colitis; FD, functional dyspepsia; NGE, noninfectious gastroenteritis; CP, constipation; COPD, chronic obstructive pulmonary disease.

TABLE 2 Heterogeneity and pleiotropy of MR analysis.

Combination	Group	Q	*p*-value of Q	I2 (%)	PRESSO-RSSobs	P-RSSobs	Egger-intercept	P-Egger
GERD-COPD	Discovery	70.65	0.0014	97.22	74.19	0.003	0.0147	0.4338
	Validation	66.56	0.0028	97.22	70.31	0.004	0.0259	0.1271
PUD-COPD	Discovery	38.89	0.2988	96.14	40.98	0.337	0.0061	0.5140
	Validation	50.37	0.0270	96.17	53.87	0.033	0.0001	0.9920
IBS-COPD	Discovery	85.70	0.0248	95.50	88.69	0.015	−0.0018	0.7999
CD-COPD	Discovery	87.11	0.0007	98.86	89.81	<0.001	−0.0046	0.5250
UC-COPD	Discovery	71.68	0.0021	98.63	76.64	0.002	−0.0131	0.1108
FD-COPD	Discovery	9.34	0.7471	95.58	10.63	0.773	0.0049	0.5759
NGE-COPD	Discovery	28.38	0.0565	95.58	31.51	0.055	0.0157	0.1664
CP-COPD	Discovery	34.05	0.0258	95.68	37.86	0.020	0.0055	0.6323
COPD-GERD	Discovery	95.86	8.26E-09	95.72	102.25	<0.001	0.0103	0.1911
	Validation	71.64	2.92E-05	95.88	76.88	<0.001	−0.0073	0.3733
COPD-PUD	Discovery	66.27	0.0880	95.44	68.57	0.118	−0.0036	0.5478
	Validation	74.43	0.3060	95.96	76.64	0.318	−0.0002	0.9753
COPD-IBS	Discovery	59.89	0.4433	95.53	63.35	0.434	0.0052	0.0676
	Validation	143.13	2.57E-06	95.94	147.18	<0.001	−0.0052	0.2249
COPD-CD	Discovery	78.17	0.0174	95.20	80.60	0.020	−0.0095	0.2375
COPD-UC	Discovery	197.05	3.71E-18	95.20	204.31	<0.001	−0.0056	0.6556
COPD-FD	Discovery	71.60	0.0924	95.47	73.99	0.097	−0.0004	0.9516
COPD-NGE	Discovery	53.32	0.5771	95.37	55.42	0.590	0.0009	0.8888
COPD-CP	Discovery	73.96	0.1061	95.51	76.30	0.126	0.0023	0.6794
	Validation	68.29	0.6950	95.93	70.11	0.697	−0.0031	0.5377

Q, Cochrane's Q; I2, I squared; PRESSO-RSSobs, RSSobs, of Global Test in MR-PRESSO; P-RSSobs, *p*-value of Global Test in MR-PRESSO; Egger-intercept, intercept of MR-Egger; P-Egger, *p*-value of Egger-intercept; GERD, gastroesophageal reflux disease; PUD, peptic ulcer disease; IBS, irritable bowel syndrome; CD, Crohn's disease; UC, ulcerative colitis; FD, functional dyspepsia; NGE, noninfectious gastroenteritis; CP, constipation; COPD, chronic obstructive pulmonary disease.

significantly differ from 0 statistically, indicating that the presence of heterogeneity did not introduce significant pleiotropic bias into the MR results. Leave-one-out analysis did not reveal any SNP that significantly influenced the causal estimates.

In the validation MR analysis, IVW results still indicated a bidirectional causal relationship between GERD and COPD (GERD-COPD, OR = 1.474, 95% CI: 1.253–1.732, p = 2.63E-06; COPD-GERD, OR = 1.078, 95% CI: 1.027–1.132, p = 0.0025). Some heterogeneity was detected by the Q-statistic and MR-PRESSO, but the Egger intercept test did not detect significant levels of pleiotropy, suggesting that heterogeneity did not introduce significant pleiotropic bias into the MR results. Leave-one-out analysis also did not identify any SNP that significantly affected the causal estimates. Therefore, the analysis results indicating a bidirectional causal relationship between GERD and COPD are considered robust.

In the discovery phase, IVW results also indicated a bidirectional causal relationship between PUD and COPD (PUD-COPD, OR = 1.165, 95% CI: 1.082–1.255, p = 5.55E-5; COPD-PUD, OR = 1.137, 95% CI: 1.070–1.207, p = 2.96E-05). The IVW estimates for PUD-COPD were supported by the MR-PRESSO and MR-RAPS methods, while MR-Egger and Weighted-Median methods did not yield statistically significant results but maintained consistency in the causal direction with IVW. The IVW estimates for COPD-PUD were supported by MR-PRESSO, Weighted-Median, and MR-RAPS, and MR-Egger also showed consistency in the causal direction with IVW. In bidirectional MR analysis, neither the Q-statistic nor MR-PRESSO detected significant heterogeneity, and an I^2 value greater than 90% suggests robust results. The Egger intercept for MR-Egger did not significantly differ from 0 statistically, indicating no significant horizontal pleiotropy. Leave-one-out analysis also did not identify any SNPs that significantly influenced the causal estimates. However, in the validation MR analysis, no evidence was found for a bidirectional causal relationship between PUD and COPD, even though the direction of the causal estimate remained consistent with the discovery phase.

In the causal analysis between IBS and COPD, no evidence was found for IBS altering the risk of COPD. However, evidence was found for COPD increasing the risk of IBS (IVW: OR = 1.042, 95% CI: 1.012–1.073, p = 0.0064). The IVW results were supported by MR-PRESSO and MR-RAPS, and MR-Egger and Weighted-Median

methods maintained consistency in the causal estimate direction with IVW. Neither the Q-statistic nor MR-PRESSO detected significant heterogeneity, and an I^2 value greater than 90% suggests robust results. The Egger intercept for MR-Egger did not significantly differ from 0 statistically, indicating no significant horizontal pleiotropy. Leave-one-out analysis did not identify any SNPs that significantly influenced the causal estimates. Causal estimates were also validated in the validation phase (IVW: OR = 1.054, 95% CI: 1.012–1.098, $p = 0.0116$). This suggests that the causal estimates of COPD on IBS are relatively robust.

In the causal analysis between CP and COPD, no evidence was found for CP altering the risk of COPD. However, evidence was found for COPD increasing the risk of CP (IVW: OR = 1.128, 95% CI: 1.066–1.193, p = 3.22E-05). These results were supported by Weighted-Median, MR-PRESSO, and MR-RAPS, and MR-Egger method maintained consistency in the causal estimate direction with IVW. Neither the Q-statistic nor MR-PRESSO detected significant heterogeneity, and an I^2 value greater than 90% suggests robust results. The Egger intercept for MR-Egger did not significantly differ from 0 statistically, indicating no significant horizontal pleiotropy. Leave-one-out analysis did not identify any SNPs that significantly influenced the causal estimates. Causal estimates were also validated in the validation phase (IVW: OR = 1.074, 95% CI: 1.023–1.127, $p = 0.0039$). This suggests that the causal estimates of COPD on CP are relatively robust.

For CD-COPD, UC-COPD, FD-COPD, NGE-COPD, COPD-CD, COPD-UC, COPD-FD, and COPD-NGE causal analysis, no positive results were found. Some analyses in these subgroups detected some heterogeneity, but MR-Egger intercept tests did not observe significant horizontal pleiotropy. Leave-one-out analysis did not identify any SNPs that significantly influenced the causal estimates. Therefore, it can be preliminarily concluded that there is no causal association between COPD and CD, UC, FD, and NGE.

Discussion

The causal relationship between gastrointestinal diseases and COPD has long been a subject of uncertainty. We conducted a bidirectional two-sample Mendelian randomization (MR) study to investigate the causal relationships between eight gastrointestinal diseases and COPD. Our results indicate a bidirectional causal relationship between GERD and COPD. COPD increases the risk of IBS and CP, but IBS and CP do not alter the risk of COPD. Limited evidence suggests a bidirectional causal relationship between PUD and COPD. Furthermore, there is no evidence of causality between CD, UC, FD, NGE, and COPD. MR results are less susceptible to confounding factors and reverse causality, which may help us better understand the causal relationship between gastrointestinal diseases and COPD.

Previous observational studies have suggested an association between GERD and COPD, but making causal inferences has been challenging due to confounding factors and reverse causality. A nationwide cross-sectional study involving 141,057 COPD patients reported a GERD prevalence of 28% among COPD patients (Kim et al., 2013). A cohort study that utilized high-resolution manometry and esophageal pH monitoring with follow-up showed a correlation between the severity of GERD and the frequency of exacerbations in COPD (Bigatao et al., 2018). A meta-analysis involving 13,245 patients suggested an increased risk of COPD exacerbations associated with GERD (OR: 5.37; 95% CI: 2.71–10.64, $p < 0.00001$) (Huang et al., 2020). However, the conclusions of these observational studies are not robust, as they are subject to various confounding factors. First, smoking is a common risk factor for both GERD and COPD (Labaki and Rosenberg, 2020; Zheng et al., 2021), which is challenging to account for in observational studies. Second, COPD and asthma have significant overlap (Leung and Sin, 2017), and asthma has been causally linked to GERD (Freuer et al., 2022; Ahn et al., 2023). Third, a considerable proportion of GERD patients do not have typical reflux symptoms, but microaspiration or silent aspiration is still suspected to contribute to the development of COPD (Lee et al., 2020), making these patients easily overlooked in observational studies. The MR method we employed partially overcomes confounding factors such as smoking and asthma by excluding SNPs associated with smoking and asthma when selecting instrumental variables for GERD and COPD. We provide evidence supporting GERD as a risk factor for COPD, which is consistent with a similar MR study conducted by Cheng et al. (2023). What sets our study apart from Cheng et al.'s findings is that we also provide evidence of a positive causal effect of COPD on GERD.

Several potential mechanisms may explain the increased risk of COPD associated with GERD. First, GERD reflux can lead to chemical or aspirational pneumonia (Sanchez et al., 2020), which can further promote the occurrence and progression of COPD (Hu et al., 2015). Studies have found that controlling GERD with proton pump inhibitors significantly reduces the risk of exacerbations in COPD (Kang et al., 2023). Second, the aspiration of gastric contents can introduce irritants into the airways, particularly gastric enzymes, which can exacerbate pulmonary inflammation by damaging lung tissues, including the bronchial wall (Iliaz et al., 2016; Iov et al., 2022). Third, the immune crosstalk related to the airway microbiome is also noteworthy. Many gut microbiota can be aspirated into the lungs with reflux, which may mediate airway inflammation in COPD patients (Kayongo et al., 2022). A typical example is the ectopic colonization of *Helicobacter pylori*, and a meta-analysis has confirmed a positive correlation between *H. pylori* infection and COPD (Wang et al., 2015). *H. pylori* exotoxins have been detected in the lungs of COPD patients, which can induce the production of interleukin-8 and interleukin-6 in human lung cells (Nakashima et al., 2015).

For the mechanism by which COPD increases the risk of GERD, several factors may explain it. First, COPD patients often experience changes in intrathoracic pressure during the breathing process, especially during exhalation. When patients exhale with more force to overcome airway narrowing, it may lead to an increase in intrathoracic pressure and secondary elevation of intra-abdominal pressure (Mancopes et al., 2020). This pressure increase may increase the risk of dysfunction of the lower esophageal sphincter, thereby increasing the risk of food and gastric acid reflux into the esophagus (Siboni et al., 2022). Second, the development of barrel chest in the middle and late stages of COPD can compress the lower esophageal sphincter, causing difficulty in closure at the junction between the lower esophagus and the stomach and leading to esophageal motility

disorders, thereby promoting esophageal reflux (Mittal and Vaezi, 2020). Third, COPD patients often need to use inhaled corticosteroids and other medications to manage their condition. Some drugs, especially corticosteroids and nonsteroidal anti-inflammatory drugs, may increase the risk of GERD because they can weaken the function of the esophageal sphincter (Mungan and Pinarbasi Simsek, 2017). Furthermore, systemic hypoxemia associated with COPD can exacerbate inflammation, malnutrition, and vascular dysgenesis, leading to the development of gastroesophageal dysfunction (Houghton et al., 2016).

PUD and COPD have also been considered to be related in observational studies. Many studies suggest that COPD may increase the risk of recurrent PUD, while others suggest that PUD could decrease the FEV1/FVC ratio and potentially serve as a risk factor for COPD (Siva et al., 2013). In another cohort study, after controlling for potential confounders, COPD patients had a significantly increased risk of PUD bleeding (HR = 1.93, 95% CI: 1.73–2.17, $p < 0.001$) (Huang et al., 2012). However, similar to the relationship between GERD and COPD, there is no appropriate randomized controlled trial (RCT) to establish the actual causal relationship between PUD and COPD due to interference from various confounding factors. Our MR study provides limited evidence of a bidirectional causal relationship between PUD and COPD, as the positive results were not validated in the validation set.

We believe that several reasons may have contributed to this result: our selection of instrumental variables for PUD and COPD using a p-value threshold of $p < 1.0E-05$ may not completely eliminate weak instrument bias, although we employed a range of sensitivity analysis methods to enhance the robustness of the results; the statistical power of the validation set we used may have been insufficient, as its total sample size was much smaller than the discovery set; there were substantial differences in population ancestry between the discovery set and the validation set. We lean towards the explanation that the statistical power of the validation set was insufficient, as our discovery analysis was conducted rigorously and did not observe significant heterogeneity and pleiotropy.

Despite the limited evidence, we still consider several possible mechanisms for the bidirectional causal relationship between PUD and COPD. Overall, both PUD and COPD are characterized by chronic inflammation. Therefore, some researchers have proposed a hypothesis that excessive pro-inflammatory mediators produced in the lungs of COPD patients may reach the intestines through the circulatory system, leading to gastrointestinal diseases; conversely, persistent gastrointestinal inflammation can exacerbate COPD due to systemic circulation (Wang et al., 2023). Regarding the increased risk of COPD associated with PUD, long-term PUD-induced inflammatory responses generate a large number of inflammatory factors, including TNF-α, IL-1, IL-6, IL-8, etc. (Fagundes et al., 2021). They can reach the lungs through the bloodstream and lymphatic system, promoting the occurrence and development of pulmonary inflammation and mutually reinforcing each other in this process (Fagundes et al., 2021). Oxidative stress induced by PUD can also exacerbate COPD inflammation (Perez et al., 2017). Malnutrition or even malnutrition in PUD patients can reduce the body's ability to resist inflammation-induced lung damage (Calder et al., 2018). Conversely, the gastrointestinal mucosa is highly reactive to hypoxia, oxidative stress, and other factors (Sverden et al., 2019), and the chronic systemic hypoxia, oxidative stress, and high inflammatory state caused by COPD become important causes of PUD (Ferrera et al., 2021).

It is worth noting that although our results suggest evidence of bidirectional causality between GERD, PUD, and COPD, it does not necessarily imply a causal relationship. This is due to the inherent limitations of Mendelian randomization and the specific limitations of our study. Despite employing various methods to mitigate potential confounding factors, we cannot completely rule out the possibility that GERD, PUD, and COPD share some genetic factors that could be influenced by common underlying factors.

Previous observational studies have indicated a certain correlation between IBS and COPD (Lai et al., 2020; Chiu et al., 2022), but this association has not been clearly established. Our MR study suggests that COPD increases the risk of IBS, while IBS does not alter the risk of COPD. There are several potential mechanisms that may explain this causal relationship. First, the long-term systemic chronic inflammation caused by COPD can disrupt intestinal immunity and the gut microbiome environment (Bowerman et al., 2020), further promoting IBS (Grayson, 2016; Canakis et al., 2020). The chronic systemic hypoxia resulting from COPD can lead to increased intestinal epithelial cell permeability and dysbiosis (Sprooten et al., 2018), further increasing the risk of IBS (Zhang et al., 2022). COPD patients often experience comorbid depression and anxiety (Pumar et al., 2014), which can be detrimental to IBS patients (Fond et al., 2014).

The association between CP and COPD has received less attention in previous research, but some studies have observed an increased risk of CP in COPD patients (Sun et al., 2013; Gursoy Coskun et al., 2021). Our MR results are consistent with observational study findings, indicating that COPD increases the risk of CP, but CP does not increase the risk of COPD. There are several possible mechanisms for this association. Firstly, COPD patients often experience symptoms such as shortness of breath and respiratory distress, which may lead to reduced physical activity levels. Insufficient physical activity can affect normal bowel motility and defecation rhythms, potentially triggering CP (Booth et al., 2012). Secondly, COPD is a chronic inflammatory disease, which may affect intestinal function, including intestinal motility, thereby increasing the risk of CP (Shatri et al., 2023). Additionally, gut microbiome dysbiosis and emotional changes induced by COPD may also contribute to an increased risk of CP (Bowerman et al., 2020; Shatri et al., 2023).

The relationship between CD, UC, FD, NGE, and COPD has also garnered attention in previous research, and observational studies have suggested a strong association between CD, UC, FD, NGE, and COPD (Brassard et al., 2015; Chiu et al., 2022; Mathews et al., 2022). However, our MR study did not find evidence of an association between them. It is important to note that the absence of evidence in our MR study does not definitively rule out the possibility of causal relationships between CD, UC, FD, NGE, and COPD. Larger MR studies or randomized controlled trials may be necessary to further investigate these potential associations.

Compared to previous observational studies, our MR study has several advantages. It is less susceptible to confounding factors and reverse causality. Each summary data set had a relatively large sample size, providing sufficient statistical power. Confirmatory

MR analysis and extensive sensitivity analyses assessed pleiotropy and potential statistical bias, enhancing the robustness of the results.

However, this study also has notable limitations. MR studies can be affected by horizontal pleiotropy, and we employed various methods to mitigate this bias. The population data selected for our study were predominantly of European ancestry, which limits the generalizability of causal relationships to other populations. Negative results in MR studies do not completely rule out causal associations because genetically determined exposures may not fully represent true exposures, and stricter selection of instrumental variables may result in negative results. Finally, due to the lack of appropriate data, we were unable to conduct gender-stratified and population-stratified analyses.

Conclusion

In summary, our results support a bidirectional causal relationship between GERD and COPD, while COPD increases the risks of IBS and CP. Additionally, our study also suggests a bidirectional causal relationship between PUD and COPD, although the evidence strength may be insufficient and further research is needed to confirm this. Furthermore, we provide evidence that there is no causal association between CD, UC, FD, NGE, and COPD. Physicians and patients should pay greater attention to the management of gastrointestinal diseases and COPD patients, as the relationship between them may not be simply susceptibility to the environment. Further exploration of the potential mechanisms of mutual interference between GERD, PUD, and COPD, as well as the mechanisms by which COPD alters the risks of IBS and CP, is warranted.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: The summary data for gastroesophageal reflux disease is available at "https://gwas.mrcieu.ac.uk/"; the summary data for peptic ulcer disease is available at "https://cnsgenomics.com/content/data"; the summary for Irritable bowel syndrome, Crohn's disease, ulcerative colitis, non-infectious gastroenteritis and constipation data are available at "https://www.ebi.ac.uk/gwas/"; data for chronic obstructive pulmonary disease (discovery) is available at "https://www.ebi.ac.uk/gwas/"; data for chronic obstructive pulmonary disease (discovery) and constipation are available at "https://www.finngen.fi/en" to obtain.

Author contributions

ZS: Data curation, Formal Analysis, Investigation, Supervision, Validation, Writing–original draft, Writing–review and editing. BQ: Data curation, Software, Writing–original draft, Writing–review and editing. LC: Conceptualization, Data curation, Formal Analysis, Investigation, Writing–original draft, Writing–review and editing. YZ: Conceptualization, Funding acquisition, Investigation, Resources, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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The causal association between smoking, alcohol consumption and risk of upper urinary calculi: insights from a Mendelian randomization study

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Background: The causal link between smoking, alcohol consumption, and upper urinary calculi remains uncertain in observational studies due to confounding factors. To uncover potential causal associations, we utilized two-sample univariable and multivariable Mendelian randomization (MR) methods.

Methods: Five risk factors related to lifestyles (cigarettes per day, lifetime smoking index, smoking initiation, drinks per week and alcohol intake frequency) were chosen from the Genome-Wide Association Study (GWAS). Upper urinary calculi were obtained from the FinnGen and United Kingdom Biobank consortium. Inverse-variance-weighted (IVW) was mainly used to compute odds ratios (OR) and 95% confidence intervals (CI). While diligently scrutinizing potential sources of heterogeneity and horizontal pleiotropy via the rigorous utilization of Cochran's Q test, the MR-PRESSO method, and MR-Egger.

Results: The summary OR for upper urinary calculi was 0.6 (IVW 95% CI: 0.49–0.74; $p = 1.31 \times 10^{-06}$) per standard deviation decrease in drinks per week. Interestingly, the genetically predicted alcohol intake frequency was associated with a significantly increased risk upper urinary calculi (OR = 1.27; 95% CI: 1.11–1.45; $p = 0.0005$). Our study found no association between smoking initiation, the number of cigarettes per day, and the lifetime smoking index and the risk of upper urinary calculi. By adjusting for body mass index and education, estimates of drinks per week remained consistent in multivariate MR analyses, while alcohol intake frequency became non-significant.

Conclusion: MR analysis showed that drinks per week was negatively associated with upper urinary calculi, whereas the effect of tobacco on upper urinary calculi was not significant and the detrimental effect of alcohol intake frequency on upper urinary calculi became non-significant after adjusting for BMI and education.

KEYWORDS

Mendelian randomization, upper urinary calculi, smoking, alcohol, risk

1 Introduction

There was a notable surge of 48.57% in the prevalence of urinary stones from 1999 to 2019, predominantly affecting the upper urinary tract-specifically, the kidneys and ureters (Zhu et al., 2021). These upper urinary calculi are highly prevalent and frequently result in debilitating conditions such as renal colic, hydronephrosis, and in severe cases, uremia (Hájková et al., 2021). Regrettably, the impact on patients' quality of life is significant, with the lifetime incidence of kidney stones standing at 14% (Marić et al., 2019). More concerning is that at least half of those affected experience recurrent stone episodes within 10 years, leading to a substantial economic and lifestyle burden (Zhang et al., 2022).

Smoking and alcohol consumption are recognized as modifiable health behaviors with potential as risk factors. Extensive research has investigated their association with urolithiasis. yet the relationship between smoking, alcohol consumption, and the risk of urinary calculi remains uncertain, exhibiting conflicting findings in the epidemiological literature (Hall et al., 2001; Liu et al., 2009; Słojewski et al., 2009; Zhao et al., 2015). Several cross-sectional studies have posited an independent role for smoking in the formation of kidney stone (Hamano et al., 2005; Liu et al., 2009), whereas another study found that the quantity and duration of smoking were not significantly associated with stone formation (Słojewski et al., 2009; Marić et al., 2019). Moreover, a meta-analysis encompassing fourteen studies reported no significant impact of alcohol intake on the incidence of kidney stones (Jones et al., 2021). In contrast, a recent cohort study indicated a notable negative association between alcohol consumption and the formation of kidney stones (Kim et al., 2022).

The establishment of causality is crucial in clinical intervention planning and the formulating of public health policies. However, observational studies often grapple with confounding factors and the risk of reverse causality bias. While randomized controlled trials (RCTs) are the gold standard for elucidating etiological relationships, they are not without limitations in design and ethical constraints. Mendelian randomization (MR) offers a solution by using single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to ascertain causality, thereby reducing bias from confounding variables (Burgess and Labrecque, 2018). Multivariate Mendelian randomization (MVMR) extends the principles of univariate MR (UVMR) by accounting for the complexity of exposure characteristics and allows for simultaneous assessment of multiple, interrelated exposures (Sanderson, 2021).

In our study, we employed both UVMR and MVMR analyses on two separate cohorts to explore the potential causal link between genetic predisposition to smoking and alcohol consumption and the susceptibility to upper urinary calculi.

2 Methods

2.1 Study design

The study adhered rigorously to the guidelines set forth in the Strengthening the Reporting of Observational Studies in Epidemiology Mendelian randomization (STROBE-MR) framework (Supplementary Table S1) (Skrivankova et al., 2021). MR is predicated on three fundamental assumptions: IVs must exhibit a robust association with exposure, they should be unaffected by confounding variables, and they should influence the outcome exclusively through the exposure (Burgess et al., 2015). Our research implemented MR analyses in strict accordance with these core principles, as illustrated in Figure 1.

2.2 Choosing instrumental variables

To ensure the stability of the causal relationship between exposure and outcome, IVs were selected based on the following principles (Zhu et al., 2021): We established genome-wide significance thresholds for exposure at $p < 5 \times 10^{-8}$ (Hájková et al., 2021). Cluster analysis was conducted to address linkage disequilibrium (LD) among the selected IVs ($r^2 < 0.001$, kb = 10,000) (Marić et al., 2019). To mitigate bias from weak IVs. The strength of the IVs was quantified using the F value (β^2/SE), with those having F < 10 being excluded. Here, β represents the effect size of exposure and SE represents the standard error of the effect size.

2.3 Data sources

Genetic summary data on smoking initiation, cigarettes per day, and alcohol consumption measured in drinks per week were acquired from the Sequencing Consortium of Alcohol and Nicotine use (GSCAN) (Saunders et al., 2022). This consortium's dataset encompasses information from 2,669,029 individuals of European descent. The genome-wide association study (GWAS) dataset on the lifetime smoking index included details on smoking duration, intensity, and quitting, which were combined to create a simulated half-life (τ) constant and a lifetime smoking index (n = 462,690) (Wootton et al., 2020). GWAS data on the frequency of alcohol intake were obtained from the questionnaire "How often do you drink alcohol?" with ordered categorical variables (n = 462,346) (Hemani et al., 2018).

Genetic association summary data for upper urinary calculi were obtained from the ninth release of the FinnGen Consortium database, encompassing 376,406 individuals of Finnish ancestry, including both males and females (Kurki et al., 2023). The analysis excluded individuals exhibiting extreme heterozygosity (±4 SD), a genotyping deletion rate (>5%), ambiguous gender, or non-Finnish ancestry. The genetic associations' effect sizes were estimated using logistic regression, controlling for age, sex, and principal genetic components. Additionally, cases of upper urinary calculi in the United Kingdom Biobank were identified based on the International Classification of Diseases, Ninth Revision (ICD-9) and 10th Revision (ICD-10) criteria. Correlation tests were adjusted for confounding variables, including age at enrolment, gender, and the first ten principal genetic components. Table 1 shows detailed information about the GWAS data.

2.4 Power calculations

We conducted an *a priori* power calculation with an α of 5% (Brion et al., 2013). Ensuring that we had at least 80% power to

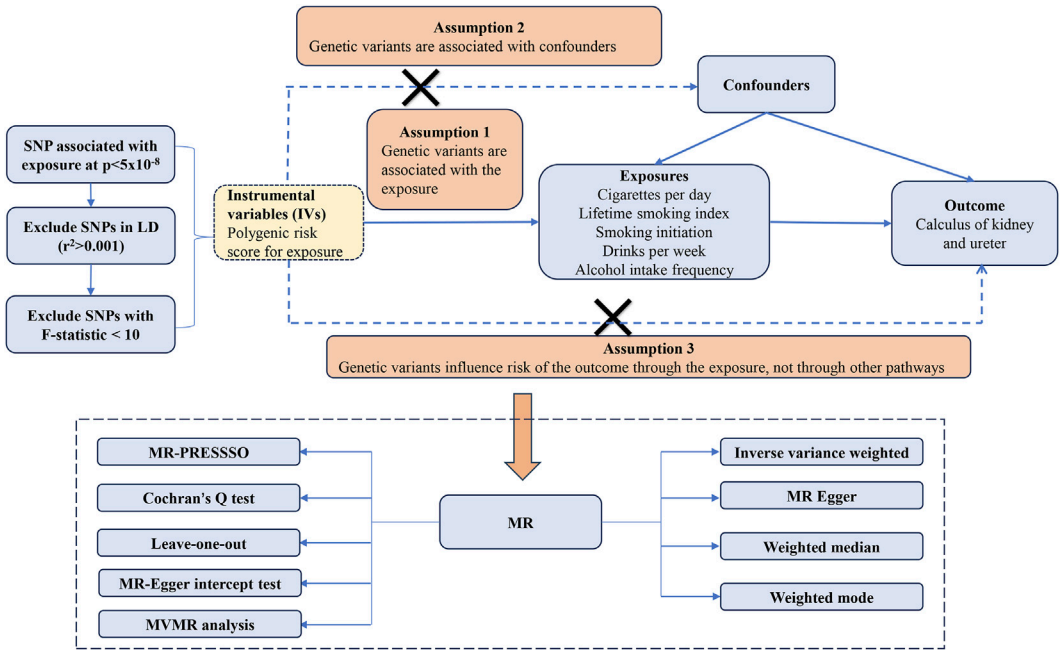

FIGURE 1
Flowchart of a MR study.

TABLE 1 Phenotypic descriptive statistics of studies included in the exposure and outcome genome-wide association study.

Exposures/outcome	Type	Sample size N)	Consortium	Year	PubMed ID	Source
Smoking initiation	Categorical	3,383,199	GSCAN	2022	36,477,530	https://genome.psych.umn.edu/index.php/GSCAN
Cigarettes per day	Continuous	784,353	GSCAN	2022	36,477,530	https://genome.psych.umn.edu/index.php/GSCAN
Lifetime smoking index	Continuous	462,690	NA	2020	31,689,377	NA
Drinks per week	Continuous	2,965,643	GSCAN	2022	36,477,530	https://genome.psych.umn.edu/index.php/GSCAN
Alcohol intake frequency	Continuous	462,346	MRC-IEU	2018	NA	https://gwas.mrcieu.ac.uk/datasets/ukb-b-5779/
Calculus of kidney and ureter	Categorical	9,713/366,693	FinnGen	2022	NA	https://r9.finngen.fi/
Calculus of kidney and ureter	Categorical	5,530/420,531	United Kingdom Biobank	2022	NA	https://www.ukbiobank.ac.uk

GSCAN GWAS, and Sequencing Consortium of Alcohol and Nicotine use.

detect Odds Ratios (ORs) for upper urinary calculi of 1.30, 1.42, 1.27, 1.25, and 1.10 for the respective variables of smoking initiation, cigarettes per day, lifetime smoking index, drinks per week, and alcohol intake frequency in the FinnGen data. Similarly, in the United Kingdom Biobank data, we could detect ORs of 1.11, 1.09, 1.51, 1.32, and 1.41 for these variables.

2.5 Other factors

To mitigate potential pleiotropy arising from indirect pathways, we utilized MVMR analyses. By consulting the PhenoScanner database, we pinpointed associations of IVs with education and obesity-related traits that met the genome-wide significance threshold ($p < 5 \times 10^{-8}$). Consequently, we meticulously selected SNPs linked to education (n = 1,131,881) (Lee et al., 2018) and BMI (n = 681,275) (Yengo et al., 2018) for our multivariate analysis.

2.6 Statistical analyses

Our primary analysis utilized the robust inverse-variance weighted (IVW) method (Burgess et al., 2013). For validation, we applied supplementary methods including weighted median, MR-Egger regression, and weighted mode. The weighted median

TABLE 2 Heterogeneity and MR-Egger test for directional pleiotropy.

Exposure/Data source	Heterogeneity (IVW)		MR-Egger test for directional pleiotropy		
	Q	*P*	Intercept	Standard error	*P*
Cigarettes initiation					
FinnGen	23.5	0.234	−0.015	0.039	0.632
United Kingdom Biobank	18.7	0.632	0.003	0.022	0.745
Cigarettes per day					
FinnGen	104.4	0.952	0.004	0.006	0.503
United Kingdom Biobank	125.2	0.567	0.001	0.009	0.956
Lifetime smoking index					
FinnGen	132.6	0.124	−0.002	0.008	0.771
United Kingdom Biobank	150.1	0.062	−0.010	0.012	0.416
Drinks per week					
FinnGen	35.2	0.598	0.015	0.012	0.227
United Kingdom Biobank	41.2	0.331	−0.003	0.025	0.913
Alcohol intake frequency					
FinnGen	74.9	0.749	0.005	0.005	0.337
United Kingdom Biobank	108.8	0.075	0.004	0.006	0.438

approach, renowned for its reliability, provided consistent results by prioritizing the influence of the most powerful instrumental variable, which carried a 50% weight (Bowden et al., 2016). To tackle potential directional pleiotropy, we further employed MR-Egger regression and weighted mode methods (Bowden et al., 2015; Hartwig et al., 2017).

We assessed the genetic correlation for upper urinary calculi between the United Kingdom Biobank and FinnGen consortium using LD Score Regression (LDSC) software, which revealed high consistency (rg = 0.80; $p = 3.75 \times 10^{-22}$) (Bulik-Sullivan BK. et al., 2015; Bulik-Sullivan B. et al., 2015). Thus, we integrated the data using a fixed-effects model. $I^2 > 50\%$ was interpreted as indicative of high heterogeneity.

Sensitivity analysis plays a critical role in evaluating heterogeneity and potential biases in MR studies. We first assessed heterogeneity using Cochran's Q test, which calculates the weighted sum of squared differences between individual study estimates and the overall IVW estimate (Burgess et al., 2017). To detect and adjust for potential outliers, we applied the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) detection method (Verbanck et al., 2018). Additionally, MR-Egger regression was utilized to test for potential horizontal pleiotropy by examining the regression intercept. Furthermore, the Steiger test was employed to rule out potential inverse associations (Hemani et al., 2017).

Statistical analyses were conducted utilizing R version 4.2.2, employing the "TwoSampleMR", "MRPRESSO", "meta" and "MVMR" packages. Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) quantified the MR analysis, with a $p < 0.05$ denoting statistical significance.

3 Results

3.1 Selection of genetic variants and F-statistics

After the initial screening of SNPs, and subsequent removal of variants in potential linkage disequilibrium (threshold: $r^2 = 0.001$, 10,000 kb) and applying Steiger filtering, a total of 433, 84, 117, 232, and 90 SNPs were used as IVs for the number of smoking initiations, cigarettes per day, lifetime smoking index, drinks per week, and alcohol intake frequency, respectively. These SNPs explained 1.5%, 1.27%, 1.05%, 0.83%, and 2.13% of the phenotypical variance, respectively. Importantly, all the included SNPs had F-values exceeding 10, indicating a minimal likelihood of weak IVs bias (Supplementary Tables S2–S11).

3.2 Heterogeneity and pleiotropy

The absence of heterogeneity and the absence of directional pleiotropy were demonstrated in all analyses, as shown in Table 2. Furthermore, the MR-PRESSO analyses did not identify any outliers, indicating a robust dataset (all p for Global test >0.05) (Table 3).

3.3 Univariate MR analysis

Genetic predisposition to increased drinks per week was found to be significantly associated with a decreased risk of upper urinary

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TABLE 3 MR-PRESSO results.

Exposure/Data source	MR-PRESSO				
	OR	95% CI	*P*	RSSobs	*p*-value for global test
Cigarettes initiation					
FinnGen	0.87	0.72–1.06	0.186	522.8	0.225
United Kingdom Biobank	1.38	1.10–1.74	0.005	484	0.053
Cigarettes per day					
FinnGen	0.99	0.76–1.28	0.94	107.2	0.559
United Kingdom Biobank	0.79	0.59–1.06	0.13	127	0.959
Lifetime smoking index					
FinnGen	1	0.80–1.26	0.95	139.1	0.052
United Kingdom Biobank	1.74	1.31–2.30	0.0001	134.8	0.143
Drinks per week					
FinnGen	0.55	0.42–0.72	1.92E-05	209.5	0.332
United Kingdom Biobank	0.68	0.49–0.94	0.02	270.3	0.06
Alcohol intake frequency					
FinnGen	1.30	1.09–1.55	0.003	122.5	0.303
United Kingdom Biobank	1.21	0.98–1.49	0.06	110.8	0.07

OR, odds ratio; RSSobs, observed residual sum of squares.

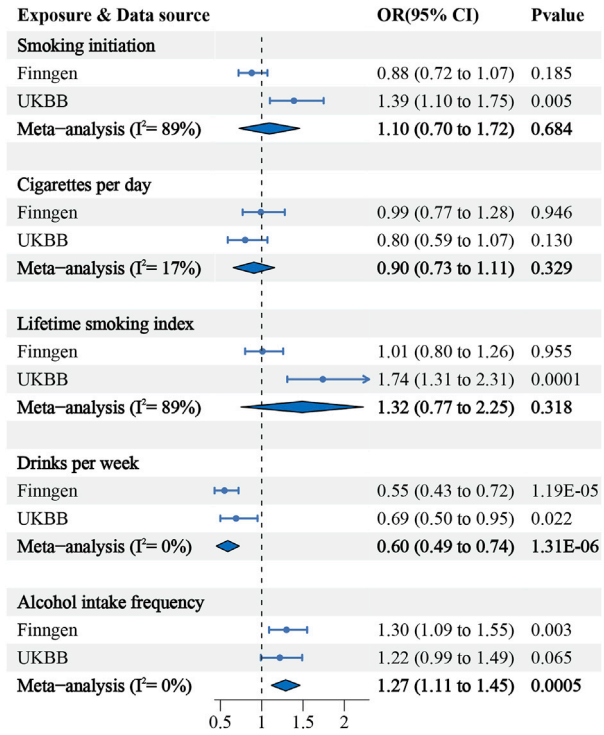

FIGURE 2
Association of smoking, alcohol consumption with the risk of upper urinary calculi in the MR.

calculi in both the FinnGen consortium and United Kingdom Biobank study. The combined OR for upper urinary calculi was 0.6 (IVW 95% CI: 0.49–0.74; $p = 1.31 \times 10^{-06}$) per standard deviation decrease in drinks per week. Interestingly, the genetically predicted alcohol intake frequency was associated with a significantly increased risk upper urinary calculi (IVW combined OR = 1.27; 95% CI: 1.11–1.45; $p = 0.0005$). Our study found no association between smoking initiation, the number of cigarettes per day, and the lifetime smoking index and the risk of upper urinary tract stones (Figure 2). These results were consistently reproduced in supplementary analyses (Supplementary Figure S1).

3.4 Multivariate MR analysis

The PhenoScanner search identified links between IVs and traits related to education and obesity. After conducting multivariate IVW analyses, considering several potentially relevant adjustments including education and BMI, we observed that drinks per week remained significantly associated with a lower risk of upper urinary calculi (IVW combined OR = 0.52; 95% CI: 0.36–0.75; $p = 0.001$). However, the association between alcohol intake frequency and upper urinary calculi became non-significant (IVW combined OR = 1.07; 95% CI: 0.97–1.17; $p = 0.174$). Furthermore, when adjusting for the lifetime smoking index, alcohol intake frequency was found to be associated with a higher risk of upper urinary calculi (IVW combined OR = 1.90; 95% CI: 1.27–2.83; $p = 0.002$) (Figure 3).

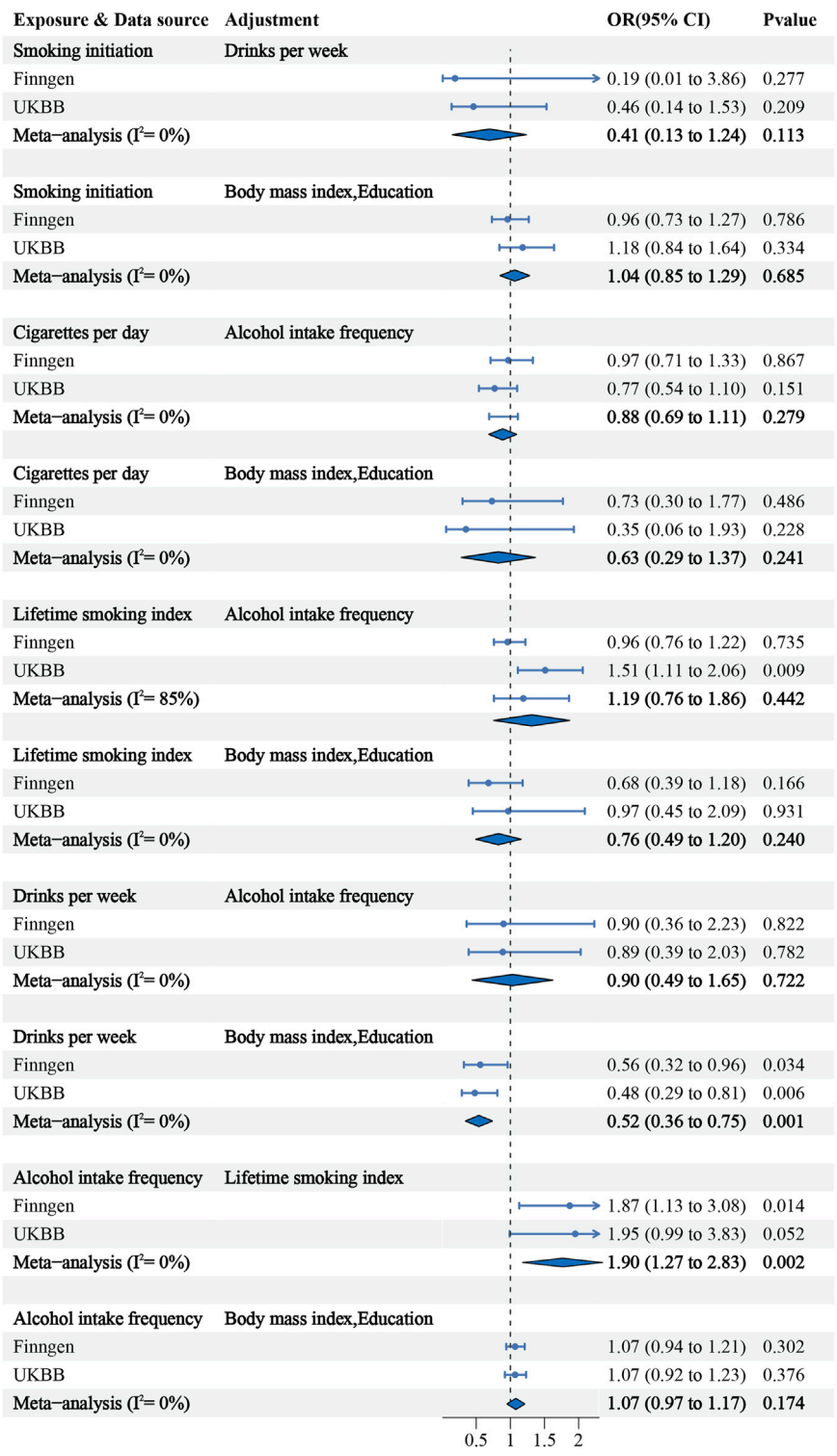

FIGURE 3
Forest plot for MVMR adjusted risk factors.

4 Discussion

Using two-sample MR, we assessed for the first time the potential causal association between smoking and alcohol consumption on upper urinary calculi, and the results revealed that drinks per week was negatively associated with the occurrence of upper urinary calculi. This finding, together with other indicators, deserves in-depth discussion.

The effect of smoking on kidney stones is a subject of controversy. On one hand, Hamano et al. (Hamano et al., 2005)

conducted a multivariate logistic regression analysis, revealing that smoking significantly increases the risk of kidney stones. Additionally, Liu et al. (Liu et al., 2009) identified smoking as an independent risk factor for the development of calcinuria. Notably, Soueidan et al. (Soueidan et al., 2015) conducted an investigative study, which indicated that patients with kidney stones had a higher prevalence of smoking (7% vs 21%, $p = 0.02$), and they were 8.5 times more likely to be current smokers. Potential mechanisms through which smoking contributes to urolithiasis formation include a considerable increase in plasma antidiuretic hormone due to smoking, leading to reduced urine output and promoting urinary supersaturation of crystals. Additionally, smoking contributes, to a lesser extent, to an increase in the production of reactive oxygen species (ROS), As signaling molecules and involved in receptor regulation, ROS activate transcription factors via P38 mitogen-activated protein kinase (-MAPK)/JNK. Thus, ROS-induced transcriptional activation leads to the production of prostaglandins and pro-inflammatory factors that impair endothelial function (Coe et al., 2005; Liu et al., 2009; Wigner et al., 2021). On the other hand, two cross-sectional studies conducted on Chinese populations found no significant association between smoking and stone formation (Dai et al., 2013; Zhao et al., 2015). Moreover, Słojewski et al. (Słojewski et al., 2009) reported an increase in urinary Hg levels in smokers, but the statistical significance was moderate. This finding does not support a possible association between smoking and urinary tract stone formation.

Currently, there is a divergence of opinions regarding the impact of drinking alcohol on the formation of urinary stones. According to Siener and others. The study discovered that individuals who consumed alcohol had higher levels of calcium in their urine, leading to temporary hypercalciuria. This could potentially raise the risk of developing calcium oxalate stones (Siener, 2006). Moreover, it is believed that alcohol enhances the production of uric acid and elevates the likelihood of developing uric acid stones. Furthermore, the consumption of alcohol causes oxidative stress on the tissue of the kidneys, which may potentially facilitate the development of kidney stones (Jones et al., 2021). It is crucial to emphasize that certain research has indicated a possible safeguarding impact of drinking alcohol. An analysis of multiple studies found that drinking alcohol was linked to a decreased overall chance of developing urolithiasis (OR = 0.683, 95%Cl: 0.577–0.808). Moreover, a correlation was observed suggesting that for each 10 g/day rise in alcohol consumption, there was a corresponding 10 percent decrease in the occurrence of urolithiasis (Wang et al., 2015).

Alcohol, on the other hand, hinders the release of vasopressin, resulting in an augmentation of urine output and dilution of urine. However, beer might also include defensive compounds discovered in hops. Studies have demonstrated that xanthohumol and humulone, which are the key components found in hops extract, possess potent abilities to suppress bone resorption (Tobe et al., 1997). As a result, these substances decelerate the release of calcium from the skeletal system and decrease the excretion of calcium. Lastly, red wine contains antioxidants that act as inhibitors of stone formation (Zecher et al., 2009). Indeed, considering the permanent harm caused by substances like acetaldehyde when alcohol is ingested beyond the limit, it is imperative to have a thorough and precise comprehension of alcohol intake.

4.1 Strength and limitation

Our MR analysis has the following advantages. Firstly, this is the first large-scale use of GWAS data to infer causal associations between smoking, alcohol consumption and upper urinary calculi, thereby reducing confounders and reverse bias. Secondly, the study population included only individuals of European origin, minimizing population stratification interference. Finally, sensitivity analyses and different model estimations were used to ensure the reliability of the results.

However, certain limitations are unavoidable. Firstly, the results of the study failed to be validated in other populations. Moreover, smoking and drinking habits are often combined, making it difficult to analyze them as independent factor. Secondly, there was a lack of data on alcohol type and consumption levels. Also, there was a failure to differentiate comparisons between genders, and multivariate analyses were unable to overcome bias because of multiplicity of effects in pathways other than education or obesity. Finally, future studies require larger sample sizes and precise stratified analyses to identify underlying physiopathological mechanisms.

5 Conclusion

There may be a causal link between drinks per week and risk of upper urinary calculi in people of European ancestry. In contrast, the detrimental effect of alcohol intake frequency on upper urinary calculi became non-significant after adjusting for BMI and education, and there is a need to further validate the potential effects and mechanisms of action of appropriate alcohol consumption on urolithiasis in the future.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

LW: Writing–original draft. SY: Data curation, Writing–original draft. KL: Data curation, Writing–original draft. EB: Methodology, Writing–original draft. J-HW: Methodology, Writing–original draft. PZ: Data curation, Software, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1268720/full#supplementary-material

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A mendelian randomization study investigates the causal relationship between immune cell phenotypes and cerebral aneurysm

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Background: Cerebral aneurysms (CAs) are a significant cerebrovascular ailment with a multifaceted etiology influenced by various factors including heredity and environment. This study aimed to explore the possible link between different types of immune cells and the occurrence of CAs.

Methods: We analyzed the connection between 731 immune cell signatures and the risk of CAs by using publicly available genetic data. The analysis included four immune features, specifically median brightness levels (MBL), proportionate cell (PC), definite cell (DC), and morphological attributes (MA). Mendelian randomization (MR) analysis was conducted using the instrumental variables (IVs) derived from the genetic variation linked to CAs.

Results: After multiple test adjustment based on the FDR method, the inverse variance weighted (IVW) method revealed that 3 immune cell phenotypes were linked to the risk of CAs. These included CD45 on HLA DR+NK (odds ratio (OR), 1.116; 95% confidence interval (CI), 1.001–1.244; $p = 0.0489$), CX3CR1 on $CD14^{-}$ $CD16^{-}$ (OR, 0.973; 95% CI, 0.948–0.999; $p = 0.0447$). An immune cell phenotype $CD16^{-}$ CD56 on NK was found to have a significant association with the risk of CAs in reverse MR study (OR, 0.950; 95% CI, 0.911–0.990; $p = 0.0156$).

Conclusion: Our investigation has yielded findings that support a substantial genetic link between immune cells and CAs, thereby suggesting possible implications for future clinical interventions.

KEYWORDS

cerebral aneurysms, immune cell phenotypes, MR analysis, causal association, NK cell

Introduction

Localized dilations resembling balloons in major branches of brain arteries, known as CAs, are found in about 3.2% of the population (Vlak et al., 2011). Aneurysmal subarachnoid hemorrhage (aSAH), which is an extremely dangerous type of stroke, can occur when aneurysms rupture. Around one-third of aSAH patients face death, while another third achieve complete recovery, and the remaining third show significant dependency, suggesting an unfavorable outlook (Nieuwkamp et al., 2009). Despite a

heritability estimate of 41% for aSAH derived from a prior twin study (Korja et al., 2010), the etiology of brain aneurysms remains inadequately comprehended.

Lately, there has been significant fascination with the connection between immune cells and various illnesses. Studies have determined a cause-and-effect relationship between the number of immune cells in the outer regions of the body and atrial fibrillation. Further analysis of specific groups has shown that increased levels of $CD4^+$ T cells are associated with a higher likelihood of developing atrial fibrillation (Feng et al., 2022). Furthermore, NKT cells play a crucial part in the development of multiple sclerosis (He et al., 2022). A recent investigation revealed that endothelial cells play a crucial role in the susceptibility to CAs. The discovery was made by conducting a meta-analysis of genome-wide association studies (GWAS) across different European and East Asian countries, involving 10,754 cases and 306,882 controls of various racial backgrounds. Additionally, the study revealed that smoking and susceptibility to blood pressure were prominent genetic risk factors for CAs (Bakker et al., 2021). Moreover, mounting proof indicates that immune cells inside CAs have a vital function in their formation and eventual bursting. According to a study, there is evidence indicating an inequilibrium in Th17 and Treg cells among people with CAs. Additionally, there is a direct relationship between the frequency of Th17 cells and the seriousness of spontaneous subarachnoid hemorrhage (SAH) (Song et al., 2021). Furthermore, impaired regulatory T cell function has been observed in CAs (Zhang et al., 2018; Zhao et al., 2018). Nevertheless, the presence of a conclusive cause-and-effect connection between CAs and the characteristics of immune cells remains ambiguous.

The utilization of genetic variation as independent variables in MR is a methodology employed to examine the causal relationship between exposure and outcome. The possible correlations between genetic variation associated with exposure and outcome may suggest the influence of exposure on the outcome (Lawlor et al., 2008). Since genetic variations are allocated randomly during conception, this effect remains unaltered by confounding factors and reverse causation. As a result, we utilized recently released combined information on immune cells and combined data from a GWAS on midbrain aneurysms to explore the causal link between immune cell characteristics and CAs using a two-sample MR method in this study.

Methods

Sources of data for exposure and outcome

Single nucleotide polymorphisms (SNPs) linked to CAs were obtained from a vast GWAS dataset comprising 945 cases and 472,738 controls of European descent (Sakaue et al., 2021). Due to the lack of personal information in this data, we do not consider the impact of age, gender, etc. on the results. The GWAS Catalog provides publicly available summary statistics for each immune trait, with accession numbers ranging from GCST90001391 to GCST90002121 (Orrù et al., 2020). The dataset includes a total of 731 phenotypes of immune cells, which consist of 118 absolute cell counts, 389 median fluorescence intensities indicating surface antigen levels, 32 morphological parameters, and 192 relative cell counts. To ensure that there were no overlapping cohorts, the initial GWAS on immune traits utilized information from 3,757 individuals of European ancestry. A Sardinian sequence-based reference panel was used to impute genotypes for around 22 million SNPs, utilizing high-density arrays. Associations were then assessed after adjusting for covariates (Sidore et al., 2015; Wang et al., 2023).

Selection of IVs

The IVs were screened using the following criteria: (Vlak et al., 2011): Choosing IVs with a significance level below: $p < 1.0 \times 10^{-5}$; (Nieuwkamp et al., 2009); Applying a clumping window size of 10,000 kb and a threshold of $R2 < 0.001$ to reduce linkage imbalance (LD) and avoid bias in the outcomes. It is essential to attribute the impact of SNPs on outcome and exposure to only one allele during MR analysis. Consequently, SNPs exhibiting a palindromic structure were excluded. The F value, calculated as (R2 (n-k-1))/(k (1-R2)), is frequently used to evaluate the degree of correlation between the exposure and IVs. In this context, "n" stands for the quantity of exposure samples in the GWAS, "k" indicates the number of IVs, and "R2" represents the degree to which IVs explain the variation in exposure. Typically, a F statistic lower than 10 is considered as a sign of a weak independent variable, which could potentially affect the results of the study. Then, 508 IVs for CAs were obtained for later analysis, Supplementary Tables S1–S22 contained details about the specific IVs. In addition, our research utilized reverse MR analysis to investigate the causal influence of CAs on phenotypes of immune cells.

Statisitical analysis

To assess the causal connection between 731 immune cell phenotypes and CAs, several techniques were employed including IVW, MR-Egger, weighted median, weighted mode and simple mode. Furthermore, the Cochran's Q statistic and its corresponding *p*-values were utilized to evaluate the heterogeneity among the chosen independent variables. In order to account for possible horizontal pleiotropy, MR-Egger was employed to assess whether the SNPs included in the study demonstrated such effects (Burgess and Thompson, 2017). However, when the MR-Egger method found significant outliers, we used the MR-PRESSO as the primary analysis because the regression accuracy of the MR-Egger was generally lower than that of the MR-PRESSO outlier test (Burgess and Thompson, 2017), and the MR-PRESSO method was utilized to address the possibility of pleiotropy by considering the MR pleiotropic residuals and outliers (Verbanck et al., 2018; He et al., 2022). We then applied the weighted median method, which can also estimate causality, but only if it comes from at least 50% of valid IVs (Bowden et al., 2016). Additionally, the accuracy of simple model is lower than that of weighted model, both of which use causal estimates from a single SNP to form clusters, but weighted model assigns weight to each SNP, but they may be superior to MR-Egger in detecting causal effects (Hartwig et al., 2017; Zuo and Li, 2023). To evaluate the correlation between specific SNPs and the obtained outcomes, a Leave-one-out (LOO) sensitivity analysis was utilized in the study (Burgess et al., 2017). This analysis aimed to determine if any single SNP was driving the association. Furthermore, scatter

diagrams were employed to evaluate the influence of anomalies on the findings, whereas funnel diagrams were used to assess the reliability of the correlation and the lack of variability. The MR Analysis was conducted using the R (version 4.2.1) software package TwoSampleMR.

Results

Genetic prediction of immunophenotypes on CAs

In order to explore the causal effect of immunophenotypes on CAs, two-sample MR analysis was used, and IVW method was used as the main analysis method. The CD8br NKT % lymphocyte was not included because of the substantial variability observed in both the Cochran Q test and MR-PRESSO global test. After multiple correction using FDR method, 10 immune cell phenotypes had causal effects on the risk of CAs (FDR < 0.06), including three immune cell phenotypes with significant association (FDR < 0.05). Figure 1 illustrates that the IVW approach revealed a correlation between 10 immune cell phenotypes and the susceptibility to CAs. For FSC-A on lymphocyte, the Cochran Q test did detect significant heterogeneity (Supplementary Table S23), although there was some heterogeneity, it did not affect the result of the IVW method, and the result was robust. However, for other immune cell phenotypes, the Cochran Q test did not detect any significant heterogeneity, thus a fixed-effect model was employed to estimate the MR Effect size (Burgess et al., 2019). A protective effect was suggested by the negative correlation of 2 immune cell phenotypes (CD3 on NKT and CX3CR1 on CD14- CD16-). Using the IVW method, the odds ratio (OR) of CD3 on NKT for brain aneurysm risk was estimated to be 0.926 (95%CI = 0.859 to 0.997, p = 0.0424, FDR = 0.0424). However, other methods such as MR Egger (OR = 1.003, 95%CI = 0.914–1.100, p = 0.9504), Weighted median (OR = 0.977,95%CI = 0.902–1.059, p = 0.5718), Simple mode (OR = 0.780, 95%CI = 0.570–1.069, p = 0.1432), and Weighted mode (OR = 0.978, 95%CI = 0.905–1.058, p = 0.5909) did not yield similar results. The disease risk was positively correlated with the phenotype of the remaining immune cell (CD45 on HLA DR+ NK). Using the IVW method, the OR value of CD45 on HLA DR+ NK for the risk of CAs was estimated to be 1.116 (95%CI = 1.001–1.244, p = 0.0489, FDR = 0.0489). MR-Egger intercept showed no significant statistical results of horizontal pleiotropy (except for CD3 on NKT (p = 0.027) and Unsw mem % B cell (p = 0.024), all p > 0.05). Despite their pleiotropy, they were not estimated to be significant after outlier-corrected. Therefore, our results are robust. No indication of genetic pleiotropy bias was found in the sensitivity analysis. Supplementary Table S23 contains the findings from the heterogeneity test, pleiotropy test and MR-PRESSO global test.

Causal effect of CAs risk on immune cell phenotypes

Following that, a two-way MR investigation was carried out to explore the possible cause-and-effect connection between the risk of CAs and the characteristics of immune cells. This study utilized the 22 immunocell phenotypic SNPs previously associated with CAs. According to the IVW method, the results showed that there is an inverse relationship between the occurrence of CAs and the presence of CD16-CD56 on natural killer (NK) cells, with an odds ratio (OR) of 0.950 and a 95% confidence interval (CI) of 0.911–0.990, indicating statistical significance (p = 0.0156, FDR = 0.0156). The findings from Figure 2A were in line with the MR Egger and weighted median methods, whereas the simple mode and weighted mode did not provide evidence for this outcome. The confirmation of the results' stability was further supported by the utilization of scatter plot and funnel plot (Figures 2B, C). The sensitivity analysis (Figure 2D) did not reveal any indication of bias towards genetic pleiotropy.

Discussion

In this study, Mendelian randomization was used to examine the causal association between 731 immune cell characteristics and CAs. Utilizing publicly available genetic data, we identified four immune traits (MFI, RC, AC, and MP) that exhibited causal associations with 10 immune cell phenotypes in relation to CAs (FDR < 0.06), and three immune cell phenotypes had significant causal effects on CAs (FDR < 0.05). Conversely, only one immune cell phenotype demonstrated a significant causal association with CAs in reverse studies (FDR < 0.05).

Natural killer cells, also known as NK cells, are a varied group of lymphocytes that have the ability to bridge the gap between innate and adaptive immune responses (Alter and Altfeld, 2006). The absence of T cell or B cell receptors characterizes these cells, which can be divided into two main subgroups depending on the varying levels of CD16 and CD56 (Kucuksezer et al., 2021). NK cells display innate cytotoxicity, antibody-mediated cytotoxicity (ADCC), and have the ability to release multiple cytokines that resemble well-known CD4 helper T cell (Th) subsets, such as Th1, Th2, and Th17 (Chin et al., 2022). Inhibitory characteristics are possessedby NK cells that generate interleukin-10, potentially contrib an inverse relationship uting to immunomodulatory responses (Tahrali et al., 2018). Moreover, it has been noted that individuals with CAs exhibit notably reduced levels of CD8+T cells in their peripheral blood in comparison to those in the normal control group (Fan et al., 2020). Several research studies have presented evidence suggesting that inflammation plays a vital part in the development of CAs. The occurrence and development of CAs are regulated by negative regulatory molecules, like Tim-3, which modulate TNF-α in $CD3^+$, $CD4^+$, and CD8+T cells (Zhang et al., 2015). We found that CD3 on NKT cell was causally associated with a reduced risk of CAs. In a previous clinical study, increased percentages of $CD3^+$ and NKT cell was found in patients with good prognosis after aSAH (Zhou et al., 2017). Our results also revealed that CAs increased $CD16^-CD56$ on NK cell levels, and then there was a positive genetic causal link between CD45 on HLA DR+ NK cell and CAs. Previous studies have shown that individuals with CAs demonstrate impaired immune function, irregularities in CD4+T cell clusters, elevated levels of B cells, and increased expression of HLA-DR in monocytes, decreased levels of CD8+T cell, DNT cell, and DPT cell, along

Exposure	Method	nSNP	P	OR(95%CI)	Heterogeneity.P	MR.Egger_intercept.P	FDR
CD25hi %CD4+	MR Egger	26	0.0339	1.181(1.022 to 1.365)	0.803	0.187	
	Weighted median	26	0.0839	1.105(0.987 to 1.237)			
	IVW	26	0.0403	1.085(1.004 to 1.173)	0.755		0.0538
	Simple mode	26	0.8986	1.010(0.864 to 1.182)			
	Weighted mode	26	0.0723	1.112(0.995 to 1.242)			
CD33br HLA DR+ CD14- AC	MR Egger	29	0.0061	1.072(1.024 to 1.122)	0.920	0.339	
	Weighted median	29	0.0092	1.071(1.017 to 1.127)			
	IVW	29	0.0029	1.058(1.019 to 1.098)	0.916		0.0573
	Simple mode	29	0.0746	1.068(0.996 to 1.145)			
	Weighted mode	29	0.0142	1.078(1.019 to 1.140)			
Naive CD4+ %CD4+	MR Egger	36	0.0895	1.132(0.985 to 1.302)	0.704	0.371	
	Weighted median	36	0.0131	1.118(1.024 to 1.222)			
	IVW	36	0.0396	1.069(1.003 to 1.140)	0.709		0.0566
	Simple mode	36	0.0849	1.137(0.987 to 1.310)			
	Weighted mode	36	0.0567	1.137(1.001 to 1.291)			
EM CD8br %T cell	MR Egger	15	0.6075	0.974(0.882 to 1.075)	0.485	0.319	
	Weighted median	15	0.2813	0.951(0.867 to 1.042)			
	IVW	15	0.0478	0.936(0.877 to 0.999)	0.480		0.0503
	Simple mode	15	0.4735	0.961(0.865 to 1.068)			
	Weighted mode	15	0.3292	0.956(0.875 to 1.044)			
CD25 on IgD+ CD24-	MR Egger	26	0.2083	0.971(0.929 to 1.015)	0.803	0.445	
	Weighted median	26	0.1886	0.968(0.922 to 1.016)			
	IVW	26	0.0427	0.962(0.927 to 0.999)	0.816		0.0502
	Simple mode	26	0.0601	0.905(0.820 to 1.000)			
	Weighted mode	26	0.1025	0.963(0.922 to 1.006)			
CD3 on NKT	MR Egger	16	0.9504	1.003(0.914 to 1.100)	0.609	0.027	
	Weighted median	16	0.5718	0.977(0.902 to 1.059)			
	IVW	16	0.0424	0.926(0.859 to 0.997)	0.258		0.0424
	Simple mode	16	0.1432	0.780(0.570 to 1.069)			
	Weighted mode	16	0.5909	0.978(0.905 to 1.058)			
CD16-CD56 on NK	MR Egger	30	0.9367	1.005(0.898 to 1.124)	0.717	0.350	
	Weighted median	30	0.1391	1.056(0.982 to 1.136)			
	IVW	30	0.0385	1.055(1.003 to 1.109)	0.718		0.0593
	Simple mode	30	0.6188	1.031(0.915 to 1.161)			
	Weighted mode	30	0.1872	1.053(0.977 to 1.136)			
CD45 on HLA DR+ NK	MR Egger	13	0.0985	1.222(0.983 to 1.520)	0.934	0.364	
	Weighted median	13	0.0902	1.138(0.980 to 1.320)			
	IVW	13	0.0489	1.116(1.001 to 1.244)	0.924		0.0489
	Simple mode	13	0.2315	1.138(0.931 to 1.391)			
	Weighted mode	13	0.1079	1.149(0.982 to 1.344)			
CX3CR1 on CD14- CD16-	MR Egger	21	0.3122	0.983(0.952 to 1.015)	0.695	0.271	
	Weighted median	21	0.2254	0.980(0.948 to 1.013)			
	IVW	21	0.0447	0.973(0.948 to 0.999)	0.672		0.0496
	Simple mode	21	0.3443	0.950(0.857 to 1.053)			
	Weighted mode	21	0.1742	0.979(0.950 to 1.008)			
CD8 on CD8br	MR Egger	30	0.4399	0.951(0.838 to 1.079)	0.358	0.740	
	Weighted median	30	0.1074	0.921(0.833 to 1.018)			
	IVW	30	0.0408	0.934(0.874 to 0.997)	0.403		0.0509
	Simple mode	30	0.1746	0.898(0.771 to 1.045)			
	Weighted mode	30	0.2398	0.930(0.826 to 1.047)			

P<0.05 was considered statistically significant

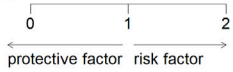

FIGURE 1
Mendelian randomization estimates of the association between immune cell phenotypes and risk of cerebral aneurysm. A total of 10 causal immune cell phenotypes were included (FDR < 0.06), three of which were significantly associated with CAs (CD3 on NKT, CD45 on HLA DR+ NK, and CX3CR1 on CD14- CD16-). OR, odds ratio; CI, confidence interval. IVW, inverse variance weighted.

with heightened expression of TLR4, p-STAT3, and the depletion marker PD1 in NK cells (Ge et al., 2022). CX3CR1 on CD14- CD16- derived from monocytes has been shown to be associated with the risk of developing CAs (Mohme et al., 2020). This is consistent with our conclusions.

B lymphocytes have been recognized as important contributors to the development of persistent inflammation (Ma et al., 2021). According to research, the cytokine IL-6 plays a role in both the inflammation and development of B cells (Dmitrieva et al., 2016). Furthermore, there has been anoted elevation in IL-6 levels within the tissues of individuals afflicted with abdominal aortic aneurysms and intracranial aneurysms (Wang et al., 2018). Furthermore, the detection of T and B lymphocytes, dendritic cells (DC), and pericytes inside the blood vessels of CAs at the individual cell level in mice implies the participation of antigen-triggered stimulation and adaptive immune reactions of inexperienced T cells in the

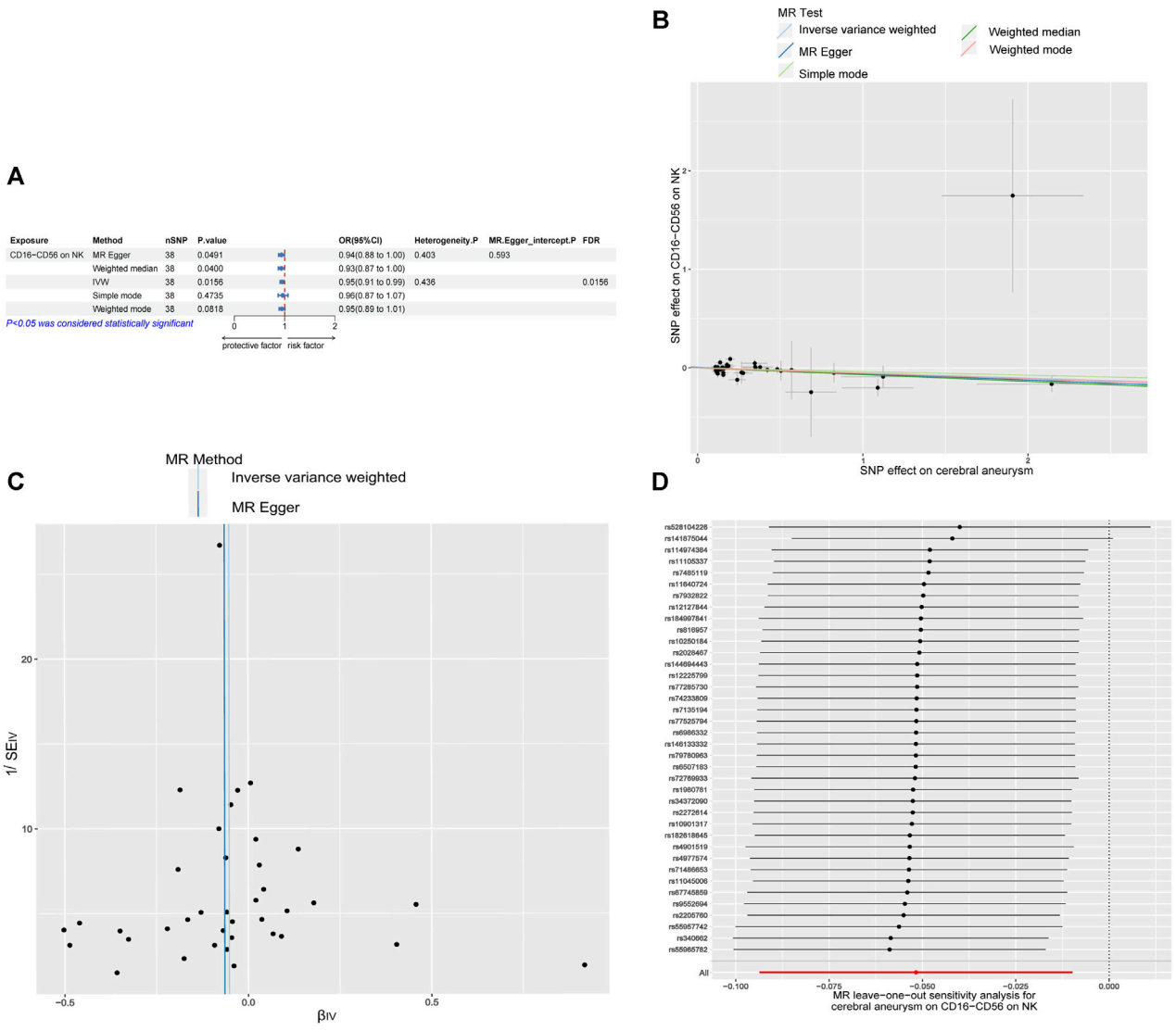

FIGURE 2
Causal relationship between immune cell phenotypes and CAs in reverse MR **(A)** Mendelian randomization estimates of the association between CD16-CD56 on NK cell and CAs. **(B)** Scatter plot of the causal effect of CD16-CD56 on NK cell on CAs risk. **(C)** Funnel plot of the causal effect of CD16-CD56 on NK cell on CAs risk. **(D)** Sensitivity analysis of the causal effect of $CD16^{-}CD56$ on NK cell on CAs risk. OR, odds ratio; CI, confidence interval; IVW, inverse variance weighted.

advancement of elastase-induced CAs. The discovery of these cells has revealed their crucial involvement in the rupture of CAs. Our results also cannot confirm that B and T lymphocytes increased the risk of CAs, and more studies are needed to investigate.

Additionally, it is important to note that neutrophils have a vital function in the context of CAs (Kushamae et al., 2020). Multiple research studies have shown that machine learning algorithms can be used to forecast the existence of CAs in blood samples from CAs patients by identifying the levels of neutrophil RNA expression (Tutino et al., 2018). Similarly, multiple research studies offer proof that supports the connection between the ratio of neutrophils to lymphocytes (NLR) and the outlook for patients with CAs, indicating the potential importance of NLR in predicting outcomes for this group (Cho et al., 2020; Zhang et al., 2021; Zhang et al., 2023). However, our results cannot show a causal effect between neutrophils and CAs, and more evidence is still needed to explore their relationship.

Our research has certain limitations. Initially, the individuals involved in this research were limited to the European population, and there was no personal information including age, sex, and consequently constraining the applicability of our discoveries to the wider population. Additional investigation is required to confirm and enhance the identified correlation. Secondly, the threshold filtering of the independent variable (IV) yielded a significance level of $p < 1.0 \times 10^{-5}$, which is relatively low. Third, we did not use multivariate MR Method and added additional data to further validate our findings, so additional evidence is necessary to further investigate the association between immune cell phenotypes and CAs. Moreover, it is crucial to recognize the intricacy of the cellular immune system, where the phenotype of immune cells functions as an indicator

or guide. Further research should focus on examining particular subsets of immune cells and their roles in the formation of CAs.

Conclusion

To summarize, our research provides preliminary proof that there is a cause-and-effect relationship between immune cell characteristics and CAs by utilizing MR techniques. The potential of this finding is to bring forth fresh viewpoints and strategies for the prevention and control of CAs. However, further inquiries are necessary to confirm and clarify the complex mechanisms that contribute to the participation of immune cells inthe development of CAs.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies involving humans because accordance with the local legislation and institutional requirements. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from the datasets processed in this study were derived from GWAS. GWAS data are publicly available abstract level data, thus, no ethical approval was required. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

XS: Software, Writing–review and editing. TW: Writing–original draft. DT: Writing–original draft. SH: Writing–original draft, Writing–review and editing. NL: Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2024.1333855/full#supplementary-material

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Coffee and the risk of osteoarthritis: a two-sample, two-step multivariable Mendelian randomization study

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Purpose: To investigate the potential causal relationship between coffee consumption and osteoarthritis (OA), and to disentangle whether body mass index (BMI) and Bone mineral density (BMD) mediate this relationship.

Methods: We performed two-sample and two-step Mendelian randomization (MR) analyses utilizing publicly available genome-wide association studies (GWAS) summary statistics to estimate the association between coffee intake and OA risk (including knee OA, hip OA, knee or hip OA, and total OA), as well as the possible mediating effects of BMI and BMD. In addition, data of different coffee types (decaffeinated coffee, instant coffee, ground coffee—including espresso, filter, etc., and other coffee types) were used to explore the effect of coffee type on the risk of OA.

Results: In two-sample MR, coffee intake increased the risk of OA in various sites, with the most significant impact observed in knee osteoarthritis (KOA) (odds ratio [OR] 2.03, 95% confidence interval [CI] 1.57–2.61, $p < 0.001$). The effect on self-reported OA was minimal (OR 1.03, 95% CI 1.01–1.05, $p = 0.006$). Further analysis of different types of coffee revealed that only decaffeinated coffee was causally associated with both KOA (OR 4.40, 95% CI 1.71–11.33, $p = 0.002$) and self-reported OA (OR 1.13, 95% CI 1.02–1.26, $p = 0.022$). In two-step MR, BMI explained over half of the coffee intake-all OA risk association, while BMD accounted for less than 5% of the mediation effect.

Conclusion: Our study suggests that coffee intake increase the risk of OA, with BMI playing a significant mediating role. Decaffeinated coffee appears to have the greatest impact on OA risk compared to other types of coffee. Therefore, managing BMI and selecting appropriate types of coffee should be included in the health management of individuals who frequently consume coffee.

KEYWORDS

osteoarthritis, coffee type, coffee intake, Mendelian randomization, mediating effects

1 Introduction

Osteoarthritis (OA) is a common chronic degenerative disease that affects the knee, hip, and hand joints, leading to chronic pain, stiffness, and movement disorders (Martel-Pelletier et al., 2016; Zhang et al., 2023). The pathogenesis of OA is complex and can be influenced by various factors such as poor lifestyle habits and health status, including excessive physical labor (Wang et al., 2020), body mass index (BMI) (Ho et al., 2022), overweight and obesity (Reyes et al., 2016).

Previous studies have reported conflicting results on the relationship between coffee consumption and OA risk (Bang et al., 2019; Lim et al., 2022). This highlights the importance of determining whether coffee intake has a causal impact on the risk of OA, especially in the context of the European League Against Rheumatology (EULAR) emphasis on the role of lifestyle in skeletal muscle diseases (Gwinnutt et al., 2023).

Coffee is one of the most popular beverage consumed worldwide and has been the subject of extensive research regarding its impact on various health conditions including cardiovascular diseases, diabetes, cancers, and mental and neurological disorders (Butt and Sultan, 2011; Alicandro et al., 2017). The complex composition of coffee contains numerous active substances that can have diverse effects on health (Terentes-Printzios and Vlachopoulos, 2022). For instance, caffeic acid, commonly found in coffee, has been shown to inhibit inflammatory reactions and may have a protective effect on OA (Huang et al., 2018). Furthermore, the flavonoids present in coffee exhibit anti-inflammatory and antioxidant properties, which could potentially be therapeutically beneficial for regulating metabolism and the progression of inflammatory bone diseases (Ortiz et al., 2022).

However, it is important to recognize that coffee as a whole cannot be equated to any single component. Moreover, there are various types of coffee available in the market, each with its own unique composition. Previous studies have shown an association between coffee and BMI (Lee et al., 2019) as well as Bone mineral density (BMD) (Tański et al., 2021). Therefore, it is worth exploring whether BMI and BMD mediate the relationship between coffee intake and OA. Observational studies are susceptible to confounding factors and reverse causality, which can lead to biased results. The emergence of Mendelian randomization provides a novel approach to address these limitations (Zhang et al., 2023).

Mendelian randomization, as a methodology employing genetic variation as an instrumental variable, serves to evaluate the causal association between exposure and outcomes. In recent years, it has been widely used in the exploration of causative factors and potential targets of OA, as well as interactions between OA and a wide range of other diseases due to its ability to mitigate the effects of confounders and avoid reverse causality bias (Zhang et al., 2022; Fu et al., 2023). Although previous Mendelian studies have suggested a potential causal association between coffee intake and OA risk (Lee, 2018; Zhong et al., 2019; Zhang et al., 2021), such conclusions should be interpreted with caution due to a limited number of single nucleotide polymorphisms (SNPs) used, low statistical power, and potential confounding factors. Additionally, there is no research yet on whether the causal relationship between different types of coffee and OA risk differs.

In this study, our primary objectives are to elucidate the causal association between various types of coffee consumption and the risk of OA. Additionally, we aim to investigate the potential mediating roles of BMI and BMD in the relationship between coffee intake and OA.

2 Materials and methods

2.1 Study design and data source

As shown in Figure 1, we commenced our study by conducting a univariate Mendelian randomization analysis. This analysis enabled us to examine the potential existence of a causative relationship between diverse forms of coffee consumption and OA. Moreover, we fortified our outcomes by incorporating external validation through the utilization of OA data extracted from the comprehensive research (Zengini et al., 2018). Subsequently, we employed two-step and two-sample Mendelian randomization analyses utilizing data on coffee intake, BMI, and BMD from relevant GWAS. These analyses sought to ascertain the potential mediating effects of BMI and BMD in the association between coffee intake and OA. Overall, our research endeavor follows a meticulous design, encompassing comprehensive assessments of distinct coffee intake types, external validation, and the examination of mediating factors such as BMI and BMD. By adopting this approach, we aim to contribute valuable insights to the existing literature on the intricate relationship involving coffee consumption, BMI, BMD, and the risk of OA.

SNPs associated with coffee intake and different coffee type choices were extracted from GWAS of more than 320,000 UKB participants. These individuals were queried about their daily coffee intake, as well as their typical choice of coffee type (decaffeinated coffee (any type), instant coffee, ground coffee - including espresso, filter, etc., and other coffee types) (Canela-Xandri et al., 2018), released by Neale lab (http://www.nealelab.is/uk-biobank/). Genetic associations with BMI with 461,460 participants were obtained from the GWAS summary data (https://gwas.mrcieu.ac.uk/). Summary-level statistic for BMD with 365,403 participants were downloaded from the GEnetic Factors for OSteoporosis Consortium website (GEFOS, http://www.gefos.org/). The primary outcome of this study was osteoarthritis (OA) and its various subtypes, which encompassed self-reported OA, knee OA (KOA), hip OA (HOA), and knee or hip OA (462,933/403,124/393,873/417,596 participants, respectively). And the data of self-reported OA used in this study were extracted from the GWAS summary data (https://gwas.mrcieu.ac.uk/). The remaining OA data were obtained from genomewide meta-analysis for OA (Tachmazidou et al., 2019). In addition to the data mentioned earlier, summary-level statistics from the study conducted by Zengini et al. (2018) were utilized for external validation purposes (63,556/22,347/11,989/32,970 participants, respectively). All participants were limited to individuals of European descent, which helped to mitigate potential biases resulting from population stratification. Detailed description of the characteristics of the GWAS related to the exposures, mediator, and outcomes can be found in Supplementary Table S1. As all the data used in this study were publicly accessible, ethical approval was not required for this research.

2.2 Selection of instrumental variables

To be considered a valid instrumental variable (IV) estimator, the following three assumptions must be met: 1) it should be strongly and consistently associated with the exposure (relevance condition); 2) it should be independent of the outcome (exclusion restriction condition); 3) it should be unrelated to any potential confounding variables with respect to both the exposures and outcomes (exchange ability condition) (Burgess et al., 2017). We extracted SNPs robustly associated with exposure ($P < 5e\text{-}8$, or relaxed to $P < 5e\text{-}6$). From this set, only independent SNPs (kb = 10,000, r2 < 0.001) were retained based on the linkage disequilibrium (LD) structure of European populations, and the palindromic SNPs with intermediate allele frequencies were excluded. In addition, the phenoscanner tool was employed to ascertain whether any of the selected instrument SNPs were associated with confounding

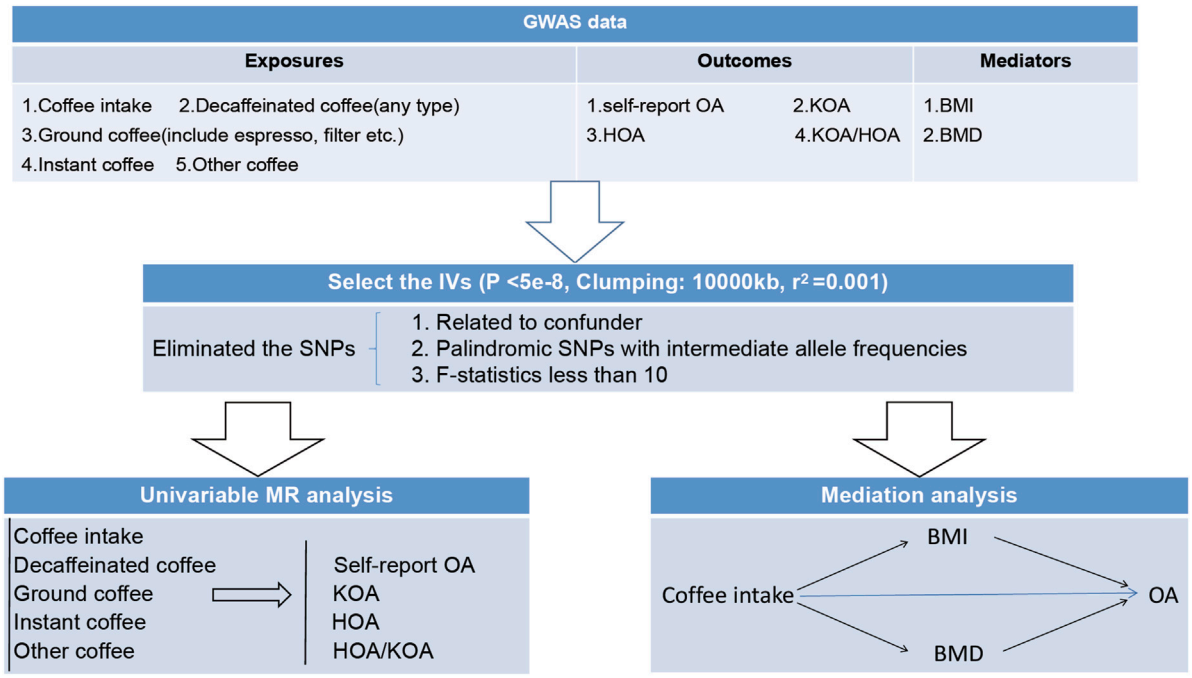

FIGURE 1
The overview design for the present study. OA, osteoarthritis. KOA, knee osteoarthritis. HOA, hip osteoarthritis. BMI, body mass index. BMD, Bone mineral density, SNPs, single nucleotide polymorphisms.

factors. To mitigate weak instrumental variable bias, we calculated the F-statistic, which was calculated using the following equation: F = R2 (SampleSize-2)/(1-R2) (Palmer et al., 2012). We applied a filter to exclude weak instruments with an F-statistic lower than 10.

2.3 Univariate MR analysis

We used the inverse variance weighted (IVW) as the main MR analysis, which utilizes a random effects model meta-analysis method to combine the outcomes from a single SNP and achieve a more precise estimate of the causal connection between the exposures and outcomes (Chen et al., 2023). The estimates were derived from a random effects model due to the p-value < 0.05 for heterogeneity based on Cochran's Q statistic, otherwise, the estimates were derived from a fixed-effects MR estimates model. Furthermore, we utilized both the MR Egger and weighted median methods to investigate the relationship between coffee intake and OA. Additionally, we evaluated the potential presence of horizontal pleiotropy through the MR-Egger intercept and the MR-PRESSO global test. The MR-PRESSO outlier test was utilized to detect any possible outliers (Verbanck et al., 2018). In case an outlier SNP was identified (p < 0.05), we recalculated the causal effects by excluding the outliers and employing the remaining SNPs.

2.4 Mediation analysis

For mediation analysis, we conducted a two-step MR. In the first step, we evaluate the causal relationship between exposure and mediation variables (beta1). Subsequently, in the second step, we estimated the causal effect of mediators on outcomes through multivariate MR (beta2). We then calculated the total effect (beta0) between exposure and outcome using two-sample MR analysis. When beta0, beta1, and beta2 were all significant, a causal relationship existed between the outcome and exposure, and the mediating variable played a partial mediational role in this causal relationship. The mediating effect was calculated using beta1 * beta2, while the mediating proportion of the causal effect between exposure and outcome was calculated using beta1 * beta2/beta0 (Chen et al., 2022). Finally, we estimated the proportion of the mediation effect in the total effect using the delta method (Carter et al., 2019). We calculated the odds ratios (OR) and 95% confidence interval (CI) to measure causal effects.

We conducted causal estimates using the "TwoSampleMR" (Hemani et al., 2018) package and detected outliers using the "MR-PRESSO" package. For MVMR analysis, we utilized the "MVMR" and "Mendelian Randomization" (Yavorska and Burgess, 2017) packages. We performed all statistical analyses using R software version 4.3.2.

3 Results

3.1 Univariate MR analysis

We utilized instrumental variables (SNPs) that were significantly correlated with the exposure for two sample MR. In addition, we excluded SNPs that showed strong correlations with BMI, type 2 diabetes, and OA, then calculated the F-statistical

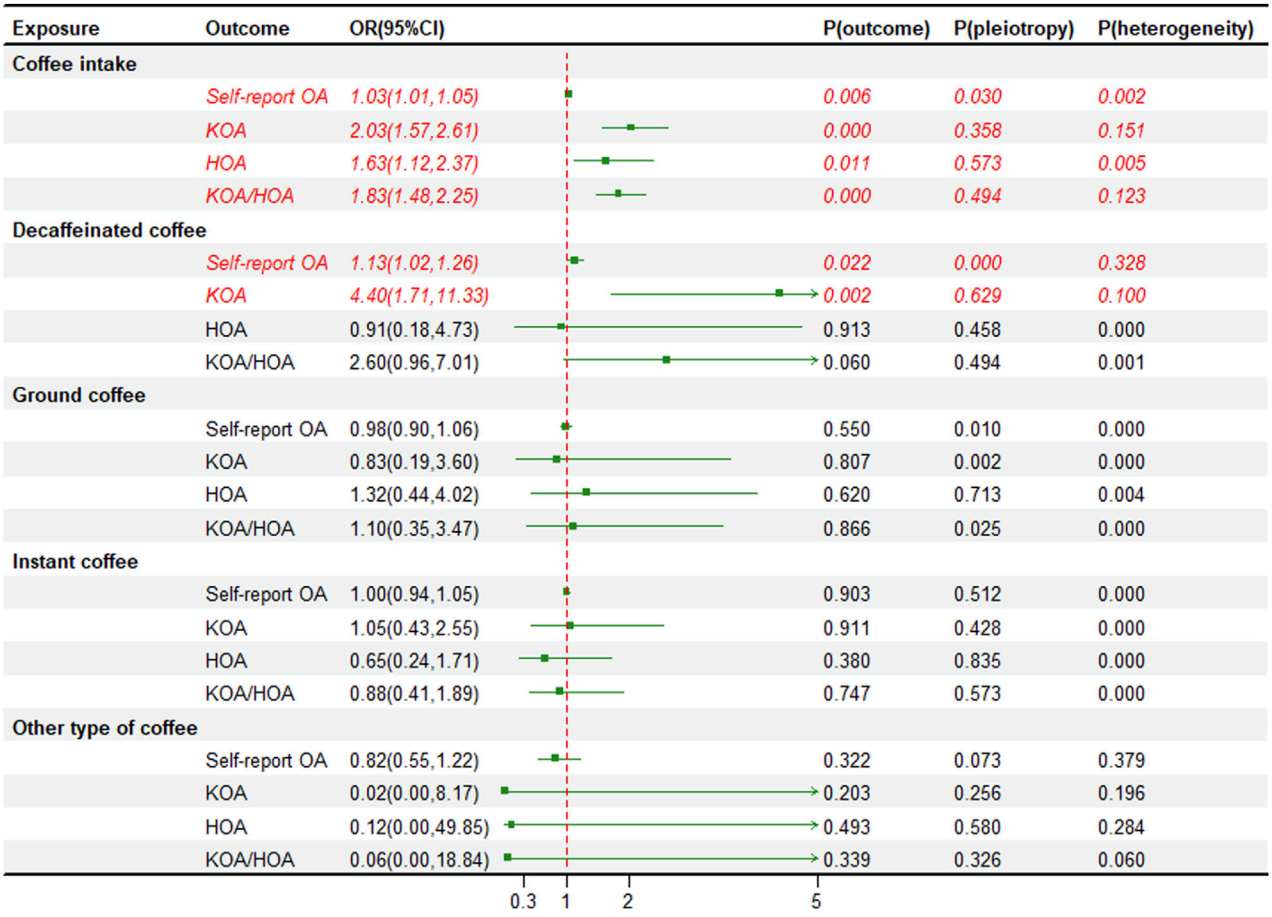

FIGURE 2
Forest plot of the main analysis of coffee intake and osteoarthritis risk. OA, osteoarthritis; KOA, knee osteoarthritis; HOA, hip osteoarthritis; OR, odds ratios; CI, confidence interval; *p*-value for pleiotropy test of univariable MR Egger. *p*-value for heterogeneity based on Cochran's Q statistic.

for the remaining SNPs (Supplementary Tables S2–S6). The univariate MR analysis indicates a causal relationship between coffee intake and the risk of OA in various parts, with the strongest impact observed in KOA (OR 2.03, 95% CI 1.57–2.61, $p < 0.001$) and minimal impact on self-reported OA (OR 1.03, 95% CI 1.01–1.05, $p = 0.006$). Interestingly, we found that only decaffeinated coffee had a significant association with KOA (OR 4.40, 95% CI 1.71–11.33, $p = 0.002$) and self-reported OA (OR 1.13, 95% CI 1.02–1.26, $p = 0.022$) (Figure 2). We also performed external validation and found a consistent causal relationship between coffee intake and OA in various parts, although there was no statistically significant correlation between coffee intake and HOA (OR 1.18, 95% CI 0.53–2.65, $p = 0.685$). Furthermore, after classifying coffee, no causal relationship was observed between classified coffee and any part of OA (Figure 3). Similar results were shown in the MR methods weighted median and MR-Egger (Supplementary Tables S7–S11). The individual effect estimates of SNPs exhibited some heterogeneity, although no outliers were identified by MR-PRESSO. Based on the results of the MR-Egger intercept test, our analysis indicated a minimal presence of horizontal pleiotropy (Figures 2, 3). Additionally, the "Leave-one-out plot" revealed that none of the SNPs had a dominant effect on the estimated causal association between coffee intake and OA (Supplementary Figures S1–S4).

3.2 Mediating effect of BMI between coffee intake and OA

We utilized a two-step MR to perform the mediation analysis. The results revealed a significant causal association between gene-predicted coffee intake and BMI (beta1 = 0.724, $p = 4.44E-05$). In addition, multivariate Mendelian analysis illustrated that BMI significantly impacted all types of OA, including self-reported OA, KOA, HOA, and HOA/KOA (beta2 = 0.036, $p = 4.49E-65$; beta2 = 0.808, $p = 1.79E-106$; beta2 = 0.483, $p = 1.99E-29$; beta2 = 0.668, $p = 1.55E-112$) (Table 1). The impact of coffee on OA was analyzed, and the results indicated significant effects on all types of OA (Figure 1). The significant values of Beta0, Beta1, and Beta2 demonstrate a causal relationship between coffee intake and OA, with BMI partially mediating this relationship. Specifically, the final causal effects mediating self-reported OA, KOA, HOA, and KOA/HOA were found to be 71.11%, 67.90%, 45.12%, and 60.56%, respectively. Furthermore, indirect effects were estimated using the detal method, further strengthens our conclusion.

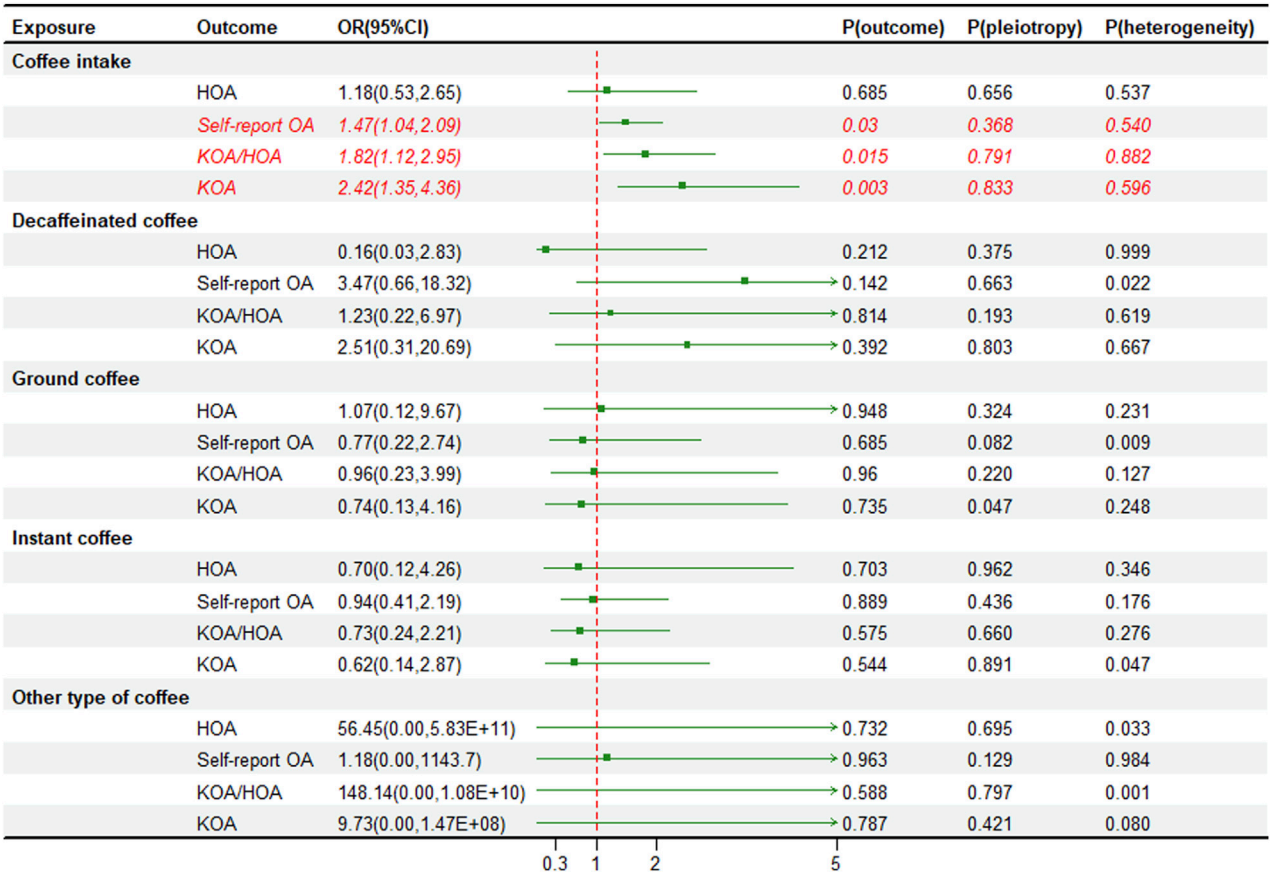

FIGURE 3
External validation forest plot of coffee intake and osteoarthritis risk. OA, osteoarthritis; KOA, knee osteoarthritis; HOA, hip osteoarthritis; OR, odds ratios; CI, confidence interval; *p*-value for pleiotropy test of univariable MR Egger. *p*-value for heterogeneity based on Cochran's Q statistic.

3.3 Mediating effect of BMD between coffee intake and OA

As shown in Table 2, we found a causal relationship between coffee intake and BMD (beta1 = 0.159, $p = 0.024$). In addition, BMD had a significant impact on OA in various sites, including self-reported OA, KOA, HOA, and HOA/KOA (beta2 = 0.003, $p = 0.033$; beta2 = 0.091, $p = 0.001$; beta2 = 0.113, $p = 0.001$; beta2 = 0.095, p = 2.89E-05). The significance of Beta0, Beta1, and Beta2 suggests the presence of a causal associations between coffee intake and OA, with BMD acting as a partial mediator. The final causal effects mediating self-reported OA, KOA, HOA, and KOA/HOA were estimated to be 1.48%, 1.69%, 2.33%, and 1.89%, respectively. However, when we calculated the standard error of indirect effects using the detal method, we found that BMD was only statistically significant in the case of KOA/HOA ($p = 0.047$).

4 Discussion

OA is a leading cause of disability among the elderly population. Since there are limited effective treatment options available, it is essential to reduce the risk of OA through lifestyle modifications. Coffee is one of the most widely consumed beverages worldwide and has been extensively studied for its potential health effects. Although some previous studies have suggested an increased risk of OA associated with coffee intake (Lee, 2018; Zhong et al., 2019; Zhang et al., 2021), it is important to note that coffee contains certain compounds, such as caffeic acids and flavonoids, which possess anti-inflammatory properties. These compounds could potentially act as protective factors against the development and progression of OA (Huang et al., 2018; Terentes-Printzios and Vlachopoulos, 2022). To further investigate the relationship between coffee consumption and the risk of OA, we conducted a comprehensive analysis using GWAS data with a larger sample size. Additionally, we carried out external validation to reinforce our conclusions. In line with previous studies, our research indicates that coffee consumption may indeed be associated with an increased risk of OA. Furthermore, we conducted a preliminary exploration of the potential pathways through which coffee intake might elevate the risk of OA. Utilizing a two-step MR analysis, we determined that BMI and BMD mediate the causal relationship between coffee intake and OA risk. It is important to emphasize that while our study provides valuable insights into the association between coffee consumption and OA risk, further research is needed to comprehend the underlying mechanisms in greater detail. Additionally, investigating the specific impact of different types of coffee on OA risk is also warranted. Nevertheless, our findings

TABLE 1 The two-step Mendelian randomization estimates for mediator body mass index between coffee intake and osteoarthritis.

Exposure	Outcome	Method	nsnp	Beta	SE	Pval	Midbeta	Mid_of_sum	*p* (detal)
Coffee intake	BMI	IVW	39	0.724	0.177	4.44E-05	0.026	0.711	7.17E-05
BMI	self-reported OA	IVW	440	0.036	0.002	4.49E-65			
Coffee intake	self-reported OA	IVW	39	0.037	0.010	1.30E-04			
Coffee intake	BMI	IVW	39	0.724	0.177	4.44E-05	0.585	0.679	5.97E-05
BMI	KOA	IVW	441	0.808	0.037	1.79E-106			
Coffee intake	KOA	IVW	39	0.862	0.132	6.43E-11			
Coffee intake	BMI	IVW	39	0.724	0.177	4.44E-05	0.349	0.451	1.24E-04
BMI	HOA	IVW	439	0.483	0.043	1.99E-29			
Coffee intake	HOA	IVW	39	0.774	0.204	1.48E-04			
Coffee intake	BMI	IVW	39	0.724	0.177	4.44E-05	0.483	0.606	5.87E-05
BMI	KOA/HOA	IVW	441	0.668	0.030	1.55E-112			
Coffee intake	KOA/HOA	IVW	39	0.798	0.125	1.95E-10			

BMI, body mass index. IVW, inverse-variance weighted. SE, standard deviation. Midbeta, mediating effect from exposure to the outcome. Mid_of_sum, the proportion of the mediating effect within the causal relationship.
The estimates were derived from a random effects model due to the *p*-value<0.05 for heterogeneity based on Cochran's Q statistic, otherwise, The estimates were derived from a fixed-effects MR, estimates model.

TABLE 2 The two-step Mendelian randomization estimates for mediator Bone mineral density between coffee intake and osteoarthritis.

Exposure	Outcome	Method	nsnp	Beta	SE	Pval	Midbeta	Mid_of_sum	*p* (detal)
Coffee intake	BMD	IVW	35	0.159	0.071	0.024	0.001	0.015	0.111
BMD	self-reported OA	IVW	413	0.004	0.002	0.033			
Coffee intake	self-reported OA	IVW	39	0.037	0.010	0.000			
Coffee intake	BMD	IVW	35	0.159	0.071	0.024	0.015	0.017	0.060
BMD	KOA	IVW	440	0.091	0.027	0.001			
Coffee intake	KOA	IVW	39	0.862	0.132	0.000			
Coffee intake	BMD	IVW	35	0.159	0.071	0.024	0.018	0.023	0.061
BMD	HOA	IVW	439	0.113	0.034	0.001			
Coffee intake	HOA	IVW	39	0.774	0.204	0.000			
Coffee intake	BMD	IVW	35	0.159	0.071	0.024	0.015	0.019	0.047
BMD	KOA/HOA	IVW	439	0.095	0.023	0.000			
Coffee intake	KOA/HOA	IVW	39	0.798	0.125	0.000			

BMD, bone mineral density; IVW, inverse-variance weighted; SE, standard deviation; Midbeta, mediating effect from exposure to the outcome. Mid_of_sum, the proportion of the mediating effect within the causal relationship.
The estimates were derived from a random effects model due to the *p*-value<0.05 for heterogeneity based on Cochran's Q statistic, otherwise, The estimates were derived from a fixed-effects MR, estimates model.

contribute to the expanding literature in this field and underscore the potential role of coffee consumption in the development of OA.

The composition of coffee is very complex and includes hundreds of different substances, of which the most familiar component is caffeine (Bang et al., 2019; Lim et al., 2022). There is numerous data suggesting that caffeine intake is associated with a high risk of osteoarthritis. In rat models, it has been observed that exposure of maternal rats to low-doses of caffeine affects the joint integrity of their foetuses (Tan et al., 2012; Tan et al., 2018), which may be due to the antagonistic effect of caffeine on adenosine receptors as well as increased osteoclastogenesis (Yi et al., 2016; Nieber, 2017). Interestingly, however, some studies have reported an inhibitory effect of caffeine on certain pro-inflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor alpha (TNF-α) (Ullah et al., 2015). Furthermore, coffee comprises a variety of polyphenolic compounds, including chlorogenic acids and caffeic acids, known for their antioxidant and anti-inflammatory properties (Heinecke et al., 2010). A study found that intra-articular injections

of chlorogenic acid not only altered the expression of inflammatory mediators, but also reduced cartilage degradation in experimental osteoarthritis (Chen et al., 2011). In addition, coffee contains a variety of flavonoids, such as quercetin, neohesperidin and hesperidin, which have a variety of biological activities (Xue et al., 2019; Ortiz et al., 2022). *In vitro* studies have shown that these flavonoids have anti-osteoclastogenic and anti-inflammatory effects. They decrease the levels of reactive oxygen species and matrix metalloproteinases and inhibit the expression of pro-inflammatory cytokines and osteoclast markers (Ortiz et al., 2022). Although past studies have revealed an association between caffeine and an increased risk of osteoarthritis, almost all of the other coffee components that have been studied have anti-inflammatory effects and restrain the development of osteoarthritis, our study found that consumption of decaffeinated coffee increased the risk of osteoarthritis. Therefore, further research is still needed to find out exactly what chemical components are responsible for the increased risk of osteoarthritis. In addition, different roasting and brewing methods and types of coffee also affect the chemical composition of coffee. By further exploring the relationship between the composition of coffee and osteoarthritis, we can better understand the effects of coffee on human health.

Importantly, there are various types of coffee available in the market, each with its own unique combination of ingredients. For instance, roasted coffee contains specific polyphenols (chlorogenic acidsCGAs and melanins) which possess antioxidant properties (Scalbert and Williamson, 2000; Yanagimoto et al., 2004). In addition, roasted coffee contains polyphenols and melanoids that play a significant role as antioxidants in diet. A study of 83 commercially available coffee varieties found that unmixed and ground coffee had the highest CGAs content, while having the lowest average caffeine/CGAs ratio (Jeon et al., 2019). However, focusing solely on coffee intake as an exposure factor may not be clinically significant. Therefore, in our study, we examined different types of coffee to better understand their impact on OA risk. We conducted two-sample MR using four types of coffee (decaffeinated coffee, ground coffee, instant coffee, and other types of coffee) as exposures, and the results indicated that only decaffeinated coffee increased the risk of self-reported OA and KOA, rather than all types of coffee exhibiting this effect. Furthermore, there was a significant difference in the upper and lower limits of the 95% CI for the results of other types of coffee. This may be due to the imprecise classification of other coffee types, including coffee types that have a completely opposite impact on OA. Overall, our findings suggest that it is advisable to minimize the consumption of decaffeinated coffee in order to reduce the risk of OA. However, it is important to acknowledge that the data utilized in this study primarily originated from the European population and may be susceptible to demographic cross-biases. Additionally, some types of coffee showed horizontal pleiotropy, indicating that further research in this area using larger samples and richer cohorts is warranted. Furthermore, a more precise classification of coffee types is necessary to minimize intra-group differences. Steps could also be taken to mitigate the potential harm of coffee consumption by reducing harmful ingredients. Therefore, it is important to carefully evaluate these results and take them into consideration when making decisions about coffee consumption.

BMI is widely recognized as an important risk factor in the occurrence and development of OA (Bouchard et al., 2010; Moran-Lev et al., 2023). Indeed, coffee has a complex composition and contains CGA, which has been associated with reducing the BMI of overweight individuals (Watanabe et al., 2019). Additionally, caffeine itself has been found to be beneficial for weight control (Kulkarni et al., 2016). However, the inclusion of artificial flavoring agents in coffee to enhance its taste has been associated with a higher BMI (Lee and Kean, 2012). It is important to note that there is still no definitive conclusion on whether coffee intake directly leads to an increase in BMI, as multiple confounding factors can influence this relationship. In our study, we conducted a two-step mediation analysis to investigate the causal relationship between coffee intake and OA risk, with BMI acting as a mediator. The results showed that coffee intake does lead to an increase in BMI, and further analysis demonstrated that BMI plays a mediating role in the relationship between coffee intake and OA risk, with a mediation ratio exceeding 50%. This suggests that closely monitoring BMI and implementing measures to control it through diet or exercise can effectively reduce the risk of OA among individuals who consume coffee frequently. It is important to consider these findings and take appropriate steps to maintain a healthy BMI in order to mitigate the risk of developing OA, especially for individuals who regularly consume coffee.

Previous studies have identified a notable association between coffee intake and 12 blood metabolites, with three of these metabolites (AFMU, 3-hydroxyhippuric acid, and triglyceride) impacting BMD (Chau et al., 2020). Furthermore, Hartley et al. (2022) reported that BMD directly affects the occurrence of OA, independent of BMI. Hence, we postulate that BMD might serve as a mediating factor in the relationship between coffee consumption and OA risk. To investigate this hypothesis, we conducted a two-step MR imaging analysis and confirmed the presence of a mediating effect exerted by BMD. However, when employing the detal method to calculate the indirect effects, we observed statistical significance (p = 0.0471) only in relation to the influence of coffee intake on KOA/HOA. Moreover, the mediating ratios were consistently below 5%. Consequently, the practical clinical significance of BMD as a mediator in the causal association between coffee and OA may be limited.

This study is subject to several limitations that need to be acknowledged. Firstly, all the GWAS data utilized in this study were derived exclusively from the European population, thus warranting caution when extrapolating the findings to other populations. Secondly, our pleiotropy analysis revealed the presence of pleiotropic effects in certain coffee varieties on OA, which partially impacts the stability of the results. Additionally, despite employing methods such as MR Egger and MR PROSSO to control for confounding factors, it is need to recognize that residual confounding factors may still exist. Lastly, our investigation solely focused on exploring the mediating effects of BMI and BMD, while neglecting various other potential factors that remain unexplored.

5 Conclusion

This study reaffirms that coffee intake is associated with an increased risk of OA, particularly highlighting the elevated risks

associated with decaffeinated coffee intake in terms of KOA and self-reported OA. Additionally, we identified BMI as a significant mediator in the causal relationship between coffee consumption and OA, whereas the mediating role of BMD appears to be relatively minor. Consequently, monitoring and managing BMI levels may potentially reduce the risk of OA among coffee consumers. Furthermore, when selecting coffee types, reducing the selection of decaffeinated variants may be preferable to mitigate OA risk.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies involving humans because the data we used were extracted from publicly available databases or studies, and ethical approvals were obtained in the original studies, so we did not need to obtain further ethical approvals. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements because we used data from publicly available databases or studies where written informed consent was obtained in the original study, so this study did not need to obtain further written informed consent.

Author contributions

WZ: Conceptualization, Formal Analysis, Investigation, Methodology, Writing–original draft. XL: Conceptualization, Formal Analysis, Investigation, Writing–original draft. YT: Funding acquisition, Methodology, Writing–review and editing. TM: Formal Analysis, Writing–review and editing. TW: Data curation, Software, Writing–original draft. TY: Formal Analysis, Investigation, Resources, Writing–original draft. LX: Data curation, Software, Writing–original draft. JJ: Investigation, Writing–original draft. HX: Conceptualization, Funding acquisition, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2024.1340044/full#supplementary-material

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Investigation of the causal relationship between inflammatory bowel disease and type 2 diabetes mellitus: a Mendelian randomization study

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Background: Type 2 diabetes mellitus (T2DM) and inflammatory bowel disease (IBD) have been associated, according to various epidemiological research. This study uses Mendelian randomization (MR) to investigate the causal link between T2DM and IBD.

Methods: To investigate the causal relationship between IBD and T2DM risk using European population data from the genome-wide association study (GWAS) summary datasets, we constructed a two-sample MR study to evaluate the genetically predicted impacts of liability towards IBD outcomes on T2DM risk. As instrumental variables (IVs), we chose 26 single nucleotide polymorphisms (SNPs) associated with IBD exposure data. The European T2DM GWAS data was obtained from the IEU OpenGWAS Project database, which contains 298,957 cases as the outcome data. The causal relationship between T2DM and IBD using a reverse MR analysis was also performed.

Results: The two-sample MR analysis, with the Bonferroni adjustment for multiple testing, revealed that T2DM risk in Europeans is unaffected by their IBD liability (odds ratio (OR): 0.950–1.066, 95% confidence interval (CI): 0.885–1.019, p = 0.152–0.926). The effects of liability to T2DM on IBD were not supported by the reverse MR analysis either (OR: 0.739–1.131, 95% confidence interval (CI): 0.651–1.100, p = 0.058–0.832). MR analysis of IBS on T2DM also have no significant causal relationship (OR: 0.003–1.007, 95% confidence interval (CI): 1.013–5.791, p = 0.069–0.790). FUMA precisely mapped 22 protein-coding genes utilizing significant SNPs of T2DM acquired from GWAS.

Conclusion: The MR study showed that the existing evidence did not support the significant causal effect of IBD on T2DM, nor did it support the causal impact of T2DM on IBD.

KEYWORDS

Mendelian randomization, type 2 diabetes mellitus, inflammatory bowel disease, irritable bowel syndrome, causal relationship

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder defined by pancreatic β-cells failure and insulin resistance in peripheral tissues. This results in impaired glucose metabolism and chronic low-grade inflammation (Zhou et al., 2022). This chronic disease is one of the leading causes of death and disability in the world, which is caused by genetic and environmental factors, such as genetic predisposition, unhealthy diet, adiposity, smoking, ambient air pollution, physical inactivity, and pre existing underlying diseases are important reasons for its continuous increase in incidence (Almigbal et al., 2023; Arsh et al., 2023; Taborda Restrepo et al., 2023). According to a report, the global prevalence of diabetes among adults exceeded 460 million in 2019, with projections indicating a substantial increase to over 700 million by 2045 (Sun et al., 2022). Given its association with detrimental microvascular and macrovascular consequences, T2DM inflicts physical and psychological distress on patients and imposes a significant financial burden on the healthcare system (Kang et al., 2022; Alemayehu et al., 2023; Hands et al., 2023). Inflammatory bowel disease (IBD), encompassing ulcerative colitis and Crohn's disease, manifests in around 1% of the population and is commonly distinguished by persistent diarrhea (with or without bleeding), stomach discomfort, and loss of body mass (Bruner et al., 2023). It is more common between the ages of 20 and 40, but can start at any age, resulting in significant differences in disease course and complications among different individuals, and the immune systems involved are also more complex, including the innate and adaptive immune systems (Caioni et al., 2021; Saez et al., 2023; Tseng, 2023). Numerous proinflammatory immune mediators, such as interleukin 17, interleukin 23, interferon gamma, and tumor necrosis factor alpha overexpression in it (Flynn and Eisenstein, 2019; Noviello et al., 2021; Parigi et al., 2022). It is linked to increasing damage to the intestine and extra-intestinal symptoms, resulting in compromised gastrointestinal function, reduced quality of life, and heightened therapeutic challenges (Dai et al., 2023). Due to the intricate and partially unknown etiological origins and development of ulcerative colitis and Crohn's disease, effectively managing these conditions can present difficulties, both in terms of clinical perspectives and resource allocation (Da Rio et al., 2023). In the literature, IBD has been documented to exhibit associations with several medical conditions, such as colorectal cancer (Gordon et al., 2023), Graves' disease (Xian et al., 2023), and metabolic disorder (Verdugo-Meza et al., 2020).

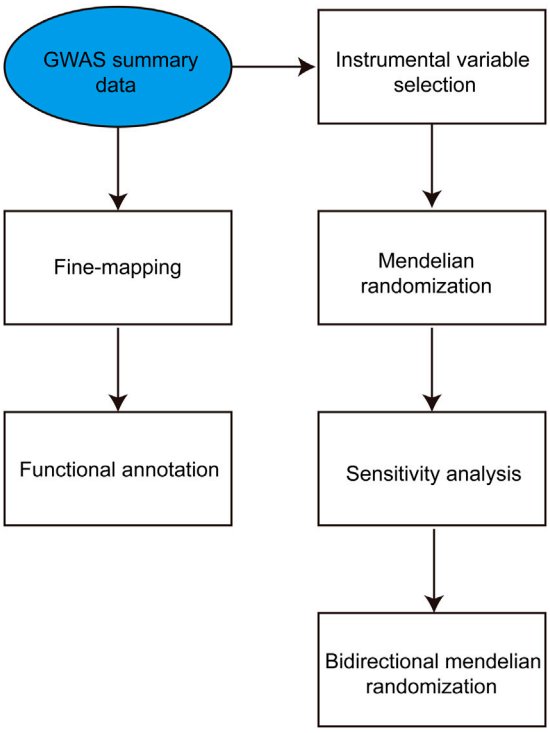

FIGURE 1
The study design and Mendelian randomization methods used in the analysis.

Previous studies have observed that IBD is a chronic and idiopathic inflammatory condition affecting the gastrointestinal system. It has also been linked to T2DM, according to various studies (Zhao et al., 2021; Allin et al., 2022; Tseng, 2022). The primary anatomical location of IBD is the large intestine, which has the highest concentration of bacterial cells. Research conducted on the gut microbiota in individuals with IBD around the globe has revealed that dysbiosis, characterized by alterations in the composition of intestinal bacteria, is associated with either an increase or reduction in certain bacterial species inside the gut of IBD patients (Al Bander et al., 2020). Alterations in microbial homeostasis in the intestine have profound implications for local and systemic immunity, hence significantly developing extra-intestinal systemic disorders such as obesity, diabetes, and atherosclerosis (Gill et al., 2022). Some epidemiological studies showed the potential relationship between T2DM and IBD. Villumsen et al. (2022)found that inflammatory bowel disease would increase the risk of type 2 diabetes. Abrahami et al. (2018) pointed out that the use of Dipeptidylpeptidase-4 inhibitors (DPP4i) in T2DM was related to the increased risk of IBD, but (Kim et al., 2015)reached the opposite conclusion that starting DPP4i in T2 diabetes could reduce the risk of IBD. But, the causality of these correlations has yet to be established. The presence of unmeasured confounding and reverse causation in this epidemiological research introduces bias, which poses challenges to establishing causal inference. However, investigating a potential causative relationship between IBD and T2DM might provide valuable insights into specific biological mechanisms and contribute to developing effective preventive measures. Mendelian randomization (MR) is an epidemiological methodology that addresses many biases commonly seen in observational research, including reverse causality and confounding (Gagnon et al., 2023). It also utilizes genetic variants strongly associated with the exposure that satisfies certain assumptions as IVs to investigate the causal relationship with an outcome. (Yin et al., 2022). Given that these variations are assigned randomly during conception, this might mitigate bias resulting from environmental confounders, provided that MR is carried out appropriately. Therefore, the MR design may be comparable to a randomized controlled trial. In this work, we use a Mendelian randomization design to investigate the causal relationship between a specific exposure (in this case, IBD) and an outcome (in this case, T2DM) by utilizing an IVs approach, as

TABLE 1 Information on instrumental variables (IVs) subjected to inflammatory bowel disease (IBD).

Rs number	Chromosome	Location	Other allele	Effect allele	EAF	*p*-value
rs10761659	10	62,685,804	A	G	0.54	4.97E-53
rs11230563	11	61,008,737	C	T	0.35	1.71E-14
rs1535	11	61,830,500	A	G	0.33	2.78E-09
rs3184504	12	111,446,804	T	C	0.51	1.29E-09
rs941823	13	40,439,840	T	C	0.75	6.19E-13
rs17293632	15	67,150,258	C	T	0.24	2.71E-20
rs744166	17	42,362,183	A	G	0.42	1.14E-22
rs1292053	17	59,886,176	A	G	0.44	9.89E-13
rs12720356	19	10,359,299	A	C	0.09	4.13E-16
rs3806308	1	19,816,373	C	T	0.38	1.08E-21
rs34856868	1	92,088,726	G	A	0.03	9.80E-09
rs10800309	1	161,502,368	A	G	0.66	6.16E-37
rs3024493	1	206,770,623	C	A	0.16	1.65E-50
rs6074022	20	46,111,557	C	T	0.75	8.32E-11
rs780094	2	27,518,370	T	C	0.61	3.88E-15
rs7608910	2	60,977,721	A	G	0.39	2.60E-36
rs1990760	2	162,267,541	C	T	0.61	3.56E-10
rs406113	6	28,515,705	A	C	0.32	4.06E-08
rs4151651	6	31,947,837	G	A	0.03	1.13E-51
rs3807039	6	32,110,596	A	C	0.11	9.67E-19
rs3806157	6	32,406,024	T	G	0.35	3.28E-13
rs1847472	6	90,263,440	C	A	0.34	6.63E-10
rs1182188	7	2,830,351	T	C	0.30	1.08E-09
rs2108225	7	107,812,658	G	A	0.44	1.27E-11
rs6651252	8	128,554,935	T	C	0.13	9.08E-10
rs10758669	9	4,981,602	C	A	0.65	4.70E-48

described in the aforementioned technique (Tseng, 2021). We utilized bidirectional MR to investigate the presence of a causal relationship between IBD and T2DM. This was accomplished using summary data from the most extensive genome-wide association studies (GWAS) conducted on European populations for the aforementioned diseases.

Materials and methods

Overall study design

Initially, the summary statistics of genome-wide association studies on IBD and T2DM were obtained from the GWAS Catalog. Following this, an extensive bidirectional two-sample MR analysis was conducted to examine the causal associations between susceptibility to IBD and T2DM. In the first phase of the analysis, the exposure variable was IBD, whereas the outcome variable was T2DM. In the second step, the exposure variable was T2DM, and the outcome variable was IBD. Subsequently, we employed IBD as the exposure while IBD and T2DM were considered the outcomes for conducting a two-sample MR analysis. The study design is depicted in Figure 1.

Data retrieval

Based on the search results of datasets from GWAS on IBD (GCST003043), it had summary statistics for a total of 126,096 single nucleotide polymorphism (SNP) (Liu et al., 2015). The present GWAS meta-analysis investigation revealed datasets consisting of 34,652 individuals of European ancestry. A replication dataset was included, comprising 6,543 individuals of East Asian descent,

TABLE 2 Estimation of causal effect of IBD on T2DM with different MR methods.

Method	Estimate	Std error	OR	95% CI		*p*-value
Simple median	−0.014	0.026	0.986	0.937	1.038	0.587
Weighted median	−0.004	0.024	0.996	0.951	1.043	0.876
Penalized weighted median	−0.002	0.023	0.998	0.953	1.045	0.926
IVW	−0.052	0.036	0.950	0.885	1.019	0.152
Penalized IVW	−0.007	0.017	0.993	0.961	1.027	0.691
Robust IVW	−0.015	0.023	0.985	0.941	1.031	0.518
Penalized robust IVW	−0.005	0.016	0.995	0.964	1.026	0.727
MR-Egger	0.064	0.088	1.066	0.898	1.266	0.464
Penalized MR-Egger	0.029	0.049	1.029	0.936	1.132	0.553
Robust MR-Egger	0.028	0.051	1.029	0.931	1.137	0.576
Penalized robust MR-Egger	0.029	0.033	1.030	0.966	1.098	0.368

* The ORs, express effects of liability to IBD, on T2DM, risk. OR, odds ratio; CI, confidence interval; MR, mendelian randomization.

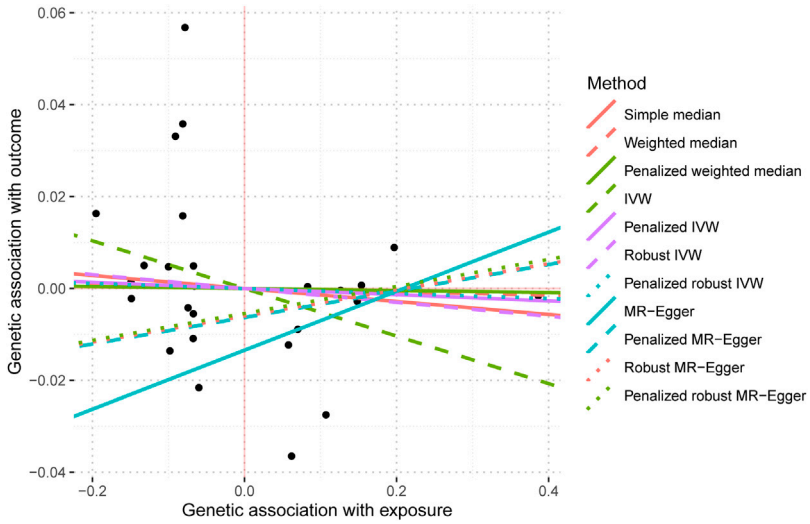

FIGURE 2
Mendelian randomization scatter plot diagram of two samples of the causal effect of IBD on T2DM. The *x*-axis represents the genetic association with IBD risk; the *y*-axis represents the genetic association with the risk of T2DM. Each line represents a diferent MR method. IBD, inflammatory bowel disease; T2DM, Type 2 diabetes mellitus; MR, Mendelian randomization. No signifcant associations were detected.

890 individuals of Greater Middle Eastern origin (including Middle Eastern, North African, or Persian populations), 51,988 individuals of European descent, and 2,413 individuals of South Asian ancestry. The GWAS dataset for T2DM (GCST007517) had summary statistics for a total of 131,218 SNP (Mahajan et al., 2018). The present GWAS meta-analysis was conducted on a sample of 298,957 people of European descent. The original publications provide comprehensive information on recruiting techniques and diagnostic criteria. The GWAS data utilized to analyze IBS (Dönertaş et al., 2021). This dataset encompassed a total of 9,689,034 SNP derived from 484,598 samples. The data was collected from the GWAS Catalog, specifically identified by the data number GCST90038626. SNPs location information using the human reference genome GRCH38 version. Information on recruitment procedures and diagnostic criteria is detailed in the original publications. Case-control association tests for IBD, IBS and T2DM were performed in each group using a linear mixed model as implemented in MMM (Pirinen et al., 2013). Moreover, as the GWAS samples were independent, no overlap is observed between the groups in GWAS populations.

Instrumental variable selection

The analysis focused on the genetic data from GWAS on IBD as the exposure factor. Initially, the selection of suitable SNPs as IVs was conducted by using the Bonferroni-corrected *p*-value obtained from multiple testing, as well as a minimum allele frequency (MAF)

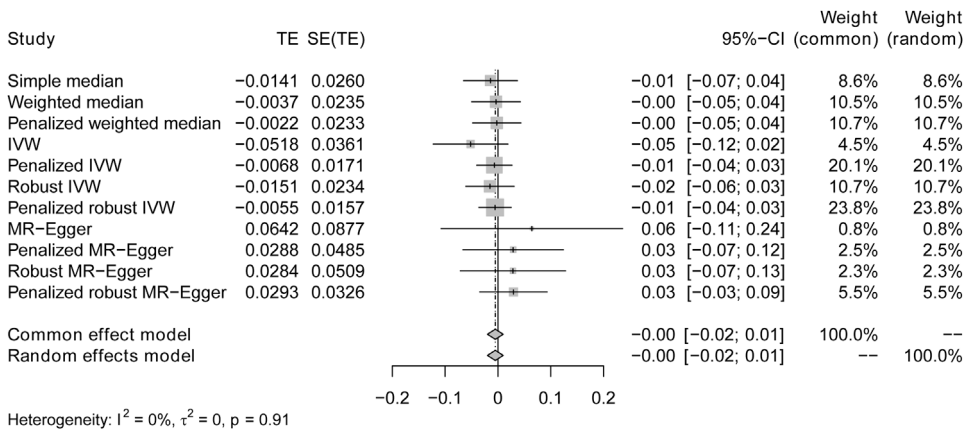

FIGURE 3
Forest plots of causal effects for IBD on T2DM. Summary of the Mendelian randomization (MR) result derived from the inverse-variance weight, MR Egger, and weighted median method, using the fixed effect common effect model.

TABLE 3 Heterogeneity test result on the IVW and MR Egger methods.

Method	Q	df	*p*-value
MR Egger	116.8911959	24	3.46E-14
Inverse variance weighted	127.0651,513	25	1.23E-15

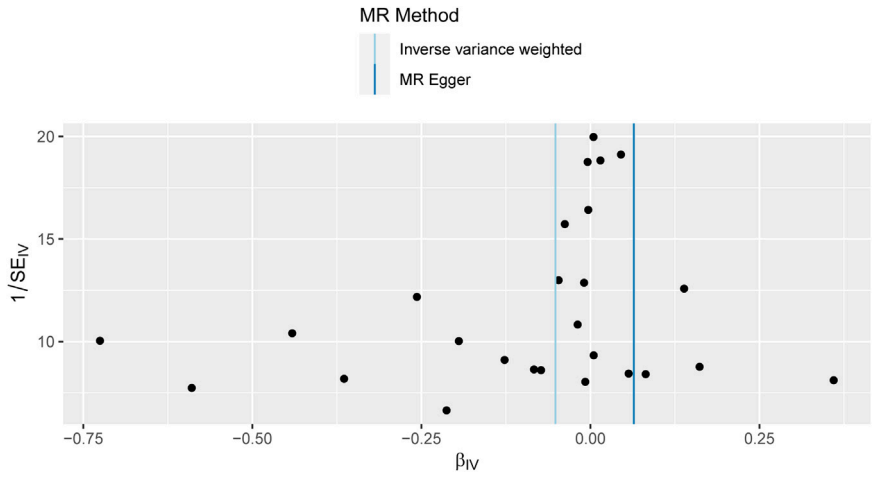

FIGURE 4
Funnel plot of Mendelian randomization results. Funnel plots for overall heterogeneity of MR estimates for the effect of IBD on T2DM. IVW, inverse-variance weighted.

threshold (p < 5e-8, MAF >0.01) (Yuan et al., 2023a). Subsequently, the genetic linkage coefficient of commonly occurring SNPs in the European population reference panel (1000G phase III EUR), as made available through the R package IEU GWAS, was employed to exclude SNPs that exhibited strong linkage. This identification process was carried out using a clumping window of 10,000 kb and an r^2 cutoff value of 0.001, as described in a prior study (Xiang et al., 2021). Ultimately, SNPs that were present in both the exposure and outcome GWAS summary statistics and had available data were selected as the final instrumental variables (Table 1). The GWAS summary statistical data for IVs is presented in Supplementary Table S1. The reverse MR was assessed following the same procedure.

Mendelian randomization (MR) analyses

The TwoSample MR R package was utilized for the bidirectional two-sample MR (Li et al., 2022). We used IBD as the exposure and T2DM as the outcome to build two-sample MR model. Then, to

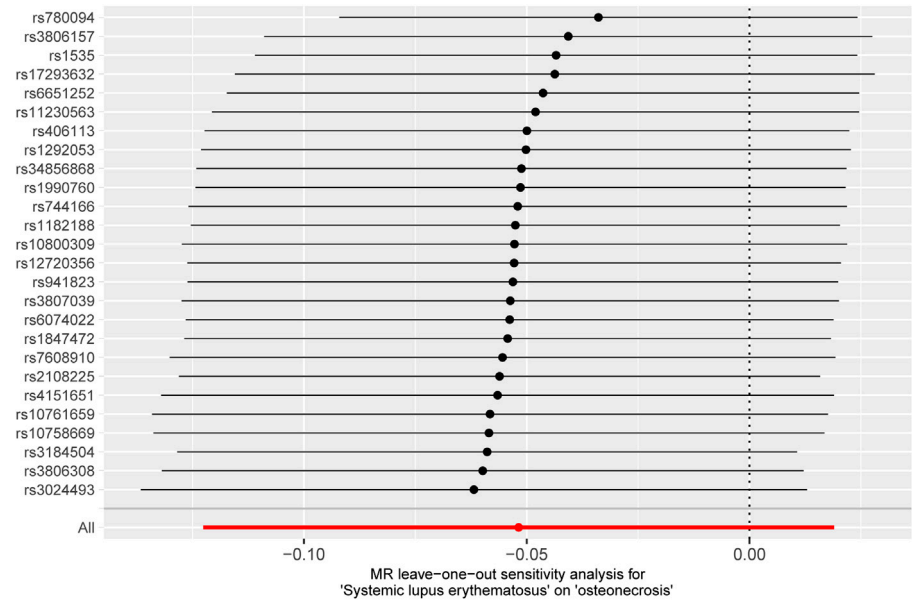

FIGURE 5
MR leave-one-out sensitivity analysis of T2DM to investigate the possibility of causal association driven by a particular SNP. Each black point represents an inverse variance weighted method for estimating the causal effect of IBD on T2DM, excluding that particular instrumental variable from the analysis. Redpoint represents the estimate using all instrumental variables. Horizontal lines denote 95% confidence intervals. OR, odds ratio; SD, standard deviation.

TABLE 4 Estimation of causal effect of T2DM on IBD with different MR methods.

Method	Estimate	Std error	OR	95% CI		*p*-value
Simple median	−0.112	0.076	0.894	0.770	1.039	0.143
Weighted median	−0.104	0.077	0.901	0.774	1.048	0.178
Penalized weighted median	−0.108	0.077	0.897	0.771	1.044	0.161
IVW	−0.167	0.134	0.846	0.651	1.100	0.212
Penalized IVW	−0.118	0.062	0.889	0.787	1.004	0.058
Robust IVW	−0.103	0.156	0.902	0.665	1.224	0.509
Penalized robust IVW	−0.103	0.095	0.902	0.749	1.086	0.276
MR-Egger	0.108	0.507	1.113	0.412	3.007	0.832
Penalized MR-Egger	−0.192	0.232	0.826	0.524	1.300	0.409
Robust MR-Egger	0.123	0.482	1.131	0.440	2.909	0.798
Penalized robust MR-Egger	−0.302	0.277	0.739	0.429	1.273	0.276

* The ORs, express effects of liability to T2DM, on IBD, risk. OR, odds ratio; CI, confidence interval; MR, mendelian randomization.

integrate the influence of distinct IVs, the inverse variance weighted (IVW) method was employed (Huang et al., 2022). The IVW technique was mainly used for fundamental causal estimations, which would yield the most accurate findings if all chosen SNPs were acceptable IVs (Xu et al., 2022). For each SNP, the Wald ratio was determined, and the individual effect of each SNP was meta-analyzed using IVW to get the final beta estimate, which was transformed into an OR (Alipour et al., 2022). To evaluate the third MR assumption, the MR Egger analysis was applied to identify any violations of IV assumptions owing to directional horizontal pleiotropy (Luo et al., 2022). We use the mr_pleiotropy_test function of R package TwoSampleMR (v0.5.7, https://rdrr.io/github/MRCIEU/TwoSampleMR/) to test the pleiotropy of SNPs.Cochran's Q test assessed heterogeneity in the IVW and MR Egger procedures. We use R package TwoSampleMR's MR_ The heterogeneity function to calculate the Cochran's Q statistic, the following formula: $Q = \sum_j w_j (\hat{\beta}_j - \hat{\beta})^2$ ($\hat{\beta}_j$ is the estimated coefficient value obtained from the *j*th IV, w_j is the corresponding

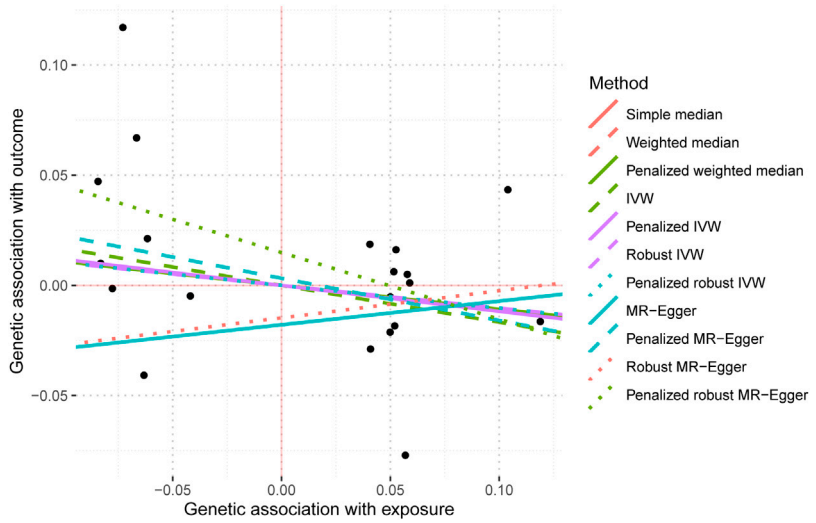

FIGURE 6
MR scatter plot diagram of the causal effect of two samples of T2DM on IBD.

TABLE 5 Estimation of causal effect of IBS on IBD with different MR methods.

Method	Estimate	Std error	OR	95% CI		*p*-value
Simple median	4.023	2.693	55.880	0.285	1.095E+04	0.135
Weighted median	4.010	2.677	55.160	0.290	1.048E+04	0.134
Penalized weighted median	3.851	2.648	47.020	0.262	8.444E+03	0.146
IVW	2.750	6.916	15.638	0.000	1.204E+07	0.691
Penalized IVW	4.133	2.025	62.377	1.179	3.301E+03	0.041
Robust IVW	5.537	2.888	253.827	0.884	7.286E+04	0.055
Penalized robust IVW	4.277	2.188	72.045	0.989	5.247E+03	0.051
MR-Egger	20.071	16.965	5.207E+08	0.000	1.438E+23	0.237
Penalized MR-Egger	13.337	4.543	6.196E+05	84.123	4.564E+09	0.003
Robust MR-Egger	13.642	6.029	8.408E+05	6.201	1.140E+11	0.024
Penalized robust MR-Egger	14.198	5.371	1.466E+06	39.318	5.465E+10	0.008

* The ORs, express effects of liability to IBS, on IBD, risk. OR, odds ratio; CI, confidence interval; MR, mendelian randomization.

weight, and $\hat{\beta}$ is the pooled estimate value obtained by combining IVW or MR Egger), where two Q statistics refer to IVW and MR Egger with p-value <0.05 were deemed to be reliable. The Weighted Median regression method, calculates a weighted median of the Wald ratio estimates and is robust to horizontal pleiotropic bias, when the majority valid assumption holds. (Bowden et al., 2016). It has been verified that the Weighted Median approach outperforms the MR-Egger regression regarding lowering the type I error and higher causal estimate power (Ding et al., 2023). Finally, a leave-one-out (LOO) analysis was undertaken to determine whether any particular SNP is disproportionately responsible for the outcome of any MR study (Gao et al., 2023). Applying Bonferroni correction for multiple testing, a p-value below 2.1E−03 was considered as signifcant.

Post-GWAS analysis of type 2 diabetes-associated SNPs

We expect to observe associations with genes involved in T2DM, so we used FUMA to perform a functional mapping of genetic associations to loci of the T2DM GWAS. Functional Mapping and Annotation (FUMA) is a comprehensive approach that combines positional mapping (Dai et al., 2022). The current study used FUMA, Expression quantitative trait locus (eQTL) mapping, and chromatin interaction mapping approaches, to perform precise mapping of SNPs identified in GWAS for T2DM. SNPs that exhibit a p-value less than 5e-8 are commonly referred to as tag SNPs. These tag SNPs are then included in the FUMA system for fine mapping (Cao et al., 2022).

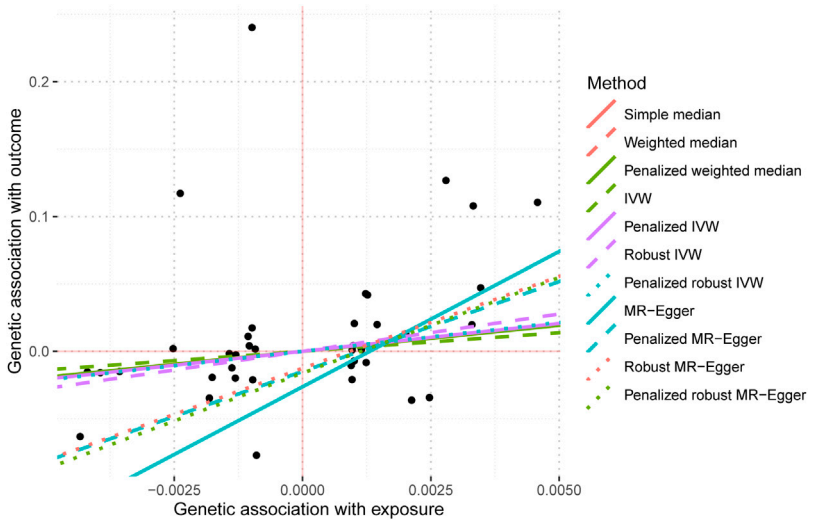

FIGURE 7
MR Scatter plot diagram of two samples of the causal Effect of IBS on IBD.

TABLE 6 Estimation of causal effect of IBS on T2DM with different MR methods.

Method	Estimate	Std error	OR	95% CI		*p*-value
Simple median	−3.573	1.988	0.028	0.001	1.380	0.072
Weighted median	−3.601	1.992	0.027	0.001	1.354	0.071
Penalized weighted median	−3.657	2.011	0.026	0.001	1.330	0.069
IVW	−3.082	2.205	0.046	0.001	3.458	0.162
Penalized IVW	−1.508	1.386	0.221	0.015	3.349	0.277
Robust IVW	−2.213	1.891	0.109	0.003	4.455	0.242
Penalized robust IVW	−2.420	2.131	0.089	0.001	5.791	0.256
MR-Egger	−1.826	5.229	0.161	0.000	4.54E+3	0.727
Penalized MR-Egger	−0.002	0.007	0.998	0.984	1.013	0.790
Robust MR-Egger	−5.690	3.239	0.003	0.000	1.932	0.079
Penalized robust MR-Egger	0.007	0.005	1.007	0.998	1.016	0.676

* The ORs, express effects of liability to IBS, on T2DM, risk. OR, odds ratio; CI, confidence interval; MR, mendelian randomization.

Furthermore, the default parameters are utilized for position mapping, eQTL mapping, and 3D Chromatin Interaction mapping. The present study employed genes derived from fine mapping using MAGMA software for enrichment analysis by enriching them in various gene function sets and tissues, categorized based on GTEx's 30 general tissues (Xu et al., 2023). Enrichment analysis (Gene Set Enrichment Analysis, GSEA) determines if a group of genes appears more frequently in a specific functional pathway than random chance (Yang et al., 2023). Using the precise test method of hypergeometric distribution, taking the enrichment analysis of differential expression as an example, the *p*-value calculation formula is: $p = 1 - \sum_{j=0}^{x-1} \frac{\binom{M}{j}\binom{N-M}{n-j}}{\binom{N}{n}}$. Among them, N represents the total number of genes, n represents the number of differentially expressed genes, M represents the total number of genes in the gene set, and j represents the number of differentially expressed genes in the gene set. The tissue-specific expression of genes acquired through fine mapping was examined using FUMA, utilizing the 30 general tissues provided by GTEx v8 (Yuan et al., 2023b). Heatmaps depicting the variations in gene expression unique to distinct tissues were produced and analyzed to identify differentially generated (Li et al., 2023). In a manner akin to the preceding enrichment analysis, additional functional datasets such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were incorporated (Zhang et al., 2023). Integrating the GWAS Catalog into FUMA enhances the enrichment of identified genes within several biological functional modules and pathways. The objective is to ascertain the presence of functional modules within the

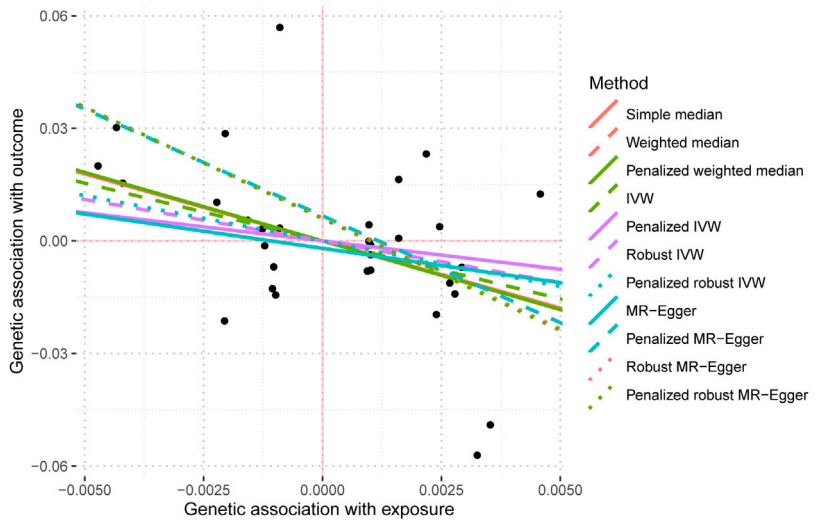

FIGURE 8
MR Scatter plot Diagram of Two Samples of the Causal Effect of IBS on T2DM.

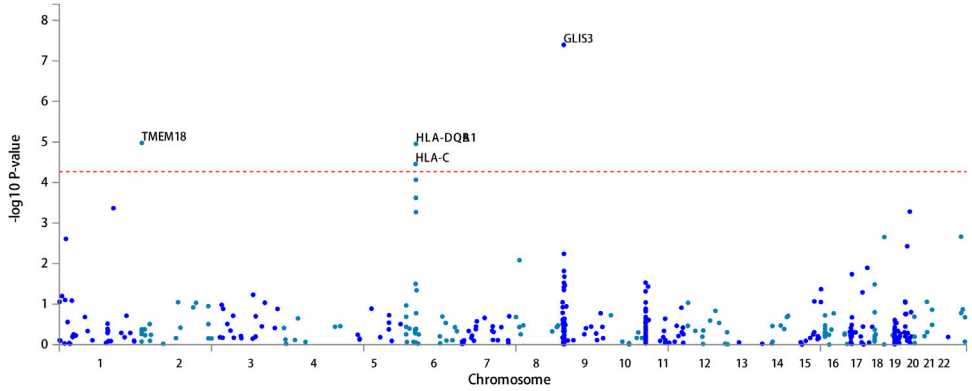

FIGURE 9
The GWAS Manhattan map of gene based for T2DM.

gene set linked to T2DM and their potential correlations with other diseases or phenotypes.

Results

The IVW analysis yielded findings indicating that the susceptibility to IBD does not have an impact on the risk of T2DM. These results align with the outcomes obtained by other MR techniques, such as MR Egger and weighted median, as presented in Table 2 and Figure 2. Table 2 displays the outcomes of the MR analyses that examined the causal association between IBD and T2DM. Correspondingly, Figure 2 presents scatter plots illustrating these findings. Based on the findings shown in Table 2 and Figure 2, it is evident that none of the approaches yielded statistically significant causal estimates when employing a significance level of 0.05. This finding can also be observed through implementing Forest Plots (Figure 3). Furthermore, the positive and negative values produced by different methods exhibited variability. In addition, the MR-Egger, the Penalized MR-Egger, the Robust MR-Egger, and the Penalized robust MR-Egger methods showed consistent positive results. Conversely, the Simple median, the Weighted median, the Penalized weighted median, the IVW, the Penalized IVW, the Robust IVW, and the Penalized robust IVW methods showed consistent negative results. The results indicate that there may be pleiotropy or heterogeneity among the Instrumental Variable used for MR Egger and IVW analysis. All in all, Mendelian randomization of two samples shows that MR analysis is insufficient to support the causal effect of IBD on T2DM.

Sensitivity studies were conducted to identify the potential existence of horizontal pleiotropy. To validate the reliability of the aforementioned study findings, a sensitivity analysis was

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TABLE 7 MAGMA gene set enrichment analysis of the 10 most significant gene set information.

Gene set	N genes	Beta	Beta STD	Se	P	P_{bon}
GOCC_ER_TO_GOLGI_TRANSPORT_VESICLE_MEMBRANE	9	2.2238	0.21887	0.43357	2.3546e-07	0.00174570044
GOCC_COPII_COATED_ER_TO_GOLGI_TRANSPORT_VESICLE	10	2.1426	0.22216	0.43095	5.0855e-07	0.00376988115
GOCC_COATED_VESICLE_MEMBRANE	19	1.6547	0.23533	0.3456	1.2202e-06	0.0090441224
WP_ALLOGRAFT_REJECTION	8	2.1954	0.20383	0.491	5.1758e-06	0.0383578538
GOCC_TRANSPORT_VESICLE_MEMBRANE	15	1.7589	0.22276	0.39617	5.9528e-06	0.044110248
GOMF_ANTIGEN_BINDING	10	2.1159	0.2194	0.4782	6.3574e-06	0.0471019766
GAURNIER_PSMD4_TARGETS	8	2.1457	0.19922	0.49165	8.2807e-06	0.0613434256
GOCC_LUMENAL_SIDE_OF_ENDOPLASMIC_RETICULUM_MEMBRANE	7	2.1309	0.18516	0.49267	9.8151e-06	0.0727004457
GOBP_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_PEPTIDE_ANTIGEN	12	2.0629	0.23406	0.47816	1.0287e-05	0.076185522
GOCC_MHC_PROTEIN_COMPLEX	7	2.1245	0.18461	0.49275	1.0408e-05	0.07707124

performed following the methodology outlined in the methods section. Initially, a pleiotropy test was performed, yielding a test statistic of p = 0.161,304, which is above the conventional significance threshold of 0.05. This outcome suggests no substantial pleiotropy, hence validating the choice of IV. Additionally, heterogeneity tests were performed on the IVW and MR Egger techniques, and the outcomes are presented in Table 3. Based on the outcomes of the heterogeneity test presented in Table 3, it was found that there was a notable presence of heterogeneity among the chosen instrumental factors ($p < 0.05$). Confirming this finding can also be observed through the funnel plot (Figure 4). These visual representations demonstrate that the estimated values derived from each SNP exhibit an asymmetrical distribution on both ends of the combined estimate. Additionally, there is a notable and statistically significant rightward deviation in the merged estimate.

Finally, a sensitivity analysis was performed on the retention method. After iteratively removing individual SNP one by one, the estimated forest plot was obtained via IVW (Figure 5). As depicted in Figure 5, the estimation results after removing a single SNP are relatively stable and exhibit minor changes after the exclusion of a single SNP. Moreover, the causal estimates derived from the IVW method, after the removal of each SNP, do not demonstrate statistical significance, further supporting our previous conclusion. In summary, this Mendelian randomisation study provides support for the absence of a substantial causal link between IBD and T2DM.

The screening criteria for IVs were kept unchanged as described in the Methods section, resulting in 20 IVs (Supplementary Table S2). The outcomes acquired by the use of identical 11 MR techniques are presented in Table 4 and Figure 6. The lack of statistical significance and inconsistent positive and negative values of the predicted coefficients produced by all MR techniques can be observed in Table 4 and Figure 6. The aggregation of findings from many methodologies reveals that using MR analysis alone does not provide sufficient evidence to establish a causal relationship between T2DM and IBD.

The present study employed irritable bowel syndrome (IBS) as the exposure variable while considering IBD and T2DM as the outcome variables to conduct a two-sample analysis. The Supplementary Material containing the MR imaging data for IBS in IBD can be accessed in Supplementary Table S3. The causative estimates derived from these data are presented in Table 5 and Figure 7. The acquired estimated values using several enhanced MR Egger techniques demonstrate statistical significance, suggesting a causal relationship between IBS and IBD.

The data used for MR imaging of IBS in T2DM can be found in Supplementary Table S4. The resulting causal estimates derived from this data are presented in Table 6 and Figure 8. It is evident that despite the negative estimated values derived from different methodologies, they lack statistical significance. Consequently, asserting that IBS may have a substantial causal impact on T2DM is unacceptable.

FUMA, a web-based bioinformatics tool that uses a combination of positional, eQTL and chromatin interaction mapping to prioritize likely causal variants and genes. The approach involved utilizing the acquired GWAS summary data for T2DM to conduct fine positional and functional analysis using the FUMA of the GWAS tool. The

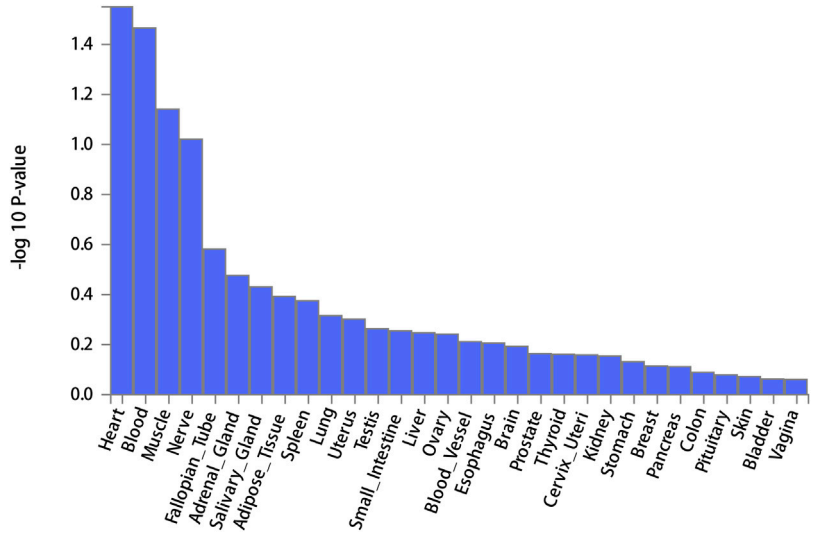

FIGURE 10
Expression of MAGMA gene in 30 general tissues.

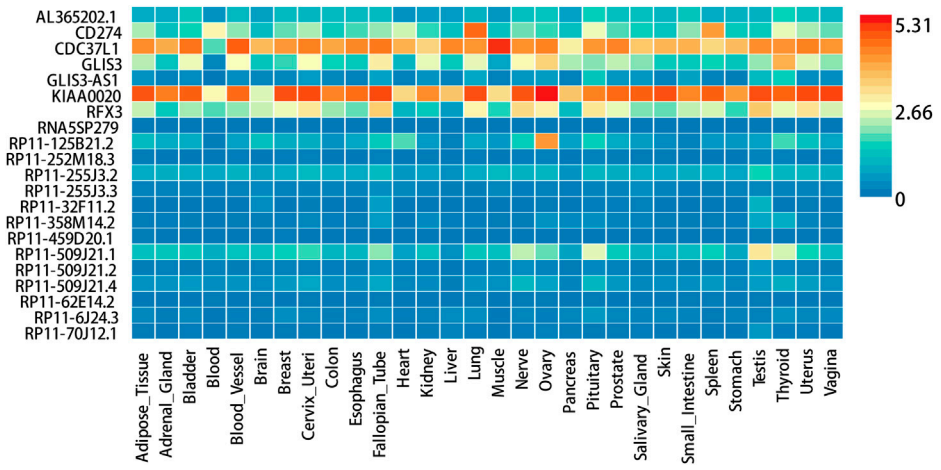

FIGURE 11
Expression heatmap of 22 fine mapped genes in 30 general tissues.

GWAS Manhattan map, which is based on genes, revealed the identification of four highly significant genes related to T2DM (Figure 9, Supplementary Table S4). FUMA precisely mapped 22 protein-coding genes utilizing significant SNPs acquired from GWAS. Table 7 displays the top 10 most significant gene sets enriched by the localized genes using MAGMA's gene set enrichment analysis. The utilization of MAGMA to enhance the inclusion of genes into various tissues, predicated on their respective expression activity, is demonstrated in Figure 10. Several genes associated with T2DM do not exhibit notable tissue specificity. To further investigate the tissue-specific expression patterns of the genes linked with T2DM, we generated a heat map depicting their expression levels across various tissues (Figure 11). Additionally, we performed an enrichment analysis to assess the differential expression of these genes in distinct tissues (Figure 12). In line with the findings of MAGMA analysis, the genes associated with T2DM identified by fine mapping did not exhibit statistically significant variations in tissue-specific expression.

This research utilizes publicly available GWAS summary information to examine the causal association between IBD, IBS, and T2DM using an MR method. Mendel's randomized analysis revealed that the available data did not provide substantial support for a significant causal relationship between IBD and T2DM. Furthermore, the analysis did not find sufficient evidence to suggest a causal relationship between T2DM and IBD.

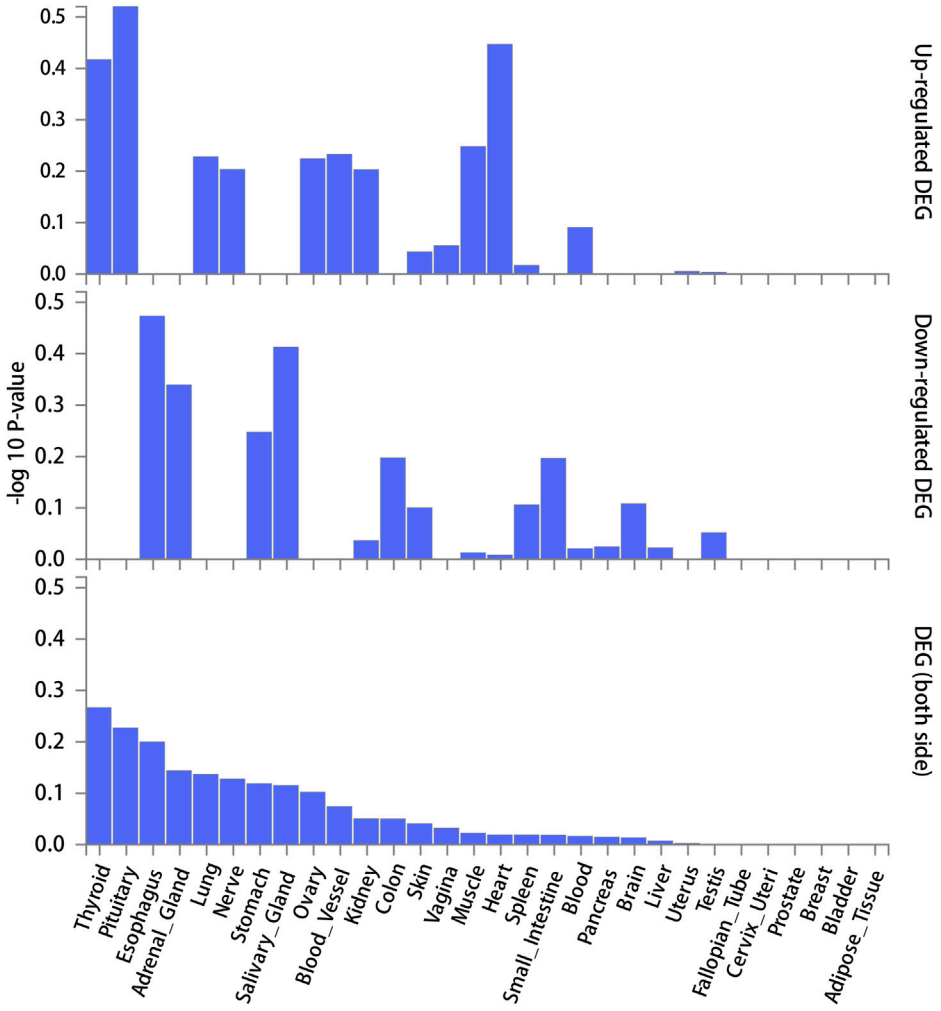

FIGURE 12
Enrichment testing of differentially expressed genes in 30 general tissues.

Discussion

This was the first study to investigate the bidirectional causal association between IBD and T2DM using a comprehensive bidirectional two-sample MR analysis method. The results of the two-sample MR analysis did not provide any evidence to substantiate the association between genetically predicted IBD and T2DM in individuals of European ancestry. The findings from the reverse MR investigation also indicated a lack of evidence supporting a relationship between genetic susceptibility to T2DM and IBD. It is implying that the reported epidemiological associations of T2DM and IBD could be the result of unmeasured confounding factors or shared genetic architecture. To differentiate between an actual negative outcome and a potential lack of validity in the MR investigations, many sensitivity analyses were conducted to verify the fulfillment of the 3 MR assumptions. Given the consistency of our MR fndings across these diferent methods, we are confdent about the validity of our MR analyses to exclude moderate to large causal efects of the exposures on the outcomes. These analyses effectively rule out the presence of substantial causal effects of the exposures on the outcomes under investigation. Previous epidemiological studies that noted a link between IBD and T2DM have been undertaken (Kim et al., 2015; Abrahami et al., 2018; Villumsen et al., 2022). The results of the current study oppose an observational study by Abdo Jurjus et al., 2015 supporting an association between liability to IBD and increased risk of T2DM (Jurjus et al., 2015). Benchimol EI et al., 2015 conducted a thorough investigation to examine the genetic link between IBD and T2DM. The findings of that study revealed a favorable genetic correlation between these two conditions. The pathogenesis of IBD is complex and involves several factors. It is characterized by chronic and extensive inflammation in the gastrointestinal tract and an imbalance in the gut microbiota. This dysbiosis leads to the upregulation of various pro-inflammatory mediators and biomolecules, ultimately contributing to the development of T2DM (Benchimol et al., 2015). One plausible explanation for the observed correlations between IBD and T2DM, as indicated by our MR results, is the presence of pleiotropy in the lack of a causal

relationship. In this study, we employed IBS as the exposure variable, while considering IBD and T2DM as the outcome variables to conduct a two-sample analysis. The results obtained using several improved MR Egger methodologies provide statistical significance (1.08; 95% CI, 1.03–1.12), suggesting a causative relationship between IBS and IBD. In a recent study, (Chen et al., 2023), showed an association between T2DM and IBS (1.08; 95% CI, 1.03–1.12). However, no substantial evidence supports a causal relationship between IBS and T2DM in our study. The differences in study results may be related to the sources of Biobank data.

In summary, the findings of the present investigation did not establish a direct relationship between genetically predicted IBD and T2DM. Similarly, there was no evidence of a causative association between genetically predicted T2DM and IBD. A potential association between IBD and T2DM may exist, wherein the gut microbiota might serve as a connecting factor. In a comparative study, untargeted metabolomics and shotgun metagenomic profiling were conducted on cross-sectional stool samples from two cohorts: a discovery cohort of 155 patients and a validation cohort of 65 patients. The patients in these cohorts were diagnosed with either Crohn's disease (CD), ulcerative colitis (UC), or non-IBD controls. The study revealed enrichments of sphingolipids and bile acids and depletions of triacylglycerol and tetrapyrrole in these patients (Franzosa et al., 2019). Patients diagnosed with IBD exhibit a decrease in bacterial diversity and abundance compared to persons without the condition. This decrease is accompanied by an increase in the presence of Firmicutes and Bacteroidetes, which is comparable to the microbial composition observed in certain cases of T2DM (Quaglio et al., 2022; Lim et al., 2023). Numerous investigations have elucidated the role of ceramides and other sphingolipids in impeding the insulin-signaling pathway in skeletal muscles and the liver, contributing to insulin resistance and T2DM (Roszczyc-Owsiejczuk and Zabielski, 2021). Furthermore, a recent study has indicated that an increase in bile acids may result in an imbalance in the gut microbiota in T2DM model rats (Tawulie et al., 2023). The findings of this study provide evidence in favor of the notion that alterations in the composition of the intestinal microbiota and its associated metabolic profile in individuals with IBD may contribute to an elevated susceptibility to developing T2DM.

There are various limitations inherent in the current investigation. The study conducted in this research is based on European population data, and it is important to note that the findings should not be extrapolated to other populations. Furthermore, a number of our MR analyses were hindered by insufficient statistical power to identify subtle effects. This may be attributed to either the restricted variability of the exposures described by the SNP instruments or the low sizes of the outcome GWAS samples. The exclusion of ambiguous or palindromic SNPs from our MR instruments may have had further implications for the statistical power of this MR research. The utilization of comprehensive summary data from large-scale GWAS pertaining to IBD and T2DM is expected to enhance the statistical power of future MR investigations in identifying potential connections. Additionally, it is imperative to do a comprehensive multivariable MR study that encompasses IBD, T2DM, and gut microbiota.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/gwas/downloads/summary-statistics.

Author contributions

L-TT: Writing–original draft, Data curation. LF: Writing–review and editing. H-YC: Data curation, Writing–review and editing. RS: Data curation, Methodology, Writing–original draft. B-BL: Formal Analysis, Validation, Writing–original draft. Y-BZ: Writing–original draft, Formal analysis, Data resources. Y-ML: Software, Visualization, Writing–review and editing. JZ: Project administration, Writing–review and editing. S-YL: Project administration, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2024.1325401/full#supplementary-material

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Association of ankylosing spondylitis with cardiovascular disease: a bidirectional two-sample mendelian randomization study

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Backgrounds: Current observational investigations hint at a potential linkage between ankylosing spondylitis and cardiovascular wellness. However, the nature of this causality remains to be elucidated. Consequently, this study is designed to evaluate the causal interconnection between ankylosing spondylitis and cardiovascular-related conditions utilizing a bidirectional two-sample Mendelian Randomization (MR) methodology.

Methods: In this study, we conducted Mendelian randomization (MR) analyses using genome-wide association study (GWAS) data. The fixed-effects inverse variance weighted (IVW) model was used as the primary analysis method, and MR-Egger regression and the weighted median method were employed as supplementary approaches. Horizontal pleiotropy and heterogeneity were evaluated using various statistical tests, including MR-PRESSO global test, MR-Egger intercept, and Cochran's Q test.

Results: The MR result demonstrated an increased risk of heart failure in individuals with ankylosing spondylitis (OR: 1.0132, 95% CI = 1.0043-1.0221, p = 0.003). The MR analysis results did not demonstrate a causal relationship between ankylosing spondylitis and other cardiovascular diseases, such as atrial fibrillation, coronary artery disease, ischemic stroke, myocardial infarction, and valvular heart disease (all $p > 0.05$). No evidence of reverse causality was found between ankylosing spondylitis and mentioned cardiovascular diseases in reverse MR analyses. Sensitivity analysis verified the reliability of the results.

Conclusion: Our MR study indicates a relationship between ankylosing spondylitis and an increased risk of heart failure. Further research is needed to confirm these findings and elucidate the underlying mechanisms involved.

KEYWORDS

ankylosing spondylitis, cardiovascular diseases, heart failure, mendelian randomization, causality

Introduction

Cardiovascular diseases (CVDs) encompass a multitude of disorders affecting the heart and vascular system (Tsao et al., 2022). Both genetic and environmental factors may play a role in the development and progression of CVDs. Despite advances in diagnosing and treating these disorders, CVDs remain the principal cause of mortality and disability worldwide (Roth et al., 2020). Given the severe societal and clinical consequences, early identification and intervention for CVDs risk factors are vital for reducing incidence and mortality rates (Roger et al., 2020). Notably, some studies suggest a possible association between ankylosing spondylitis and CVDs (Ding et al., 2022; Kwon et al., 2022).

Ankylosing spondylitis (AS) is classified as an autoimmune disease leading to bone remodeling and spinal rigidity (Toussirot, 2021). Previous studies have not definitively established the risk of CVDs in patients with AS. While some studies indicate AS as an independent risk factor for CVDs (Setyawan et al., 2021; Kwon et al., 2022), conflicting results have been published (Tsai et al., 2015), and whether these relationships are causal and their directionality remain unclear. Recently, a large meta-analysis indicated that CVD comorbidities are common in AS, providing crucial evidence of an association and attracting significant attention (Zhao et al., 2020). Due to the potential limitations of confounding factors and reverse causality in current observational studies, there is an urgent need to explore the causal relationship between AS and CVDs using robust research methods.

Mendelian randomization (MR) is a research method that utilizes genetic variations associated with the exposure of interest as instrumental variables (IVs) to infer the linkages between exposure and disease consequences (Burgess and Thompson, 2015). Mendelian randomization (MR) analyses are less susceptible to confounding, reverse causality, and measurement error compared to conventional observational studies due to the random allocation of genetic variants at conception, which precedes the onset of disease (Lawlor et al., 2008; Yarmolinsky et al., 2019). The applicability of MR studies has been demonstrated in the evaluation of diverse causal relationships between behavioral exposures, educational attainment, socioeconomic statuses, and various diseases (Tillmann et al., 2017; Harrison et al., 2020). Furthermore, prior MR explorations have scrutinized the causal implications of AS on stroke (Mei et al., 2022) and atrial fibrillation (Chen et al., 2022). Nonetheless, these endeavors have largely focused on specific forms of CVDs. Therefore, our research engaged a MR design to ascertain the existence of a causal association between AS and CVDs, thus providing a scientific groundwork for the primary prevention of CVDs.

Materials and methods

Study design and GWAS datasets

We adopted a two-sample bidirectional Mendelian Randomization (MR) approach to assess the causal relationship between AS and CVDs, the latter specifically encompassing Heart Failure (HF), Coronary Atherosclerosis, Valvular Heart Disease (VHD), Atrial Fibrillation (AF), Myocardial Infarction (MI), and Ischemic Stroke (IS). Concurrently, a reverse analysis was conducted to investigate the potential causal relationship between CVDs and AS. The core premises of our MR design are predicated on three assumptions (Tsao et al., 2022): genetic variations display significant associations with the exposure (Roth et al., 2020); genetic variations are independent of any confounding variables (Roger et al., 2020); genetic variations link to the clinical outcome solely via the exposure path (Davies et al., 2018; Tsao et al., 2022) (Figure 1).

Summary statistic data for AS and VHD were obtained from the FinnGen database (https://www.finngen.fi/en). The datasets (Table 1) for AS and VHD respectively included 166,144 participants (consisting of 1,462 cases and 164,682 controls) and 218,792 participants (including 38,209 cases and 180,583 controls). Data on HF was collected from the HERMES consortium, encompassing 977,323 individuals of European descent (with 47,309 cases and 930,014 controls) (Shah et al., 2020). The summary statistics for AF were derived from a large-scale GWAS meta-analysis, incorporating 60,620 AF cases and 970,216 controls (Nielsen et al., 2018). The summary statistics for Ischemic Stroke were sourced from a large-scale GWAS, including 440,328 individuals of European descent (comprising 34,217 Ischemic Stroke cases and 406,111 controls) (Malik et al., 2018). The GWAS summary statistics for Coronary Atherosclerosis were procured from the UK Biobank, screening 361,194 Europeans (with 14,334 cases and 346,860 controls). The summary statistic data for Myocardial Infarction was obtained from a large-scale GWAS that involved a total of 395,795 participants (includes 14,825 MI cases and 380,970 controls) (Hartiala et al., 2021). To address potential biases resulting from population stratification, the GWAS datasets utilized in this study exclusively included individuals of European ancestry. The current analysis did not require ethical approval as all GWAS statistical data in current analysis are publicly available and have already received approval from relevant ethics review boards.

Statistical analysis

This study was orchestrated in accordance with the STROBE-MR guidelines (Skrivankova et al., 2021a). Initially, we designated single nucleotide polymorphisms (SNPs) with substantial associations with AS as IVs ($p < 5.0 \times 10^{-8}$). Subsequently, to ensure the independence of genetic variants, a further pruning step was conducted on SNPs based on linkage disequilibrium (LD) (distance threshold = 10,000 kb, $r^2 < 0.001$). The F statistic was used to assess the strength of the Ivs in the analysis. An F statistic >10 (Burgess and Thompson, 2011; Pierce et al., 2011) indicates a low risk of weak instrument bias in the MR analysis (the calculation formula can be found in (Figure 2). If the chosen SNP was not included in the GWAS of the outcome variable, LD proxy SNPs from the 1000 Genomes Project ($r^2 > 0.8$) were selected as substitutes. Additionally, any palindromic SNP with a minor allele frequency (MAF> 0.3) was removed to ensure the effects of the SNP on exposure and disease are attributed to the same allele (Davey Smith and Hemani, 2014; Burgess et al., 2016). Moreover, to minimize the influence of confounding factors on the outcome variable, we excluded potential instrumental variables (IVs) that

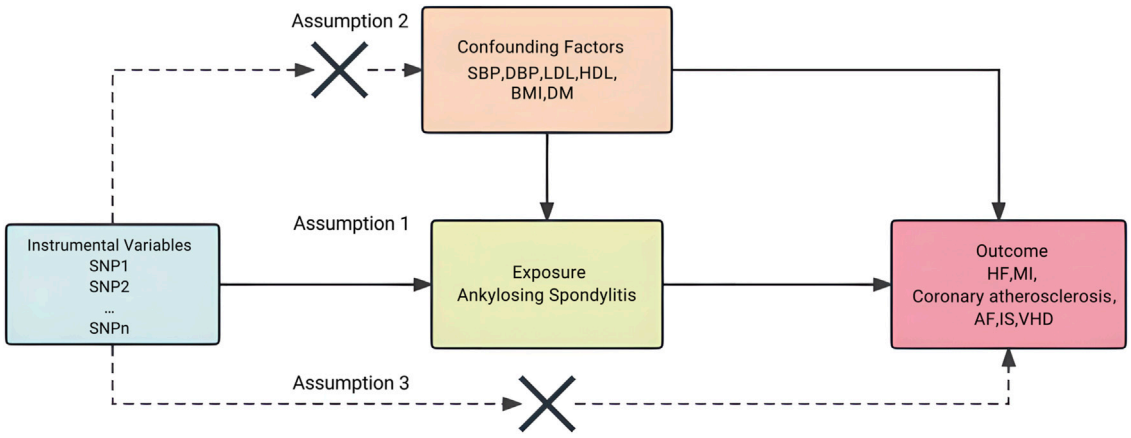

FIGURE 1
Study design of forward Mendelian randomization. Conceptual schematic of the two-sample Mendelian randomization for the association between ankylosing spondylitis and the risks of cardiovascular disease.

TABLE 1 Sample characteristics for exposures and outcomes in the Mendelian randomization analysis.

Trait	Sample size case/Control	Years	Population	Consortium	Data sources
Ankylosing spondylitis	1,462/164,682	2021	European	FinnGen	finn-b-M13_ANKYLOSPON
Heart Failure	47,309/930,014	2020	European	HERMES	ebi-a-GCST009541
Coronary atherosclerosis	14,334/346,860	2018	European	United Kingdom Biobank	ukb-d-I9_CORATHER
Ischemic stroke	34,217/406,111	2018	European	Malik et al.	ebi-a-GCST006908
Valvular heart disease	38,209/180,583	2021	European	FinnGen	finn-b-I9_VHD_EXNONE
Atrial fibrillation	60,620/970,216	2018	European	Nielsen et al.	ebi-a-GCST006414
Myocardial Infarction	14,825/380,970	2021	European	Hartiala et al.	ebi-a-GCST011364

$$F = \frac{R^2 \times (N-2)}{1-R^2}$$

$$R^2 = \frac{2 \times \mathrm{EAF} \times (1-\mathrm{EAF}) \times \beta^2}{2 \times \mathrm{EAF} \times (1-\mathrm{EAF}) \times \beta^2 + 2 \times \mathrm{EAF} \times (1-\mathrm{EAF}) \times N \times \mathrm{se}^2}$$

FIGURE 2
Calculation formula. F: F-statistic, which measures the overall significance of the regression model. R2: Coefficient of determination, which represents the proportion of the variance in the dependent variable explained by the independent variable(s). N: Sample size, the number of observations in the dataset. EAF: Exposure allele frequency, the frequency of the allele associated with the exposure variable in the population. Beta: Regression coefficient, represents the change in the dependent variable associated with a one-unit change in the independent variable. se: Standard error, which measures the variability or uncertainty associated with the estimated regression coefficient.

were associated with known confounders referring to the human genotype-phenotype association database (PhenoScanner-V2, http://www.phenoscanner.medschl.cam.ac.uk/) (Staley et al., 2016; Kamat et al., 2019). These rigorous steps were taken to ensure the validity and integrity of the IVs used in MR analysis (Figure 3).

The Inverse Variance Weighted (IVW) method was the main analytical approach used in this study. This method combines the Wald ratio estimates of each SNP on the outcome to obtain a summary causal estimate and provide maximum statistical power (Smith and Ebrahim, 2003; Pagoni et al., 2019). In conjunction, we applied the MR-Egger method (Burgess and Thompson, 2017) and the Weighted Median (WM) method (Bowden et al., 2016) as supplementary analysis tools, acknowledged as some of the most scientifically robust and commonly employed methods in this field (Skrivankova et al., 2021b). The assessment of horizontal pleiotropy was conducted by estimating the intercept term derived from the

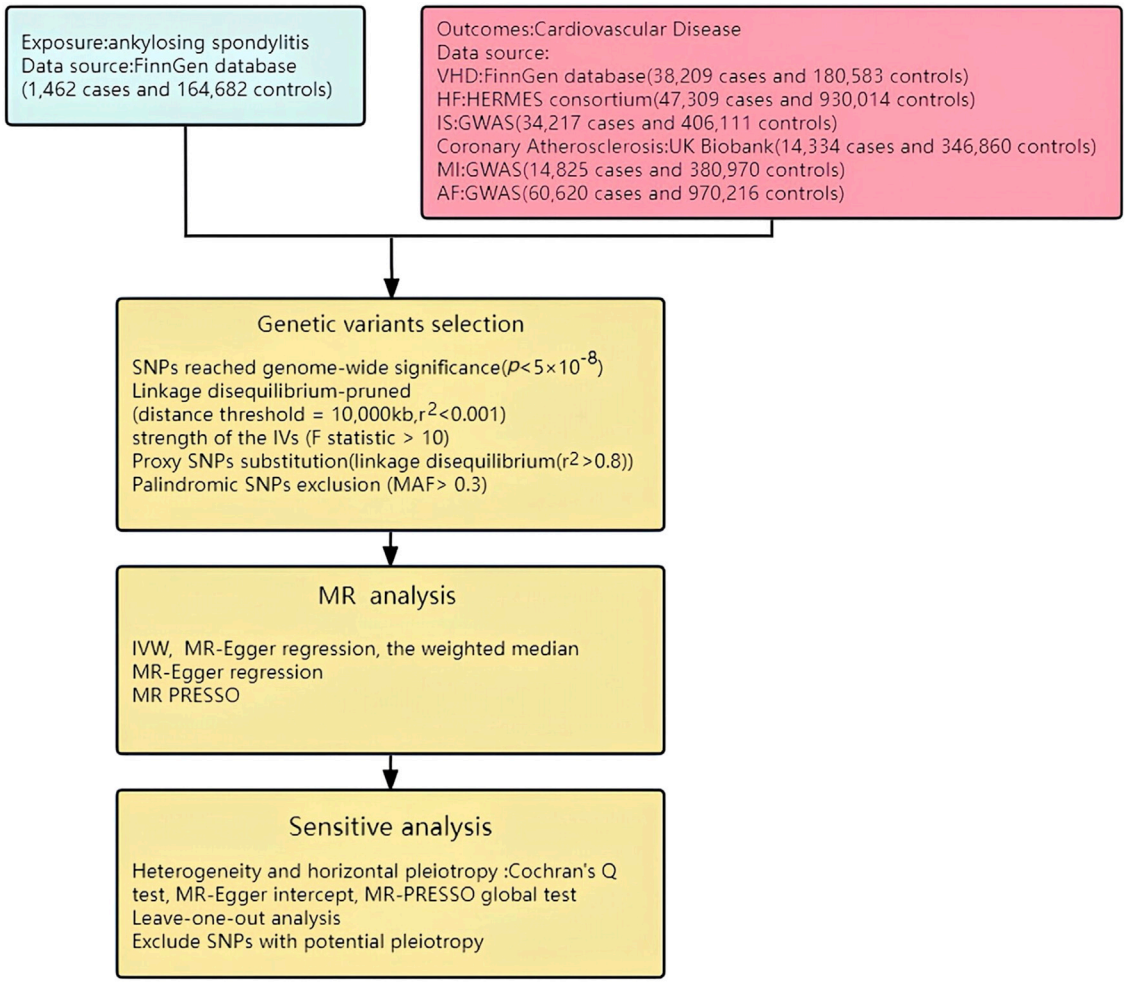

FIGURE 3
Study flow chart. HF: Heart Failure; MI: Myocardial Infarction; AF: Atrial fibrillation; IS: Ischemic stroke; VHD: Valvular heart disease.

MR-Egger regression, indicating potential bias when the intercept deviates from 0 (Bowden et al., 2015). This method maintains the capacity to generate valid causal estimates even when all IVs are invalid. Compared to the MR-Egger method, the weighted median estimator can identify causal effects even if up to half of the IVs are invalid.

Consequently, the Cochran's Q test was utilized to assess heterogeneity, with a significance level of $p < 0.05$ indicating the presence of heterogeneity. (Bowden et al., 2019). Horizontal pleiotropy was examined using the MR-Egger intercept test and MR-PRESSO global test (Rees et al., 2017; Verbanck et al., 2018). Prior to MR analysis, the MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) method is utilized to discard any outliers suspected of potential pleiotropy, ensuring the reliability of MR estimates (Bowden et al., 2017; Verbanck et al., 2018). Moreover, to evaluate the reliability and stability of the MR results, we carried out a sensitivity analysis using the "leave-one-out" method (Hemani et al., 2018). The significance threshold in MR analysis was set as a two-sided p-value <0.008 (0.05/6), according to the Bonferroni correction method. p values <0.05 but higher than the Bonferroni correction threshold are deemed as potential associations. A reverse MR study assessed the relationships between six CVDs and AS using identical methodologies. R software version 4.0.5, utilizing the "TwoSampleMR" and "MR-PRESSO" packages, was employed for data processing and visualization.

IV selection and verification

In this study, we obtained 13 significant and independent SNPs as AS SNPs. No SNPs related to cardiovascular disease confounders were found after retrieval on the PhenoScanner website. We found related proxy SNPs in GWAS data to replace a small number of SNPs. In addition, palindromic SNPs with intermediate allele frequencies and SNPs with incompatible alleles were excluded in the harmonization process to eliminate any potential bias. This left 10, 11, 11, 12, 11, 13 SNPs as IVs for causal inference of AS to HF, MI, Coronary Atherosclerosis, AF, IS, and VHD, respectively. Furthermore, the estimation of the F statistic suggests that we did not use weak IVs in the MR analysis (all F statistics >10). The remaining SNPs were chosen as IVs (Supplementary Table S1).

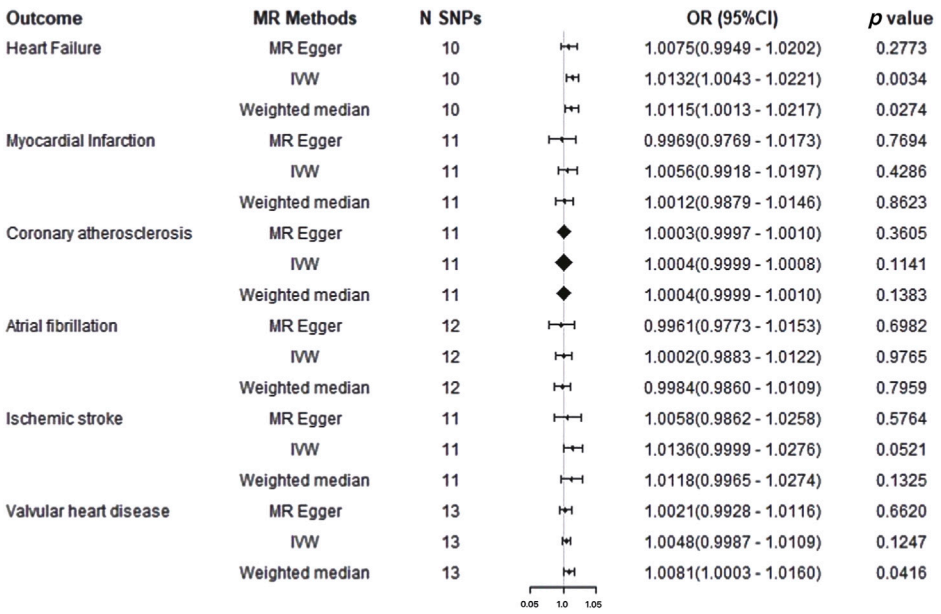

FIGURE 4
Mendelian randomization results for cardiovascular disease on ankylosing spondylitis risk. IVW: Inverse Variance Weighted method; MR, Mendelian randomization; SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

Results of the MR study

In the forward two-sample MR analysis, we utilized IVW, WM, and MR-Egger methods to assess the presence of a causal association between AS and CVDs. Using the fixed effect IVW model as the primary analytical standard an applying Bonferroni correction, we found that Ankylosing Spondylitis amplified the genetic susceptibility risk of HF in the European population (OR = 1.0132, 95% CI 1.0043-1.0221, p = 0.0034) (Supplementary Figures SF1–SF3). The WM method (OR = 1.0115, 95%CI = 1.0013–1.0217, p = 0.0274) indicated a potential association with a consistent direction. However, the MR-Egger regression model (OR = 1.0075, 95%CI = 0.9949–1.0202, p = 0.2773) did not exhibit statistically significant differences (Figure 4). The WM results also suggested a potential association between AS and VHD risk in the European population (OR = 1.0081, 95% CI 1.0003–1.0160, p = 0.0416), while IVW and MR-Egger indicated an insignificant association, albeit in a consistent direction. Furthermore, no evidence was found for a potential causal relationship between the genetic susceptibility of Ankylosing Spondylitis and the risk of MI, Coronary atherosclerosis, AF, IS (all $p > 0.05$), with IVW, WM, and MR-Egger results demonstrating consistency (Supplementary Table S2). However, this analysis might lack sufficient statistical power to detect such a weak association.

Results of sensitivity analysis in MR study

In MR analysis, Cochran's Q test demonstrated no significant heterogeneity among the IVs for HF, MI, Coronary Atherosclerosis,AF, IS, and VHD, indicating that a fixed effect IVW model is appropriate for analysis. Importantly, no evidence of horizontal pleiotropy was found based on the results of the MR-Egger intercept test and MR-PRESSO test. Furthermore, the leave-one-out analysis demonstrated the robustness of our study, as the exclusion of any individual IV did not significantly impact the overall findings (Supplementary Figure SF4). The MR-PRESSO test did not detect any outliers, which further strengthens the reliability of the results. In summary, the sensitivity analysis affirmed the robustness of our conclusions (Table 2). Furthermore, the funnel plot analysis revealed that, apart from HF and AF, the variation in effect sizes around the point estimates was symmetrical, indicating the absence of substantial evidence for horizontal pleiotropy (Supplementary Figure SF5).

Results of reverse MR study and sensitivity analysis

The results of the fixed effect IVW model did not show potential statistical differences between Heart Failure (OR: 0.8361, 95% CI = 0.4816-1.4517, p = 0.5249), Myocardial Infarction (OR: 1.0936, 95% CI = 0.8894-1.3448, p = 0.3960), Coronary atherosclerosis (OR: 5.4668, 95% CI = 0.0650-459.6306, p = 0.4525), Atrial fibrillation (OR: 0.9994, 95% CI = 0.8962-1.1145, p = 0.9917), Ischemic stroke (OR: 0.8816, 95% CI = 0.6030-1.2888, p = 0.5153), Valvular heart disease (OR: 0.8449, 95% CI = 0.4052-1.7619, p = 0.6532) and AS. The outcomes derived from the MR-Egger regression model and the WM model align with these conclusions (Supplementary Table S3).

In sensitivity analysis, apart from MR-PRESSO, the MR-Egger regression test did not reveal any evidence of pleiotropy effects (Supplementary Table S4). Notably, according to Cochran's Q test, the causal relationship between MI and AS showed significant heterogeneity; however, random effects IVW produced ineffective results (OR: 1.0936, 95% CI = 0.8894-1.3447, p = 0.3960). No significant correlations were found between any other

TABLE 2 Sensitivity analysis of the MR analysis results of exposures and outcomes.

Outcomes	Pleiotropy test MR-egger			Heterogeneity test MR-egger		IVW[c]		MR-PRESSO global test	
	Intercept	SE[a]	*p*-value[b]	Cochran's Q	*p*-value	Cochran's Q	*p*-value	RSS obs[d]	*p*-value
HF[e]	0.0076	0.0062	0.253	1.518	0.992	3.034	0.963	9.752	0.715
MI[f]	0.0109	0.0095	0.282	14.327	0.111	16.409	0.089	21.604	0.144
Coronary atherosclerosis	4.073×10^{-5}	0.0003	0.902	9.151	0.423	9.168	0.516	9.729	0.726
AF[g]	0.0043	0.0078	0.595	18.986	0.040	19.559	0.052	22.242	0.151
IS[h]	0.0092	0.0087	0.315	11.031	0.274	12.420	0.258	13.919	0.409
VHD[*9]	0.0041	0.0057	0.487	10.709	0.468	11.226	0.510	14.288	0.603

[a]SE, standard error.
[b]*p*-value <0.05 was considered as with statistical differences in both heterogeneity and horizontal pleiotropy tests.
[c]IVW, inverse variance weighting.
[d]RSS, residual sum of squares.
[e]HF, Heart Failure.
[f]MI, Myocardial Infarction.
[g]AF, Atrial fibrillation.
[h]IS, Ischemic stroke.
[i]VHD, valvular heart disease.

cardiovascular disease and AS. The reverse MR analysis did not yield a significant causal impact of CVD on AS.

Discussion

In the present investigation, we carried out a bidirectional two-sample MR analysis, utilizing large-scale, publicly available genomic summary data to scrutinize the potential causal impacts of Ankylosing Spondylitis on six distinct CVDs. Our data provide evidence of a significant causal association between AS and the risk of HF, but no causal association between AS and susceptibility to other CVDs. Additionally, the reverse SVMR study suggests no relevance of high risk of HF, MI, Coronary atherosclerosis, AF, IS, and VHD to AS. In summary, the current study results suggest that patients with ankylosing spondylitis should be vigilant about the increased risk of HF and take preventive measures as early as possible. Furthermore, this topic should be further validated in large-scale studies in the future, deepening the disease typing and severity of heart failure, as well as assessing treatment options for patients with cardiac involvement.

Our study findings strengthen or extend the existing observational evidence. A longitudinal cohort study involving 77,928 participants pointed out an increased incidence of congestive HF in patients with AS (Bae et al., 2019), which is largely consistent with our results. Another study showed that the correlation between AS and heart failure varies among populations, with the risk of HF significantly higher in the male AS cohort aged 60 to 69 than in the general population cohort (Hung et al., 2016). It is noteworthy that these cohort studies can only provide associative evidence, unable to determine the causality between the two. In addition, another systematic review of echocardiographic features in patients with AS indicates an increased risk of diastolic left ventricular dysfunction in AS patients, with specific electrocardiographic manifestations of poorer E/A ratio, longer deceleration time, and prolonged average isovolumic relaxation time (Heslinga et al., 2014). Further research found that TNF blockade therapy may improve several echocardiographic parameters of cardiac function in AS patients, and the level of NT-proBNP improves after using TNF blockade therapy in AS (Heslinga et al., 2015). However, these results focus on echocardiographic parameters and biochemical indicators, the clinical significance of which is unclear.

In the past decade, several observational studies exploring the relationship between chronic AS and CVD have yielded conflicting results. For example, while most cohort studies found an increased risk of ischemic heart disease in patients with AS (Szabo et al., 2011; Huang et al., 2013; Wright et al., 2015), a study by Essers et al. did not support this conclusion (adjusted HR 1.20 (95% CI 0.97–1.48)) (Essers et al., 2016). A meta-analysis published by Turina et al., in 2011 showed that the incidence of MI in AS patients was 7.4%, compared to 4.6% in the control group, and the incidence of stroke in AS patients was 2.2%, compared to 2.3% in the control group, with none of the meta-analysis results reaching a significant level (So et al., 2017). In addition, a retrospective cohort study in 2012 involving 1,686 AS patients found a risk ratio of 1.28 for MI and 1.0 for CVD/stroke, again not reaching a significant level (Brophy et al., 2012). Moreover, non-ischemic cardiac manifestations such as arrhythmias (Forsblad-d'Elia et al., 2013; Bengtsson et al., 2019), valvular diseases (Palazzi et al., 2008), and diastolic dysfunction (Heslinga et al., 2014) have also been observed in AS. Finally, AS has been associated with cardiovascular risk factors such as diabetes (Chen et al., 2014; Chou et al., 2014), hypertension (Chen et al., 2014; Chou et al., 2014), dyslipidemia (Chen et al., 2014; So et al., 2017), obesity (Catapano et al., 2016), and metabolic syndrome (Haroon et al., 2014), which interact with AS and affect the occurrence of CVD complications in AS patients. Our MR analysis did not provide evidence for a causal effect of AS on AF, MI, Coronary atherosclerosis, Ischemic stroke, and

VHD, which is consistent with previous MR study results (Chen et al., 2022; Mei et al., 2022).

Considering that heart failure typically arises as a consequence of various cardiovascular conditions, including atrial fibrillation, coronary artery disease, ischemic stroke, myocardial infarction, or valvular heart disease, the outcomes may be expounded upon through a multidimensional lens encompassing pathophysiological, genetic, pharmacotherapeutic, and methodological perspectives. For instance, AS may induce myocardial electrical and structural remodeling via processes such as inflammation, immune response, and myocardial fibrosis (Roth et al., 2020; Tsao et al., 2022), thereby elevating the risk of HF. Similar pathophysiological mechanisms are commonly observed in conditions like myocardial infarction (Roger et al., 2020), stroke (Toussirot, 2021; Ding et al., 2022; Kwon et al., 2022), arrhythmias (Tsai et al., 2015; Setyawan et al., 2021), and valvular heart disease (Burgess and Thompson, 2015; Zhao et al., 2020). Furthermore, it is well recognized that genetic factors play a crucial role in the development and progression of AS (Tsao et al., 2022). The presence of these specific genetic variants in AS patients may predispose them to an increased susceptibility to HF(Malik et al., 2018; Skrivankova et al., 2021a; Hartiala et al., 2021). Moreover, pharmacological treatment for AS may also influence the occurrence of HF. Guidelines suggest that reducing the inflammatory burden of AS through medication may have a favorable impact on patients' CVD risk (Roth et al., 2020). However, a meta-analysis indicated that non-selective NSAIDs and selective cyclooxygenase-2 inhibitors may have adverse effects on cardiovascular outcomes in patients with inflammatory joint diseases. Finally, research findings may be constrained by the limited statistical power of Mendelian randomization studies, as well as the heterogeneity and confounding factors present in clinical research on disease subtypes. Therefore, larger-scale studies are needed to clarify the role of AS in specific disease subtypes and to determine whether biases in previous observational studies are due to confounding factors.

Prior clinical research on AS and VHD has been somewhat limited, primarily focusing on aortic valve regurgitation, with most studies supporting a correlation between AS and VHD. Prospective studies have shown a significantly increased hazard ratio for aortic valve regurgitation in AS compared to general population cohorts (Azevedo and Pecoits-Filho, 2010). In longitudinal retrospective cohort studies, AS subjects had a 1.63-fold increased risk of developing VHD compared to non-AS subjects, with statistically significant differences (Kim and Choi, 2021). Subgroup analyses for types of VHD revealed significantly elevated risks of mitral valve disease, aortic valve disease, and tricuspid valve disease in AS subjects compared to non-AS subjects (Kim and Choi, 2021). Moreover, valvular heart involvement appears to be more common in patients with longer disease duration (Haroon et al., 2015). Studies have found that among AS patients with a 15-year disease duration, 3.5% developed aortic valve insufficiency, while among those with a 30-year disease duration, 10% developed aortic valve insufficiency (Divecha et al., 2005). Additionally, retrospective cross-sectional studies indicate a significant increase in the risk of VHD with aging among AS patients after adjusting for gender and ethnicity (Papagoras et al., 2013). Consistent with previous MR studies (Fauci, 2015; Bhattad et al., 2022), this study did not suggest a significant causal relationship between AS and VHD, possibly due to limitations in sample size and testing statistical power of MR studies. Additionally, related research has found that the relationship between serum lipid levels and CVD risk in inflammatory joint diseases is nonlinear and may be contradictory (Zhang et al., 2018; Jiménez-Balderas et al., 2001; Toms et al., 2011). Traditional regression methods adjusting for confounding factors may not be applicable, necessitating the introduction of statistical methods such as polynomial regression and segmented regression in subsequent studies. Furthermore, studies have found that controlling disease activity may influence the onset of AS (Bengtsson et al., 2018; Siao et al., 2021), with most observational studies not incorporating this modifiable factor, posing challenges to research outcomes.

Many studies have shown that AS increases the morbidity and mortality of cardiovascular diseases (Graham and Smythe, 1958; Lui et al., 2011; Ward, 2018). However, the mechanism behind this association is still unclear. Research has shown elevated levels of pro-inflammatory cytokines such as IL-6 and CRP in AS (Zhong et al., 2023), suggesting that inflammation may play a role. Studies have shown that AS can lead to obliterative endarteritis in small vessels supplying the atrioventricular node and the aortic root, leading to a prolongation of the PQ interval and an increased risk of atrial fibrillation, first-degree atrioventricular block, and aortic valve insufficiency (Haroon et al., 2015). IL-6 induces the acute phase response, leading to increased levels of CRP and fibrinogen and monocyte activation, the activated monocytes deposit fibrinogen in the vessel wall, leading to atherosclerosis (Cai et al., 2024). Moreover, when the endothelium is damaged, foam cells and smooth muscle cells release IL-6 and other inflammatory cytokines, causing more vascular damage and exacerbating this process, ultimately leading to the formation and progression of atherosclerosis (Myasoedova et al., 2011). In addition, during the course of AS, IL-6 interacts with the hypothalamic-pituitary-adrenal (HPA) axis, leading to traditional cardiovascular risk factors including decreased insulin sensitivity, increased BMI, and the occurrence of hypertension (Boyer et al., 2012), exacerbating atherosclerosis.

It is worth noting that in the investigation of the association between AS and conduction abnormalities, a meta-analysis incorporating eight studies revealed a significantly heightened risk of atrioventricular conduction block among AS subjects compared to controls (Kerekes et al., 2009). Similarly, observational and retrospective cohort studies have reported such findings (Forsblad-d'Elia et al., 2013; Raterman et al., 2013; Morovatdar et al., 2021; Wu et al., 2016). However, another meta-analysis did not indicate a significant correlation between AS and cardiac conduction abnormalities (Hung et al., 2016). Regarding specific types of conduction abnormalities, prospective studies have shown a significantly elevated hazard ratio for II-III degree AV block and pacemaker implantation in AS compared to general population cohorts (Azevedo and Pecoits-Filho, 2010). Furthermore, research suggests that AS patients with arrhythmias exhibit higher disease activity indices compared to those without arrhythmias, with statistically significant differences, indicating a potential association between arrhythmias and AS disease activity (Dik et al., 2010). Mechanistically, arrhythmias often reflect the presence of cardiac conduction reentry resulting from inflammatory processes, fibromuscular proliferation and fibrosis, as well as increased automaticity and triggered activity (So et al., 2017). Additionally, the human leukocyte antigen B27 is recognized to

potentially contribute to arrhythmogenesis by increasing platelet adhesion to vascular walls (Dalecky et al., 2024). Moreover, drug-induced arrhythmias, such as those caused by non-steroidal anti-inflammatory drugs (NSAIDs) and chloroquine, are commonly observed in AS (Moyssakis et al., 2009; Aksoy et al., 2016; Gawałko et al., 2020). NSAIDs, in particular, may affect cardiac electrophysiological properties by influencing various cardiac ion channels (Stas et al., 2008). As for myocardial disease, only limited literature exists on this topic (Haroon et al., 2015; Bakhriansyah et al., 2019), indicating the need for further investigation.

However, there are some limitations to this pilot study: At first, this study included individuals primarily of European descent, so extrapolating the study results to other populations is limited. Additionally, the study uses summary-level data, providing only binary variables, and the relationship between AS severity and CVD severity is still unclear. Furthermore, the odds ratio values obtained in this study are relatively lower compared to other studies, and further confirmation is needed in subsequent research. Therefore, we need to treat the results of MR with caution.

Conclusion

In summary, this study suggests an increased risk of HF in patients with AS. The findings provide new genetic evidence for the causal relationship between AS and CVDs risk. Clinically, it is important to assess the cardiac function of AS patients and facilitate early prevention of cardiac dysfunction. Additionally, further exploration of the mechanisms underlying the development of HF in AS patients can aid in early diagnosis and treatment of cardiovascular complications.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

PL: Data curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing–original draft. JS: Funding acquisition, Methodology, Resources, Supervision, Writing–original draft, Writing–review and editing. ZQ: Formal Analysis, Methodology, Supervision, Writing–review and editing. SQ: Supervision, Writing–review and editing. XL: Funding acquisition, Resources, Writing–review and editing. LS: Investigation, Methodology, Writing–review and editing. ZZ: Software, Writing–review and editing. ML: Data curation, Methodology, Writing–review and editing. LY: Data curation, Formal Analysis, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2024.1260247/full#supplementary-material

SUPPLEMENTARY FIGURE F1
Inverse Variance Weighted outcome of Mendelian randomization for the association between ankylosing spondylitis and the risks of cardiovascular disease. IVW: Inverse Variance Weighted method; MR, Mendelian randomization; SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

SUPPLEMENTARY FIGURE F2
Mendelian randomization results for heart failure on ankylosing spondylitis risk.

SUPPLEMENTARY FIGURE F3
The scatter plot of Mendelian randomization methods for ankylosing spondylitis on cardiovascular disease. **(A)**, atrial fibrillation; **(B)**, coronary atherosclerosis; **(C)**, heart failure; **(D)**, ischemic stroke; **(E)**, myocardial infarction; **(F)**, valvular heart disease. Regression lines represent the causal effect of the ankylosing spondylitis on cardiovascular disease risk using IVW, MR-Egger, and Weighted median to estimate. MR: Mendelian randomization; SNPs: single nucleotide polymorphisms.

SUPPLEMENTARY FIGURE F4
Leave-one-out analysis for ankylosing spondylitis on cardiovascular disease. **(A)**, atrial fibrillation; **(B)**, coronary atherosclerosis; **(C)**, heart failure; **(D)**, ischemic stroke; **(E)**, myocardial infarction; **(F)**, valvular heart disease. MR: Mendelian randomization; SNPs: single nucleotide polymorphisms.

SUPPLEMENTARY FIGURE F5
Funnel plot from genetically predicted ankylosing spondylitis on cardiovascular disease. **(A)**, atrial fibrillation; **(B)**, coronary atherosclerosis; **(C)**, heart failure; **(D)**, ischemic stroke; **(E)**, myocardial infarction; **(F)**, valvular heart disease.

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Elucidating the role of hepatic enzymes in spontaneous abortion: a Mendelian randomization approach

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Background: While the hepatic enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) are crucial for liver function, their role in Spontaneous Abortion (SA) has not been thoroughly explored. Utilizing Mendelian Randomization (MR), this study aims to clarify the putative causal relationship between AST/ALT levels and SA.

Methods: Genome-wide association study (GWAS) summary data for SA (finn-b-O15_ABORT_SPONTAN), AST (ukb-d-30650_raw), and ALT (ukb-d-30620_raw) were acquired from the Integrative Epidemiology Unit OpenGWAS database. Bidirectional MR analysis was conducted using MR-Egger, Weighted Median, Simple Mode, Weighted Mode, and Inverse Variance Weighted (IVW) algorithms, and the robustness of MR results was assessed through sensitivity analyses including Heterogeneity, Horizontal Pleiotropy, and Leave-One-Out (LOO) tests. The causal role of AST and ALT's coaction in SA was explored via multivariable MR (MVMR) analysis.

Results: The MR results via the IVW algorithm revealed a causal relation between both AST and ALT and SA (AST: P = 0.013; ALT: P = 0.017), identifying them as risk factors for SA (AST: odd ratio (OR) = 1.019; ALT: OR = 1.012). Sensitivity analysis substantiated the reliability of these results. Moreover, not notably causality was found between SA and AST/ALT (P > 0.05). Through MVMR analysis, AST and ALT demonstrated functional complementarity in the occurrence of SA, attributable to counterbalanced causalities (AST: P = 0.128; ALT: P = 0.899).

Abbreviations: AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; SA, Spontaneous Abortion; MR, Mendelian Randomization; GWAS, Genome-wide association study; IVW, Inverse Variance Weighted; LOO, Leave-One-Out; MVMR, multivariable MR; OR, odd ratio; IEU, Integrative Epidemiology Unit; SNPs, single nucleotide polymorphisms; IVs, instrumental variables; CI, confidence interval; SE, standard error.

Conclusion: The study substantiates a causal linkage between transaminase levels and SA, enhancing our understanding of their biological interaction and the regulatory mechanisms at play. These insights could have implications for identifying novel biomarkers and therapeutic targets for SA.

KEYWORDS

spontaneous abortion (SA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Mendelian randomization (MR), hepatic enzymes

1 Introduction

Pregnancy loss, also known as miscarriage or spontaneous abortion (SA), is an unintended pregnancy loss that occurs before the embryo reaches viability, usually within the first 20 weeks of pregnancy (ACOG, 2018), and our definition of SA refers to termination of pregnancy within 28 weeks of gestational age, with a fetal mass of no more than 1 kg (NICE, 2023). Clinical manifestations are bleeding and spasmodic pain. SA can be divided into uncomplicated and accompanied complications, with common complications being massive bleeding and infection. Uncomplicated bleeding and cramping pain are the most common symptoms in patients with pregnancy loss. Other symptoms of uncomplicated pregnancy loss may include loss or reduction of pregnancy reactions, such as decreased breast tenderness and/or decreased nausea and vomiting (DeVilbiss et al., 2020). The occurrence of SA can be influenced by a number of factors, including genetic factors, embryo abnormalities, maternal health problems, chronic diseases, substance abuse, exposure to harmful substances, and intrauterine infections (Nguyen et al., 2009). Miscarriage is usually accompanied by vaginal bleeding and abdominal pain, but there may be no noticeable symptoms (Deng et al., 2022; Alves and Rapp, 2023). About 10% of women of childbearing age experience SA (Kostova et al., 2023), and about 1%–3% experience recurrent miscarriage (van Wely, 2023), which has a huge impact on women's physical and mental health, as well as a serious economic burden on the family and society (Quenby et al., 2021), so it is important to find the risk factors for RA prevention and intervene early.

Some studies have indicated a potential association between altered levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the occurrence of SA. A study of women patients at risk for miscarriage and at risk for preterm labor found that AST levels were significantly higher in patients at risk for miscarriage than in a group of normal pregnant women, but there was no significant difference in ALT levels (Micle et al., 2012). ALT is produced primarily in the plasma of hepatocytes, and is a specific marker of liver injury, whereas AST is found in the liver, heart, and other tissues (Cooper et al., 2023), and elevated levels of both in the blood are indicative of liver injury or disease (Tamber et al., 2023). High levels of AST and ALT may reflect an underlying inflammatory state, and inflammation has been recognized to be strongly associated with the development of SA (Gao et al., 2020). Severe liver injury may affect maternal metabolism and hormonal balance, which may affect embryonic development (Obiegbusi et al., 2023). AST and ALT are involved in oxidative stress, which has been implicated in the development of SA (García-Romero et al., 2019). Raising a noteworthy question: Do the level changes of AST and ALT directly influence the risk of SA? We hypothesize that an abnormal increase in AST and ALT may be related to some pathophysiological processes associated with SA, such as inflammation, oxidative stress, or vascular dysfunction. Furthermore, considering that AST and ALT are typically biomarkers of liver function impairment, it is also worth exploring whether they indirectly affect the risk of SA by influencing maternal metabolism or immune function.

Mendelian randomization (MR) is an analytical method used to study the causal relationship between observed biomarkers or environmental factors and a specific outcome, such as morbidity risk (Birney, 2022). In the study of disease mechanisms, with the discovery of a large number of genetic variants closely associated with specific traits in biology and the public release of data from many large-sample Genome-Wide Association Study (GWAS) (Wang et al., 2022), it has helped to reveal the intrinsic mechanisms of diseases (Bowden and Holmes, 2019).

To investigate the potential causal relationship between AST, ALT, and the risk of SA, this study conducted univariate and multivariate MR analyses on two independent samples.

2 Materials and methods

2.1 Data sources and summary

The summary data from GWAS for SA, AST, and ALT were obtained from the Integrative Epidemiology Unit (IEU) OpenGWAS database (https://gwas.mrcieu.ac.uk/). The data of SA (finn-b-O15_ABORT_SPONTAN) was comprised of 9,113 cases and 89,340 controls, with a total of 16,379,138 single nucleotide polymorphisms (SNPs). The numbers of SNPs for AST (ukb-d-30650_raw) and ALT (ukb-d-30620_raw) was 13,586,009 and 13,586,000, respectively. The MR method is based on three fundamental assumptions (ACOG, 2018): The genetic variants have a significant and direct association with the exposure being studied (NICE, 2023); The genetic variants are not influenced by any factors that could confound the relationship between the exposure and the outcome; and (DeVilbiss et al., 2020) The genetic variants solely affect the outcome through their impact on the exposure, without any additional effects on other outcomes. To simplify, these assumptions state that the genetic variants are strongly related to the exposure, unaffected by confounding factors, and have no other effects on the outcome.

2.2 GWAS data pre-processing

The "extract_instruments" function in R package "TwoSampleMR" was adopted to perform the data reading for

exposure factors and filtering of instrumental variables (IVs), then the default p-value threshold of less than 5×10^{-8} in the MR analyses was applied to identify SNPs significantly associated with the exposure (Hemani et al., 2018) and to remove IVs with linkage disequilibrium (clump = TRUE, r2 = 0.001, kb = 10,000). Simultaneously, the IVs markedly relevant to the outcome were also rejected, and then the "harmonise_data" function was utilized to harmonize the effect equipotential with effect size. The exposure factors—IVs—outcome was matched for the following analysis. Finally, IVs were retrieved through PhenoScanner database, SNPs that might affect the outcome through other confounding factors were excluded to ascertain the ultimate SNPs as IVs. The IVs was evaluated by calculating the F-statistic, which was typically recommended to be 10 or higher to ensure the reliability of the genetic instruments used in MR analysis.

2.3 Bidirectional Mendelian randomization (MR) analysis and sensitivity analysis

Five algorithms uniting with the "mr" function of the R package "TwoSampleMR" were employed to execute the bidirectional MR analysis, including MR Egger (Jha et al., 2023), Weighted median (Soares et al., 2018), fixed effects Inverse variance weighted (IVW) (Zhang et al., 2023), Simple mode and Weighted mode (Llovet et al., 2019). Primarily, the MR results were referred to IVW and evaluated utilizing the Cochran Q value, derived from subsequent heterogeneity analysis. The scatter plots, forest plots and funnel plots were created to exhibit the results of causality between two aminotransferases and SA. Ultimately, the sensitivity analysis comprised of the Heterogeneity, Pleiotropy and Leave-One-Out (LOO) sensitivity test was executed to evaluate the reliability of the above MR results. First, Cochran's Q test was used to evaluate the heterogeneity among SNPs, with p values greater than 0.05 indicating no heterogeneity. Secondly, horizontal pleiotropy was used to assess whether confounding factors existed between exposure factors and outcome sample data. The mr_pleiotropy_test function was used to test for the presence of horizontal polytropy in the evaluation, and the absence of horizontal polytropy indicated that the results were reliable. When the p-value is greater than 0.05, it indicates that there is no horizontal polytropy. Third, we conducted a LOO sensitivity test to determine whether there was an abnormal SNP that was sensitive to the outcome effect.

2.4 Multivariable MR (MVMR) analysis

The "mv_extract_exposures" function in R package "TwoSampleMR" was adopted to perform the data reading for multivariable exposure factors and filtering of IVs ($P < 5 \times 10^{-8}$), followed by the elimination of IVs with linkage disequilibrium (clump = TRUE, r2 = 0.001, kb = 10,000). The "extract_outcome_data" function was also employed with proxies = TRUE and rsq = 0.8, and then the "mv_harmonise_data" function was utilized to harmonize the effect equipotential with effect size for MVMR analysis. The F statistic was calculated using the strength_mvmr function, with an F statistic greater than 10 indicating that genetic variation had sufficient power to correlate the exposure factor with the outcome. For analysis, this study mainly used the IVW method to explore the influence of two exposure factors on the outcome. In addition, pleiotropy_mvmr function was used to calculate horizontal pleiotropy in MVMR. The flow chart of this study is shown in Figure 1.

3 Results

3.1 Positive causal relationships of AST and ALT on SA occurrence based on bidirectional MR analysis

After filtrating, there were 82 and 112 SNPs of AST and ALT successively acquired as IVs. All of these genetic variants had F statistics higher than 20, indicating a low likelihood of weak IVs (Supplementary Table 1).The forward MR results were presented in Supplementary Table 2. The causalities were detected between two aminotransferases and SA (AST: $P = 0.013$; ALT: $P = 0.017$), and all exposures were risk factors for SA (AST: odd ratio (OR) = 1.019; ALT: OR = 1.012) based on IVW method. The scatter plot also revealed that these exposures were risk factors on SA occurrence in accordance with the above MR results, less affected by confounding factors because of intercepts near zero (Figure 2A). Meanwhile, the diagnostic efficiency of each SNP of AST and ALT on outcome SA was estimated through MR Egger and IVW methods. The forest plots showed that the holistic points of AST and ALT were located in the right of baseline, supporting positive effects on the outcome (Figure 2B). The MR analysis of two exposures was in accord with Mendel's second law random grouping (Figure 2C). In addition, reverse MR analysis Supplementary Table 3 showed that SA had no significant causal relationship with either of the two aminotransferases, in which AST and ALT as outcomes and SA as an exposure factor ($P > 0.05$).

3.2 Sensitivity analysis illustrated the reliability of the MR results

Furthermore, the reliability of the above MR results was demonstrated by the sensitivity analysis. There were no heterogeneity for AST and ALT via fixed effects IVW method (AST: Q_pval = 0.172, ALT: Q_pval = 0.086) (Table 1). The Pleiotropy test suggested there was no horizontal pleiotropy for two exposures (AST: $P = 0.786$; ALT: $P = 0.370$) with the help of the mr_pleiotropy_test function (Table 2). Finally, the influence of the remaining IVs on the outcome was evaluated after removing the IVs one by one. We found that there were no points of severe bias by LOO method (Figure 3). In conclusion, AST and ALT were risk factors on SA occurrence with the proven dependability.

3.3 Mutually connected with AST and ALT on SA occurrence

We estimated mutually the effects of AST and ALT on SA using multivariable MR. A total of 126 SNPs were acquired as IVs after filtrating, including 69 for ALT and 57 for AST. In addition, the F

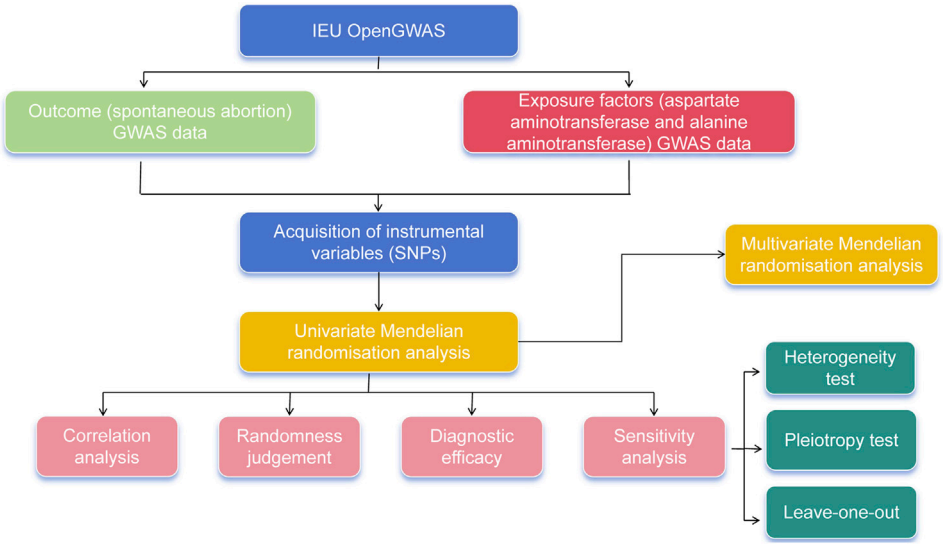

FIGURE 1
Flow chart of this study.

statistics for both exposure factors were greater than 20 (Table 3). The multivariable MR results were presented in Supplementary Table 4. In MVMR analysis, the relationship between ALT, AST and SA was also evaluated, and only ALT (OR = 1.018, P = 0.035) had a strong potential causal relationship with SA. Besides, no horizontal pleiotropy was found in the IVW-MVMR sensitivity analysis (p = 0.061) (Table 4).

4 Discussion

This study identified a causal relationship between AST and ALT levels and SA. These two transaminases are risk factors for the development of SA, and their functional complementarity mitigates this causal relationship. This suggests an interconnected causality between AST and ALT concerning the outcome, indicating that they tend to function more complementarily rather than independently. Furthermore, sensitivity analyses confirmed the reliability of the MR results. It has been shown that nearly 3% of pregnancies are complicated by liver disease, which may result in adverse outcomes for mother and child, but more studies are needed to understand the epidemiology of pregnancy-related liver disease and to assess the long-term prognosis of maternity (Hemani et al., 2018). Additionally, obesity-induced liver function abnormalities can affect embryonic development and have long-term adverse effects on the offspring (Jha et al., 2023). Elevated liver enzymes due to cytomegalovirus infection during pregnancy can lead to miscarriage and congenital malformations in newborns (Soares et al., 2018). Previous studies have also examined related topics, such as, studies have confirmed that significantly elevated serum transaminase levels in patients with recurrent miscarriage are an independent risk factor for early pregnancy failure (Zhang et al., 2023).

The pregnancy outcomes in women with hepatitis C are closely tied to liver enzyme levels (Llovet et al., 2019). Specifically, ALT (alanine aminotransferase) serves as a critical prognostic indicator in pregnant women with COVID-19 (Carrión-Nessi et al., 2023), and serum ALT levels are a key factor in determining pregnancy outcomes in women with hepatitis B virus infection during pregnancy (Nguyen et al., 2009; Alves and Rapp, 2023). The underlying mechanisms appear to involve two primary aspects. First, the AST (aspartate aminotransferase)/GOT (glutamate oxaloacetate transaminase) enzyme facilitates the transfer of amino groups from aspartate to alpha-ketoglutarate, resulting in the formation of glutamate and oxaloacetate. Substantial tissue destruction leads to the release of AST/GOT and ALT/GPT into the serum. Secondly, elevated serum levels of AST/GOT in pregnant women indicate that both AST and ALT play roles in oxidative stress. It has been determined that infection with the hepatitis E virus (HEV) initiates an immune and inflammatory response in the body, resulting in damage to hepatocytes and elevated levels of AST and ALT. This inflammatory response may also compromise placental blood perfusion and alter the environment in which the fetus grows and develops, thereby increasing the risk of miscarriage (Qian et al., 2023).

This study's main finding is the identification of a causal link between ALT and AST levels and spontaneous abortion (SA). Fluctuations in ALT and AST levels appear to elevate the risk of SA. However, after conducting additional statistical analyses through univariate and multivariate Mendelian randomization (UVMR and MVMR) and assessing the multivariate level, the inverse variance weighted (IVW) algorithm confirmed a positive causal relationship between AST/ALT levels and spontaneous abortion (SA). Conversely, the reverse MR analysis revealed no significant causality between SA and either aminotransferase. This result is not contradictory but rather illustrates the utility and limitations of MR analysis in determining the direction of causal inference. ALT remained a significant risk factor for SA, whereas AST's significance diminished. This outcome may be due to a covariance or interaction between ALT and AST, obscuring AST's independent effect. Additionally, other unaccounted-for confounders or potential common causes might interfere with

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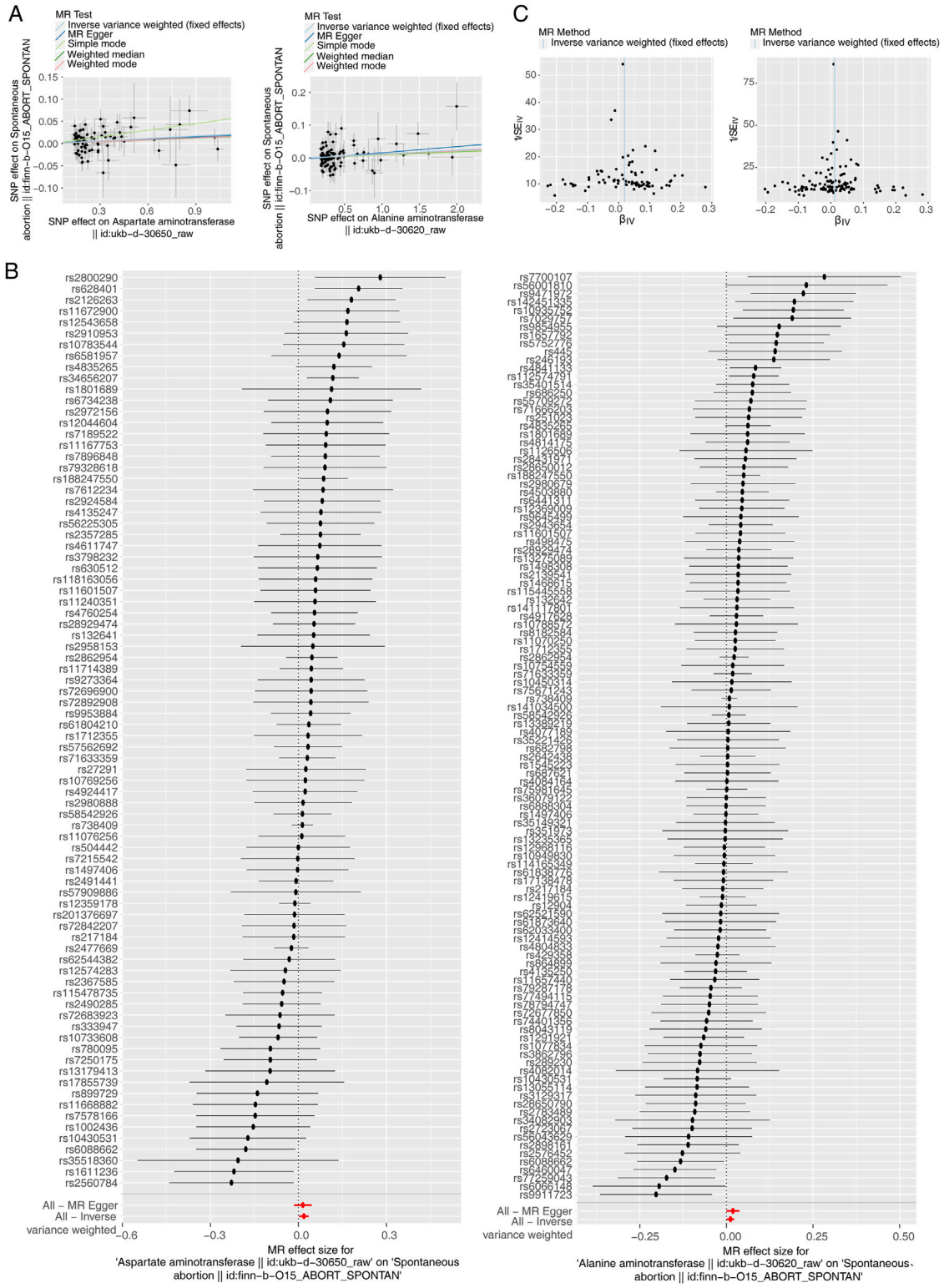

FIGURE 2
Causal effect of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) on Spontaneous Abortion (SA) in forward Mendelian randomization (MR) analysis. **(A)** Scatter plot of genetic associations between exposure and outcome. Left: AST and SA. Right: ALT and SA. **(B)** Left: AST-SA funnel plot. Right: ALT-SA funnel plot. It is found that based on inverse variance weighted (IVW) method, almost left-right symmetry. **(C)** Left: The forest plot for the single nucleotide polymorphism (SNP) analysis of AST increasing on SA risk. Right: The forest plot for the SNP analysis of ALT increasing on SA risk. The *x*-axis shows the MR effect size for AST and ALT increasing on SA, while the *y*-axis illustrates the analysis for each of the SNPs. The dot and bar indicate the causal estimate and 95% confidence intervals (CI) of the association between AST and ALT increasing on SA risk.

TABLE 1 The heterogeneity test results of AST and ALT causal influence on SA.

id.exposure	id.outcome	Outcome	Exposure	Method	Q	Q_df	Q_pval
ukb-d-30650_raw	finn-b-O15_ABORT_SPONTAN	Spontaneous abortion || id:finn-b-O15_ABORT_SPONTAN	Aspartate aminotransferase || id:ukb-d-30650_raw	MR Egger	92.86228	80	0.154
ukb-d-30650_raw	finn-b-O15_ABORT_SPONTAN	Spontaneous abortion || id:finn-b-O15_ABORT_SPONTAN	Aspartate aminotransferase || id:ukb-d-30650_raw	Inverse variance weighted	92.94872	81	0.172
ukb-d-30620_raw	finn-b-O15_ABORT_SPONTAN	Spontaneous abortion || id:finn-b-O15_ABORT_SPONTAN	Alanine aminotransferase || id:ukb-d-30620_raw	MR Egger	130.8819	110	0.085
ukb-d-30620_raw	finn-b-O15_ABORT_SPONTAN	Spontaneous abortion || id:finn-b-O15_ABORT_SPONTAN	Alanine aminotransferase || id:ukb-d-30620_raw	Inverse variance weighted	131.8441	111	0.086

Q, Q-statistic; Q_df, degrees of freedom.

TABLE 2 The horizontal pleiotropy test results of AST and ALT causal influence on SA.

id.exposure	id.outcome	Outcome	Exposure	egger_intercept	se	pval	Judge
ukb-d-30650_raw	finn-b-O15_ABORT_SPONTAN	Spontaneous abortion || id:finn-b-O15_ABORT_SPONTAN	Aspartate aminotransferase || id:ukb-d-30650_raw	0.001172	0.004295	0.785652	NO
ukb-d-30620_raw	finn-b-O15_ABORT_SPONTAN	Spontaneous abortion || id:finn-b-O15_ABORT_SPONTAN	Alanine aminotransferase || id:ukb-d-30620_raw	−0.00326	0.003623	0.370465	NO

se, Standard Error; pval, P-value.

the effects of ALT and AST, rendering the impact of AST insignificant.

The findings underscore the importance of monitoring ALT levels in assessing the risk of SA, particularly in individuals with related risk factors. The study also sheds light on the interconnected causal relationship between AST and ALT, suggesting a collaborative role in the pathogenesis of SA and paving the way for future mechanistic research. These insights advocate for the routine monitoring of ALT levels, especially in those at risk, to better understand and mitigate SA risk.

However, it is important to acknowledge that this study has certain potential limitations and pitfalls. Firstly, whether the population studied is representative of the population as a whole, and if the study sample is not representative, then the external generalizability of the findings may be limited. Hence, future studies should consider expanding sample size and including more diverse populations to ensure that findings are more generalizable. Another important consideration is the potential influence of confounding factors. By analyzing multiple exposures simultaneously, multivariate Mendelian randomization (MVMR) can partially control for confounders linked to various exposures. Although our study has diligently attempted to account for the effects of multiple exposure factors on outcomes through MVMR methods, it remains true that several potential confounders might not have been directly measured. These unmeasured confounders could include, but are not limited to, non-genetic environmental factors such as lifestyle, dietary habits, socioeconomic status, measurement errors, and possible gene-environment interactions. Furthermore, in our multivariate analyses, the influence of the two transaminases on the outcome appeared diminished. This attenuation may result from the multivariate context, where interactions and covariance among variables may diminish the apparent effect of any single variable. To more comprehensively assess the impact of potential confounders, we plan to further investigate and control for these factors in future studies, employing advanced techniques that incorporate broader genetic and environmental data, as well as more refined statistical models. The databases used in this study predominantly encompass genetic information from European men and women. Given that genetic variations across different races and ethnicities, as well as environmental factors such as dietary habits, lifestyle, and exposure to pollutants, can significantly affect liver function indicators and overall health status, this may restrict our ability to generalize the findings concerning the relationship between AST/ALT levels and spontaneous abortion (SA). To overcome this limitation, future studies will aim to incorporate more diverse datasets from various races, ethnicities, and geographic regions to more thoroughly evaluate the combined effects of genetic variants and environmental factors on AST/ALT levels and SA.

Additionally, issues such as pleiotropy and linkage disequilibrium could potentially complicate Mendelian Randomization (MR) analyses, affecting the accuracy of causal inferences. To address these concerns, comprehensive sensitivity analyses were conducted, and their results supported the reliability of our primary conclusions. We anticipate further refining MR analysis techniques in subsequent studies to enhance both the accuracy and reliability of causal determinations.

While this study offers insights into biomarker discovery and potential therapeutic targets, the absence of direct clinical validation and longitudinal data means we cannot definitively ascertain the clinical significance of AST/ALT or other potential biomarkers in managing spontaneous abortion. This represents a significant research gap and will be a primary focus moving forward.

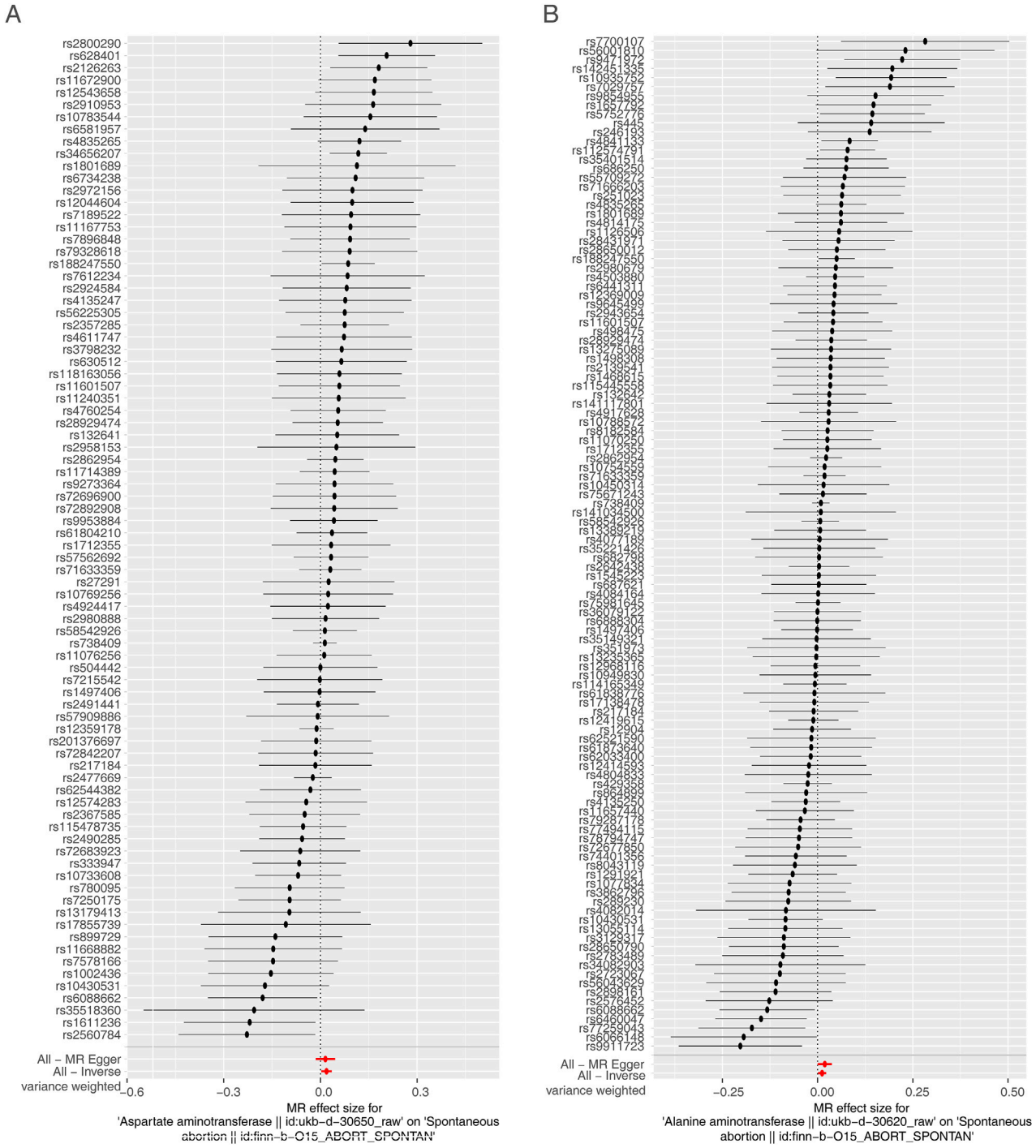

FIGURE 3
Sensitivity analysis. **(A)** Presentation of the leave-one-out sensitivity analysis for the effect of AST increasing SNPs on SA risk in the context of MR. **(B)** Presentation of the leave-one-out sensitivity analysis for the effect of ALT increasing SNPs on SA risk in the context of MR. The dot and bar indicate the estimate and 95% CI when a specific SNP is removed. OR, odds ratios; IV, instrumental variant; SE, standard error.

Future efforts could include more extensive clinical validation and longitudinal studies. Present basic research remains crucial for understanding the pathophysiological mechanisms behind spontaneous abortion, discovering new biomarkers, and identifying therapeutic targets. This work lays a vital theoretical foundation and provides a direction for upcoming clinical research and interventions. Finally, there is the long-term follow-up. This study may have only covered data over a period of time, whereas SA may be associated with long-term lifestyle and health factors. Therefore, the need for long-term follow-up and research also needs to be emphasized. Long-term follow-up studies are essential to verify the durability of research findings over time and to gain a deeper understanding of the enduring effects of relevant variables on health outcomes.

TABLE 3 The F-statistics of IVs in multivariate Mendelian randomization (MVMR).

	Exposure 1	Exposure 2
F-statistic	25.48706186	32.36263158

TABLE 4 The horizontal pleiotropy test results of AST and ALT on SA in Multivariate MR analysis.

Qstat	Qpval
110.5527388	0.060582766

Research has indicated that levels of AST/ALT and HSI are linked with the increased risk of hypertensive disorders of pregnancy (HDP) (Liu et al., 2024). Additionally, the serum levels of biochemical markers such as TBA, DBIL, and ALT in pregnant women diagnosed with intrahepatic cholestasis of pregnancy (ICP) subtypes at the onset of pregnancy are subject to continual variation, mirroring changes in the subtypes and severity of ICP. These fluctuations can result in numerous adverse pregnancy outcomes (Feng et al., 2024).

Moreover, either isolated or widespread elevations in liver enzyme levels, particularly notable increases in gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) during early pregnancy, have been identified as three crucial independent predictors for the onset of gestational diabetes mellitus (GDM) (Cui et al., 2024). Variations in these liver enzymes are associated not only with GDM but can also significantly influence the long-term risk of chronic conditions such as metabolic syndrome in pregnant women. We aim to investigate the specific mechanisms behind elevated liver enzymes during pregnancy, including genetic and environmental factors and their interactions. We will also analyze how changes in liver enzymes affect the mother's future risk of metabolic syndrome, diabetes mellitus, and other chronic illnesses. Additionally, we plan to evaluate whether these changes heighten the risk of metabolic or cardiovascular diseases in the offspring, thereby elucidating the complexity and diversity of liver enzyme alterations during pregnancy.

In response to the current findings, it is known that there may be an interaction between AST and ALT in the pathogenesis of SA, and future studies could further explore the mechanism of this interaction to understand their mutual effects in inflammation, fat metabolism, and liver injury, which could theoretically provide a more in-depth theoretical basis for this finding.

5 Conclusion

In conclusion, the present study provides important preliminary evidence for a causal relationship between transaminases (AST and ALT) and SA and highlights their interrelationship. However, further studies are needed to address potential limitations, and in future studies, potential constraints need to be addressed, potential roles of genetic and environmental factors between aminotransferase levels and SA need to be explored, and biological mechanisms linking aminotransferase levels to SA need to be studied in depth. In the meantime, continued attention to progress in the study of SA and these two aminotransferases will have significant clinical and SA reduction implications.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

YZ: Writing–original draft, Writing–review and editing. ZL: Writing–original draft, Writing–review and editing. XL: Writing–original draft, Writing–review and editing. CW: Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2024.1336728/full#supplementary-material

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Assessment of the causal association between obstructive sleep apnea and telomere length: a bidirectional mendelian randomization study

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Background: A plethora of observational studies has established a significant correlation between Obstructive Sleep Apnea (OSA) and Telomere Length (TL). Nevertheless, a universal consensus on precise causal association and its directionality has not yet been achieved. To shed light on this, we employed Mendelian Randomization (MR) to investigate the bidirectional causal association between OSA and TL.

Method: Utilizing publicly accessible Genome-Wide Association Studies (GWAS) datasets, we procured genetic data pertinent to MR analysis. The study incorporated samples from both the OSA (n = 217,955) and TL (n = 472,174) cohorts. In the forward MR analysis, OSA served as the exposure variable and TL as the outcome. Conversely, the reverse MR analysis treated TL as the exposure and OSA as the outcome. We employed the Inverse variance weighted (IVW) as the primary methodology for MR analysis. To ensure the robustness of our MR findings, multiple sensitivity analyses were performed.

Results: In the forward MR analysis, a negative correlation was indicated between OSA and TL (IVW: odds ratio (OR) = 0.964, 95% confidence interval (CI): 0.939–0.980, P = 0.006 < 0.05). However, no significant association was identified between TL and the risk of OSA in the reverse MR analysis (IVW: OR = 0.965, 95% CI: 0.870–1.070, P = 0.499 > 0.05).

Conclusion: Our study indicated a potential association between OSA and the increased risk of shorter TL, offering vital academic support for future clinical studies on this association.

KEYWORDS

obstructive sleep apnea, telomere length, causality, genetic association, mendelian randomization

1 Introduction

OSA is a sleep-associated respiratory disorder (Schütz et al., 2021). Its prevalence has surged recently (Peppard et al., 2013; Ghavami et al., 2023), affecting approximately 100 million adults worldwide (Benjafield et al., 2019). OSA primarily arises from the obstruction of the upper respiratory pathway, which restricts airflow. Those afflicted with

OSA often experience apnea or hypoventilation during sleep, leading to consistent intermittent hypoxia and repeated sleep interruptions (Wallace et al., 2022). This sequence of pathophysiological reactions can induce chronic inflammation and oxidative stress, potentially resulting in cellular damage and early cellular aging (Gaspar et al., 2017; Turkiewicz et al., 2021). Persistent exposure to these conditions might compromise the integrity of various physiological systems, and in severe instances, pose mortal threats (Kapur et al., 2017; Bandi et al., 2021). Therefore, comprehensive research into OSA's implications for human health is of paramount importance.

Telomeres, situated at the termini of eukaryotic chromosomes, are specific structures whose length is commonly assessed in white blood cells (O'Sullivan and Karlseder, 2010). Their fundamental role is to maintain chromosome integrity and stability. Owing to intrinsic constraints of cellular replication, telomeres aren't comprehensively replicated with each cellular division, resulting in their consistent attrition. Upon reaching a specific diminutive length, cells might suspend division, potentially precipitating premature senescence or apoptosis (Blackburn et al., 2015; Wang et al., 2018). Consequently, telomeres are recognized as pivotal biological markers of cellular aging, often termed the aging "timer" (Fumagalli et al., 2012). Existing research underscores the profound correlation between telomere attrition and elevated disease incidence and mortality (Aung et al., 2023; Wang et al., 2023; Zhu S. et al., 2023). Thus, preserving telomere stability is imperative for disease prevention and the deceleration of cellular aging.

In recent years, the relationship between OSA and TL has garnered considerable attention, particularly the contentious debate over OSA as a potential risk factor for telomere shortening (Turkiewicz et al., 2021). The majority of studies highlighted a negative correlation between OSA severity and TL (Barceló et al., 2010; Bhatt et al., 2021; Pinilla et al., 2021). Contrarily, a few studies suggested that children diagnosed with OSA might experience telomere elongation instead of reduction (Kim et al., 2010). Another study proposed a J-shaped relationship between TL and the severity of OSA, suggesting that patients with moderate to severe OSA might have longer telomeres (Polonis et al., 2017). Interestingly, research indicated shorter TL in high-risk female OSA patients, and this trend was independent of income, age, obesity, smoking, hypertension, alcohol consumption, and education level, but such a trend was absent in their male OSA counterparts (Riestra et al., 2017). In contrast, another study emphasized OSA as a significant factor leading to telomere shortening in middle-aged men (Boyer et al., 2016). Clarifying the exact relationship between OSA and TL is crucial for a deeper understanding of OSA and its impact on human health. However, given the conflicting findings on TL variations in OSA patients and the absence of definitive evidence on whether TL changes occur before or after OSA onset, discerning a clear causal relationship remains elusive.

MR analysis is an analytical approach that leverages genetic variations (single nucleotide polymorphisms, SNP) as instrumental variables (IVs) to infer the causal relationship between exposure and outcome (Xu et al., 2022). Given its capacity to circumvent confounding factors and reverse causality, the findings derived from MR are deemed more credible. In this study, we employed a bidirectional two-sample MR strategy to probe the potential causal association between OSA and TL.

2 Methods

2.1 Research design

In alignment with the guidelines of the STROBE checklist for MR studies (STROBE-MR), we undertook a bidirectional MR analysis to assess the bidirectional association between OSA and TL (Skrivankova et al., 2021). For the validity of this analysis, it was essential that three core assumptions be met (Davies et al., 2018): ① IVs must be strongly associated with the exposure; ② IVs must be independent of any confounders that could affect the outcome; ③ IVs affect the outcome only through their association with the exposure, and not through any other pathways.

2.2 Data source

For this study, we utilized summary data from two GWAS (https://gwas.mrcieu.ac.uk/) of European ancestry. Genetic information for OSA was sourced from the publicly available GWAS data in the FinnGen database, featuring 16,761 cases and 201,194 controls (Table 1). The diagnosis of OSA relied on ICD codes (ICD-10: G47.3; ICD-9: 3472A), which were obtained from the Finnish National Hospital Discharge Registry and the Cause of Death Registry. This diagnosis was based on subjective symptoms, clinical examination, and sleep registration, with a threshold of AHI ≥5 events·h^{-1} or a respiratory event index ≥5 events·h^{-1} serving as key indicators for confirmation (Strausz et al., 2021). The GWAS data for TL was derived from the United Kingdom Biobank (UKB), comprising 472,174 adults with specific traits (Codd et al., 2021) (Table 1). This research strictly relied on samples of European ancestry, effectively eliminating confounding factors associated with racial variations. As the data we employed are publicly available, no supplementary ethical approval was required.

2.3 Selection of IVs

In accordance with the objective of identifying SNPs significantly correlated with exposure, we adopted a genome-wide significance threshold of $P < 5 \times 10^{-8}$. However, under this criterion, many SNPs related to OSA were not identified. Thus, we adjusted our criterion to a more lenient $P < 5 \times 10^{-7}$ for isolating OSA-associated SNPs (Zhu Q. et al., 2023). Employing the PLINK clustering technique, we excluded SNPs in linkage disequilibrium (r2 > 0.001; aggregation window: 10,000 KB), retaining only the SNPs with the most significant P-values. During data integration, we discarded palindromic SNPs. To ascertain potential biases within weak IVs, the strength of IVs was assessed using the F-statistic. Previous research indicated that an F-statistic exceeding 10 signifies a reduced likelihood of IV bias (Zhu Q. et al., 2023).

TABLE 1 Summary of the GWAS.

Trait	GWAS ID	Sample size	N SNPs	Population	PubMed ID
OSA	finn-b-G6_SLEEPAPNO	217,955	16,380,465	European	33,243,845
TL	ieu-b-4879	472,174	20,134,421	European	34,611,362

OSA, obstructive sleep apnea; TL, telomere length; N SNPs, Numbers of single nucleotide polymorphisms.

2.4 Statistical analysis

In version 4.3.0 of R software, the "TwoSampleMR" package was utilized for causality assessment. Four MR methods were selected for analysis: IVW, Weighted Median (WM), weighted mode, and MR-Egger regression. IVW evaluates the variance of each SNP's effect estimation and assigns more weight to the SNPs considered to be more stable and precise, enhancing the statistical robustness of the overall effect estimation (Burgess et al., 2013). Thus, we primarily adopted IVW for our MR analysis. However, caution is required as associations between certain genetic variants and non-exposed confounders may introduce bias into IVW results (Bowden et al., 2017). To ensure the stability of our results, we also implemented MR-Egger regression, WM, and weighted mode methodologies. MR-Egger regression can adjust for potential confounders and is somewhat tolerant to weaker IVs, but it generally requires a large sample size (Burgess and Thompson, 2017). WM yields consistent estimates even when up to 50% of the genetic variations prove ineffective (Bowden et al., 2016). Additionally, the weighted mode has a relatively lenient assumption regarding the efficacy of genetic variations.

In MR analysis, when a specific SNP affects the outcome but this influence is independent of the exposure's causal relationship, it's termed horizontal pleiotropy. This phenomenon could introduce biases in MR results. To comprehensively assess potential horizontal pleiotropy, we employed several analytical methodologies: Firstly, we used MR- Pleiotropy RESidual Sum and Outlier (MR-PRESSO) to detect outliers that might violate the causal effect (Ong and MacGregor, 2019). Subsequently, MR-Egger regression was conducted. A substantial deviation of its intercept from zero indicates horizontal pleiotropy. Lastly, by adopting a"leave-one-out" study, we systematically eliminated each SNP to compare the MR findings of the residual SNPs with the aggregate MR results. Furthermore, given the potential varying impact of distinct SNPs on exposure, such variations can lead to heterogeneity. Thus, Cochrane's Q value was utilized to gauge the heterogeneity among SNPs.

3 Results

3.1 Forward MR analysis: causality of OSA on TL

In our forward MR analysis, we adopted 8 SNPs linked with OSA ($P < 5.00 \times 10^{-7}$) to explore their latent effects on TL. The F-statistic for each SNP exceeded 10, with values ranging from 26.162 to 66.586. Details were provided in Supplementary Material: Supplementary Table S1. Based on the IVW method, we found a significant negative causal relationship between OSA and TL (OR = 0.964, 95%CI: 0.939–0.989, P = 0.006 < 0.05, Table 2). This relationship was reinforced by results from both WM (OR = 0.954, 95%CI: 0.926–0.983, P = 0.002 < 0.05, Table 2) Weighted Mode (OR = 0.951, 95%CI: 0.914–0.989, P = 0.032 < 0.05, Table 2). Yet, findings from the MR-Egger regression did not confirm a distinct causal association between OSA and TL (OR = 0.916, 95%CI: 0.802–1.050, P = 0.246 > 0.05, Table 2). We have visualized these causal estimates in a scatter plot (Figure 1A).

To evaluate the stability of the aforementioned findings, we conducted sensitivity analyses and heterogeneity tests. The MR-PRESSO test revealed no outliers that could disrupt the causal relationship (P = 0.241 > 0.05). The MR-Egger regression further confirmed that the study results were uninfluenced by horizontal pleiotropy (P = 0.475 > 0.05, Table 3). Using the "Leave-one-out" technique for sensitivity analysis revealed that the stepwise exclusion of individual SNPs exerted no substantial influence on the causal relationship estimates (Figure 1B). Additionally, Cochran's Q test demonstrated no evidence of heterogeneity in either IVW (P = 0.178 > 0.05, Table 3) or MR-Egger regression (P = 0.158 > 0.05, Table 3).

3.2 Reverse MR analysis: causality of TL on OSA

In reverse MR analysis, SNPs associated with TL were employed as the IVs to evaluate their influence on OSA. After data consolidation, four palindromic SNPs (rs2276182, rs2306646, rs56178008, and rs670180) were excluded, and 130 validated SNPs were chosen as IVs ($P < 5.00 \times 10^{-8}$). See Supplementary Material for specifics: Supplementary Table S2. According to the IVW results, there was no significant causal relationship between TL and OSA. This finding was corroborated by other MR analyses (Table 4). Moreover, no horizontal pleiotropy or heterogeneity was observed (Table 5).

4 Discussion

In this study, we utilized an open-access GWAS dataset and bidirectional two-sample MR methods to comprehensively evaluate the relationship between OSA and TL. The results of forward MR analysis, using IVW, WM, and weighted mode MR methodologies, demonstrated a negative association between OSA and TL. This suggests a close association between OSA and telomere depletion. Although the MR-Egger findings were not statistically significant, possibly due to the method accommodating horizontal pleiotropy, resulting in wider confidence intervals and potential biases (Burgess and Thompson, 2017; Rees et al., 2017). This underscores the importance of considering methodological limitations when

TABLE 2 Forward MR analysis of OSA on TL.

MR methods	N SNPs	β	SE	OR (95%CI)	p-value
IVW	8	−0.037	0.013	0.964 (0.939–0.989)	0.006
MR Egger	8	−0.087	0.068	0.916 (0.802–1.050)	0.246
Weighted median	8	−0.047	0.015	0.954 (0.926–0.983)	0.002
Weighted mode	8	−0.050	0.019	0.951 (0.914–0.989)	0.032

OSA, obstructive sleep apnea; TL, telomere length; N SNPs, Numbers of single nucleotide polymorphisms; MR, mendelian randomization; SE, standard error; β, causal effect coefficient; OR, odds ratio; IVW, inverse variance weighted.

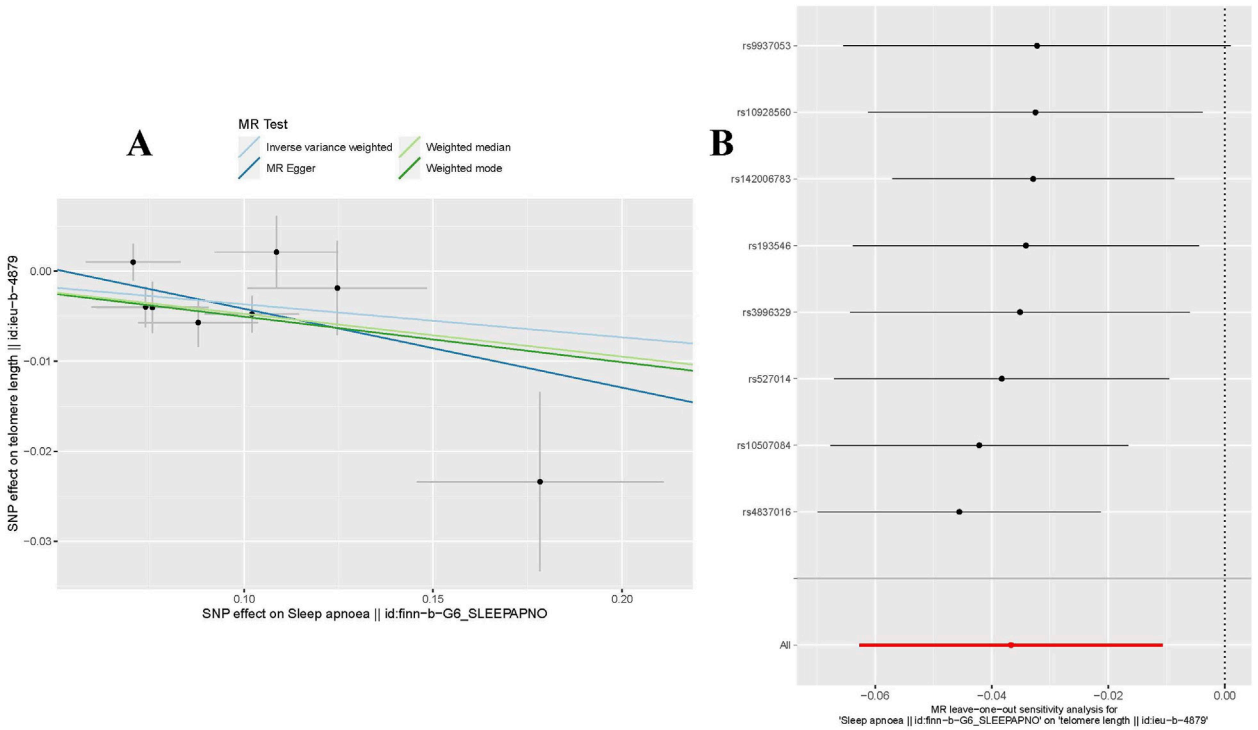

FIGURE 1
MR analysis for OSA on TL. **(A)** The scatter plot illustrated an intuitive depiction of the relationship between OSA-related IVs and TL. **(B)** The "Leave-one-out" sensitivity analysis enables the identification of bias-inducing SNPs, further elucidating their potential impact on the overall causal estimation. OSA, Obstructive Sleep Apnea; TL, Telomere Length; instrumental variables, IVs; SNPs, single nucleotide polymorphisms.

TABLE 3 Analysis of heterogeneity and pleiotropy in forward MR.

MR methods	p-value for heterogeneity	egger_intercept	p-value for pleiotropy
IVW	0.178		
MR Egger	0.158	0.005	0.475

MR, mendelian randomization; IVW, inverse variance weighted.

making causal inferences. On the other hand, reverse MR analysis substantiated the absence of a causal relationship between TL and OSA risk, indicating that a reduction in telomere length does not directly cause the onset of OSA. Sensitivity analyses and heterogeneity tests further affirmed the robustness of our findings. In summary, this study suggests that while OSA may expedite the depletion of TL, no causal association exists between TL and OSA risk.

Based on the literature search to date, this research is the inaugural study employing MR techniques to scrutinize the causal relationship between OSA and TL. Our results substantiated a negative causal linkage between OSA and TL,

TABLE 4 Reverse MR analysis of TL on OSA.

MR methods	N SNPs	β	SE	OR (95%CI)	p-value
IVW	130	−0.036	0.053	0.965 (0.870–1.070)	0.499
MR Egger	130	−0.115	0.0891	0.890 (0.742–1.070)	0.219
Weighted median	130	−0.068	0.083	0.934 (0.797–1.095)	0.412
Weighted mode	130	−0.119	0.095	0.888 (0.743–1.061)	0.211

OSA, obstructive sleep apnea; TL, telomere length; N SNPs, Numbers of single nucleotide polymorphisms; MR, mendelian randomization; SE, standard error; β: causal effect coefficient; OR, odds ratio; IVW, inverse variance weighted.

TABLE 5 Analysis of heterogeneity and pleiotropy in reverse MR.

MR methods	p-value for heterogeneity	egger_intercept	p-value for pleiotropy
IVW	0.067		
MR Egger	0.069	0.003	0.302

MR, mendelian randomization; IVW, inverse variance weighted.

which aligns with previous research outcomes. For instance, a meta-analysis incorporating seven case-control studies along with one cohort study, involving a total of 2,639 participants, revealed that individuals with OSA have significantly shorter TL compared to their healthy counterparts (mean difference: −0.03; 95%CI: −0.06 to −0.00; P = 0.003) (Huang et al., 2018). Subgroup analyses based on age and sample size further reinforce this observation. Moreover, after accounting for demographic and lifestyle factors, cross-sectional studies demonstrated a significant association between severe OSA symptoms and reduced TL (P = 0.007) (Carroll et al., 2019). Additionally, a pilot study revealed that after 6 months of Continuous Positive Airway Pressure (CPAP) treatment, significant alleviation of hypoxia symptoms was observed in OSA patients, accompanied by an increase in TL (P = 0.03) (Madaeva et al., 2022).

While the mechanisms underlying the association between OSA and TL remain to be conclusively elucidated, extant research supports the hypothesis that oxidative stress and inflammation serve as foundational elements in establishing a negative causal relationship between the two (Kim et al., 2016; Turkiewicz et al., 2021). As such, this reinforces our findings that OSA serves as a risk factor for TL shortening. Specifically, the prevalent conditions of chronic intermittent hypoxia and sleep fragmentation in OSA patients disrupt the oxygen equilibrium in the bloodstream, thus precipitating oxidative stress. This sequence of events culminates in the production of a plethora of Reactive Oxygen Species (ROS), which inflict harm upon proteins, lipids, and DNA, thereby accelerating the shortening of TL (Cadet and Wagner, 2013). Moreover, heightened levels of various inflammatory indicators like Tumor Necrosis Factor-α, Interleukin-6, and C-Reactive Protein are frequently detected in the bloodstream of OSA patients, possibly triggering systemic inflammation and establishing a biological nexus between OSA and TL (Zhang et al., 2016). Concurrently, OSA is often accompanied by endocrine imbalances, notably fluctuations in cortisol levels, which may further modulate telomerase activity, thus indirectly affecting TL. Research by TempAku PF also suggested that OSA may affect telomerase activity by inhibiting the expression of KLOTHO protein, thereby connecting OSA and TL (Tempaku et al., 2021). TL serves as a key biomarker for biological aging and is connected to various age-related diseases, whereas OSA is intimately linked with a multitude of health challenges, including but not limited to cardiovascular disorders (Labarca et al., 2018; Huang et al., 2020; Ooi and Rajendran, 2023). Given that these phenomena may interact through complex biochemical mechanisms and genetic regulations, it becomes particularly crucial to gain a deeper understanding of the impact of OSA on biological aging. Consequently, our research sheds light on the potential negative causal relationship between OSA and TL, providing new perspectives for comprehending their interplay. This suggests that alleviating OSA symptoms may be significant for delaying cellular aging and maintaining telomere stability. Timely treatment of OSA may not only emerge as a vital strategy for combating aging but also afford novel insights for the prevention of age-related diseases.

The present study is characterized by multiple noteworthy strengths. First, we employed a two-sample MR design based on large-scale GWAS data, effectively minimizing the bias introduced by unobserved confounding variables and thus more accurately establishing the causal link between OSA and TL. Second, we conducted a bidirectional causality analysis to comprehensively ensure the exclusion of misleading causal effects when exploring the relationship between OSA and TL. Lastly, to holistically evaluate the causal effects, we used a variety of advanced statistical methods, including IVW, WM, Weighted Mode, and MR-Egger regression.

This study is subject to several limitations. The reliance on GWAS data from European populations limited the global applicability of our findings. Further MR studies involving diverse ethnic groups are warranted to corroborate these findings. Second, there may be differential effects on TL among OSA patients based on gender, age, and severity level. Unfortunately, the absence of stratified GWAS data precludes a more comprehensive analysis. Third, the IVs currently available for causal inference were relatively limited. However, as GWAS research evolves, we expect to identify a greater number of genetic markers strongly associated with OSA. To summarize, the study did shed light on the relationship between OSA and

diminished TL. Nonetheless, the exact mechanisms contributing to this correlation warrant further in-depth investigation.

5 Conclusion

Overall, our study definitively demonstrated that OSA substantially hastens the deterioration of telomeres, which bears significant implications for clinical practice, particularly given that accelerated telomere degradation is linked to numerous ailments and shortened lifespan. Nevertheless, we found no causal relationship between TL and the risk of OSA onset. This insight offers a novel direction for subsequent studies, implying that TL may not be a dependable indicator for assessing the risk of OSA.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

RX: Conceptualization, Formal Analysis, Methodology, Writing–original draft. SC: Data curation, Software, Writing–original draft. XL: Data curation, Writing–review and editing. ZL: Writing–review and editing, Resources.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2025.1294105/full#supplementary-material

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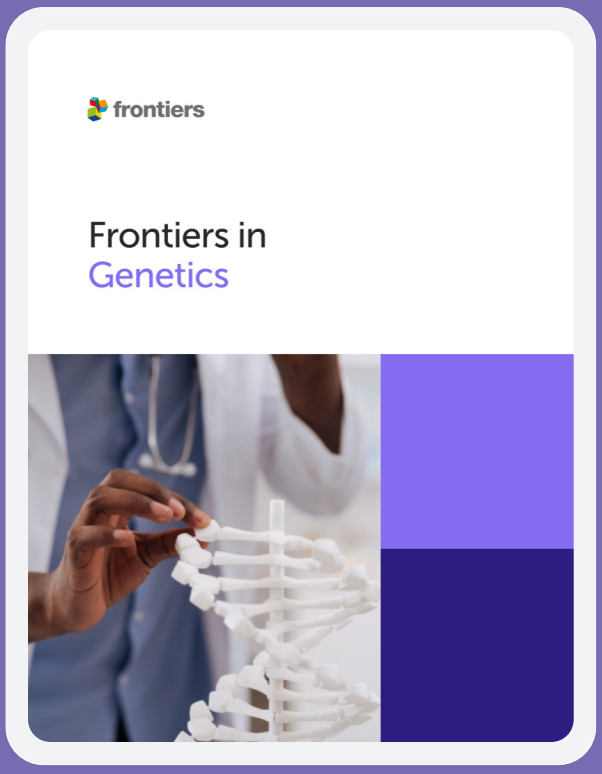

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