



APOB and CCL17 as mediators in the protective effect of SGLT2 inhibition against myocardial infarction: Insights from proteome-wide mendelian randomization

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ARTICLE INFO

Keywords:

Sodium-glucose cotransporter 2 inhibition
Myocardial infarction
Circulating protein
Mendelian randomization

ABSTRACT

Aims: Sodium-glucose cotransporter 2 (SGLT2) inhibitors offer a novel therapeutic avenue for myocardial infarction (MI). However, the exact nature of this relationship and the underlying mechanisms are not fully understood.

Methods: Utilizing a two-sample Mendelian Randomization (MR) analysis, we elucidated the causal effects stemming from the inhibition of SGLT2 on MI. Then, The pool of 4907 circulating proteins within the plasma proteome were utilized to explore the mediators of SGLT2 inhibitors on MI. Protein-protein network and enrichment analysis were conducted to clarify the potential mechanism. Finally, employing MR analysis and meta-analysis techniques, we systematically assessed the causal associations between SGLT2 inhibition and coronary heart diseases (CHD).

Results: SGLT2 inhibition (per 1 SD decrement in HbA1c) was associated with reduced risk of MI (odds ratio [OR] = 0.462, [95% CI 0.222, 0.958], P = 0.038). Among 4907 circulating proteins, we identified APOB and CCL17 which were related to both SGLT2 inhibition and MI. Mediation analysis showed evidence of the indirect effect of SGLT2 inhibition on MI through APOB ($\beta = -0.557$, 95%CI [-1.098, -0.155]) with a mediated proportion of 72%, and CCL17 ($\beta = -0.176$, 95%CI [-0.332, -0.056]) with a mediated proportion of 17%. The meta-analysis result showed that SGLT2 inhibition was associated with a lower risk of CHD.

Conclusion: Based on proteome-wide mendelian randomization, APOB and CCL17 were seen as mediators in the protective effect of SGLT2 inhibition against myocardial infarction.

1. Introduction

The sodium-glucose cotransporter 2 (SGLT2), encoded by the SLC5A2 gene, exhibits predominant expression in the kidney, playing a pivotal role in the reabsorption of glucose(Luo et al., 2021). SGLT2 inhibitors are standard in diabetes management, extending beyond glycemic control to comprehensive cardiorenal protection(Scheen, 2020; Jiang et al., 2022). Cardiovascular disease (CVD) encompasses disorders causing heart and blood vessel damage, marked by rapid onset and high

mortality rates(Mozaffarian et al., 2015). Myocardial infarction (MI), linked to coronary heart disease, contributes to 75% of sudden cardiac deaths, emphasizing an urgent health concern(Hayashi et al., 2015). MI incidence often correlates with reduced coronary artery blood flow, triggering myocardial hypoxia and subsequent ischemic necrosis(Zasada et al., 2021).

Multiple large-scale randomized controlled clinical trials (RCTs) have substantiated the potential of SGLT2 inhibitors in ameliorating MI. The EMMY trial, characterized by its multicenter, double-blind, placebo-

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controlled design, enrolled patients following acute MI. Analytical findings reveal that administration of empagliflozin within 72 h post-percutaneous coronary intervention exhibits beneficial effects on cardiac biomarkers, as well as structural and functional cardiac parameters, compared to the placebo in the context of AMI(Benedikt et al., 2023). In an extensive meta-analysis of 54 RCTs and 32 cohort studies, involving 6 distinct SGLT2 inhibitors and a collective cohort of 3,394,423 individuals, the findings reveal a significant reduction in myocardial infarction incidence in both RCTs (Relative Risk 0.9, 95% CI 0.84–0.96) and cohort studies (Relative Risk 0.89, 95% CI 0.83–0.94). Numerous animal experiments have elucidated the impact of SGLT2 inhibitors on myocardial infarction. Regardless of diabetes status, these inhibitors demonstrate the capacity to reduce infarct size, impede myocardial fibrosis, and enhance cardiac function(Li et al., 2021; Jiang et al., 2022; Lim et al., 2019). This series of studies holds promise for MI treatment, concurrently sparking a wave of mechanistic research aimed at deepening our understanding of this drug category.

In light of the absence of SGLT2 expression in the myocardium, our hypothesis posited that the ameliorative effects of SGLT2 inhibitors on MI are mediated through the modulation of specific mediators. Multiple extensive proteomic analyses have unveiled substantial alterations in circulating protein levels among MI patients undergoing SGLT2 inhibitor treatment(Cowie and Fisher, 2020). The mechanism by which SGLT2 inhibitors improve myocardial infarction remains unclear, specifically in terms of whether it involves the regulation of circulating plasma proteins. Further exploration is warranted.

Mendelian randomization (MR), rooted in the principles of Mendel's laws of inheritance, represents a genetic epidemiological methodology utilizing genetic variants as instrumental variables (IVs) to systematically evaluate the prospective causal association between an exposure and the outcomes(Birney, 2022; Holmes et al., 2017). MR analysis can mitigate confounding and reduce bias from reverse causation, as genetic variants are randomly allocated at conception(Xie et al., 2023) and consistently before the onset of outcomes(Skrivankova et al., 2021a,

2021b). Observational studies frequently suffer from biases and confounding, prompting us to assess the causal relationship between SGLT2 inhibition and MI. Furthermore, the two-step MR is employed to investigate whether certain factors mediate the effects of SGLT2 inhibition on MI.

Here, we conducted a comprehensive proteome-wide MR analysis utilizing large-scale aptamer-based plasma protein measurements. Employing multiple sensitivity analyses and mediation analysis, our objective was to determine whether plasma proteins serve as mediators in the impact of SGLT2 inhibition on MI.

2. Methods

2.1. Study design

Fig. 1 outlines the study framework. Initially, a two-sample MR was conducted to explore the causal link between SGLT2 inhibitors and MI. Subsequently, a two-step MR analysis evaluated whether plasma circulating proteins mediate the protective effects of SGLT2 inhibitors against MI. The MR analysis relies on three critical assumptions: (1) selected genetic variants demonstrate robust and reliable associations with the exposure, (2) chosen genetic variants are independent of known confounders, and (3) selected genetic variants solely impact outcomes through exposure. Adhering to STROBE-MR guidelines, the study maintains ethical standards, with essential characteristics of summary-level genome-wide association studies (GWASs) data detailed in [Supplementary Table 1](#), publicly accessible from recent publications, and ethical approvals secured from original studies.

2.2. Selection and validation for genetic predictors of SGLT2 inhibition

To obtain IVs for SGLT2 inhibition, a few processes were performed as previously described(Dai et al., 2023) and exhibited in **Fig. 1**: (1) Use data from Genotype-Tissue Expression ([The GTEx Consortium atlas of](#)

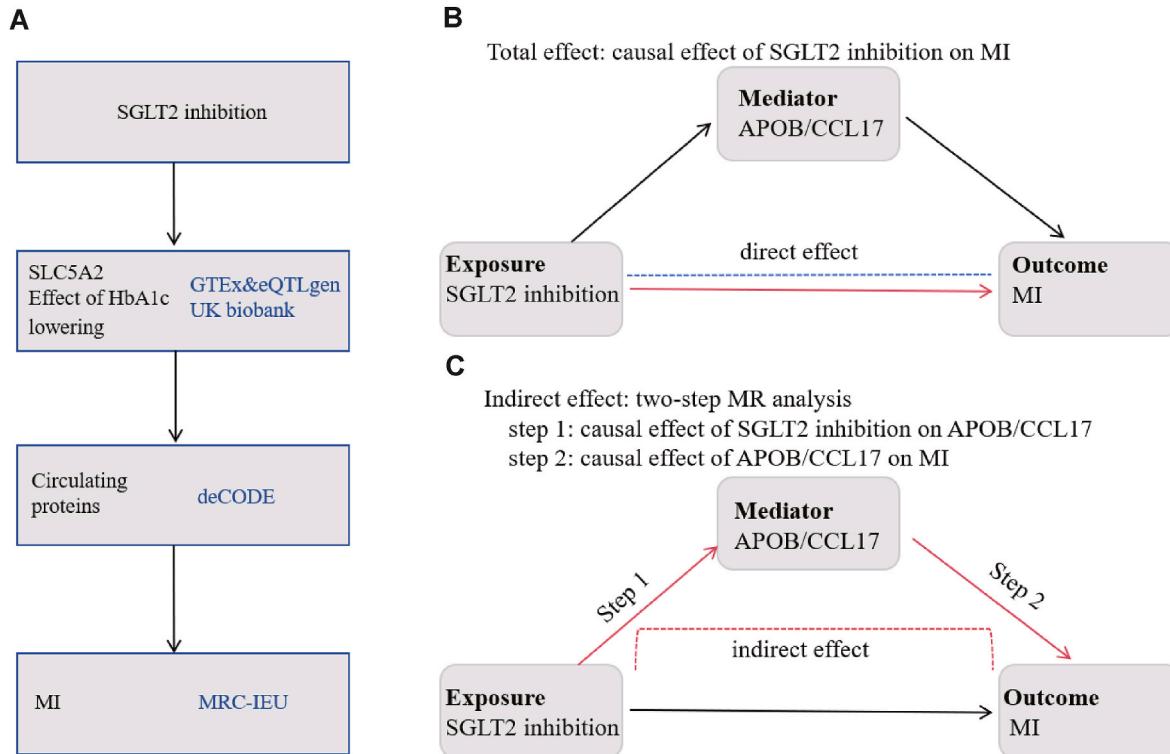


Fig. 1. Study design and summary.

We applied a two-sample MR to reveal the causality of SGLT2 inhibition on MI and a two-step MR approach to identify circulating proteins mediating this causality.

genetic regulatory effects across human tissues, 2020) and the eQTLGen Consortium(Võsa et al., 2021) to identify SNPs linked to mRNA expression of the SLC5A2 gene and investigate the possible function in SGLT2 inhibition agents. (2) The assessment of the glycemic-lowering effects of SGLT2 inhibitors relies on the association of each SLC5A2 variant with HbA1c levels, estimated through MR methodology(Au Yeung and Schooling, 2019; Luo et al., 2020). Solely opting for genetic variations exhibiting a broad regional connection with HbA1c (p-value <1 × 10⁻⁴), our attention centered on a subset of unaffiliated individuals of European descent lacking diabetes within the UK Biobank (n = 344, 182). (3) Applying genetic colocalization, we validated the common occurrence of a consistent causal variation within the SLC5A2 vicinity in both SLC5A2 and HbA1c expressions, with a probability surpassing 0.7. (4) A conventional clumping process was implemented, filtering out IVs with correlations exceeding 0.8. The strength of associations for each SNP was evaluated using F-statistics to measure the statistical power of the genetic variants. Ultimately, six SNPs were chosen as IVs for SGLT2 inhibition in the following MR analysis (Supplementary Table 2).

2.3. Proteomic data source

Genetic correlations with the concentrations of 4907 circulating proteins in the plasma proteome were derived from an extensive study on protein quantitative trait loci (pQTL), involving a cohort of 35,559 individuals of Icelandic heritage(Ferkingstad et al., 2021). All plasma samples underwent processing using the SomaScan version 4 assay (SomaLogic). Adjustments for age, sex, and sample age were applied to the 4907 tested aptamers using rank-inverse normal transformed levels. Residuals were re-standardized through rank-inverse normal transformation, and the standardized values served as phenotypes in genome-wide association testing conducted under the BOLT-LMM linear mixed model. For MR analysis, independent and significant IVs for each circulating protein pQTL were selected based on criteria: P < 5e-8, r²<0.001, minimum allele frequency>0.01, and absence of linkage disequilibrium within 1 MB, utilizing the European 1000 Genomes Project reference panel(Clarke et al., 2012).

2.4. Outcome data sources in MR

Summary-level data for MI were obtained from IEU OPEN GWAS publicly available and comprised of individuals of European ancestry. In addition to MI, a range of other coronary heart diseases (CHD) were incorporated. Detailed information regarding all the outcome data can be found in Supplementary Table 1.

2.5. MR analysis

The primary method used in MR analysis is the Inverse Variance Weighted (IVW) method, evaluating the causal relationship between exposure and outcomes through a weighted average of effect sizes derived from instrumental variable estimates(Burgess et al., 2015). In cases where there was only one SNP for a particular exposure, we utilized the Wald ratio method. Additionally, the weighted-median method was applied, accommodating deviations of up to 50% from the assumptions in instrumental variable estimates in the MR analysis. Supplementary analyses were further performed using the simple mode and weighted mode methods. Scatter plots illustrated the causal direction, while forest plots visually conveyed the effect size and statistical significance. Multiple analyses were evaluated employing the false discovery rate (FDR) criterion, with a significance threshold set at P = 0.1. We used R v.4.3.1 software (<https://www.r-project.org/>) and the "TwoSampleMR" package (<https://mrcieu.github.io/TwoSampleMR/>) to conduct MR analysis.

2.6. Validation of MR assumptions and sensitivity analysis

We utilized a Phenoscanner search to eliminate IVs showing associations with confounding factors or outcome measures. (<http://www.phenoscanner.medschl.cam.ac.uk/phenoscanner>). The F-statistic was calculated to assess the IVs' strength(Mao et al., 2023; Yao et al., 2022). $F = \frac{(n-k-1)}{k} \times \frac{R^2}{1-R^2}$, n = sample size, k = number of IVs, the coefficient of determination (R²) served as a metric to measure the proportion of variation explained by individual SNPs, R² is calculated using the following formula : $R^2 = \frac{2 \times \beta^2 \times EAF \times (1-EAF)}{2 \times \beta^2 \times EAF \times (1-EAF) + 2 \times SE^2 \times n \times EAF \times (1-EAF)}$. IVs with an F-statistic below 10 were excluded to minimize potential result bias. Pleiotropy was assessed using the intercept P-value from MR Egger regression and the global test P-value from MR Pleiotropy RESidual Sum Outlier (MR-PRESSO), with a P-value exceeding 0.05 indicating no pleiotropy associated with IVs. Heterogeneity was evaluated using Cochran's Q statistic, with a P-value above 0.05 and an I² value below 50% denoting no heterogeneity. Outlier detection and exclusion were performed using the MR radial and MR-PRESSO methods. In the presence of heterogeneity, a random-effects IVW model was applied; in its absence, a fixed-effects IVW model was employed. To gauge the impact of each unique single nucleotide polymorphism (SNP) on the overall assessment of causal linkages, a "leave-one-out" study was conducted.

2.7. Mediation MR analysis linking SGLT2 inhibition with MI

Investigating the potential mediating role of plasma proteins in the link between SGLT2 inhibition and MI, a two-phase MR analysis was carried out. The assessment of the indirect mediation effect utilized the "coefficient product" method(Yao et al., 2022). The proportion of the complete effect governed by plasma protein levels was computed by dividing the indirect impact ($\beta_1 \times \beta_2$) by the overall effect (β_3). In this context, β_1 , β_2 , and β_3 represent the effects of SGLT2 suppression on plasma proteins, circulating proteins on MI, and SGLT2 inhibition on MI, respectively. Estimates of effects were obtained through a two-sample MR analysis, and standard errors were calculated using the "delta method".

2.8. Protein-protein interaction (PPI) network and enrichment analysis

To delve into the intricate mechanisms connecting APOB (apolipoprotein B) or CCL17 (Chemokine C-C motif ligand 17) and MI, we extended our exploration using protein correlation data sourced from the STRING database. Building a PPI network, we curated proteins from three distinct origins: (1) APOB or CCL17, (2) proteins associated with MI obtained from the Disgenet website, and (3) first neighbors of APOB or CCL17 from the protein list. The PPI was accomplished using the stringApp plugin within the Cytoscape platform. Additionally, we executed pathway enrichment analysis using R software to pinpoint enriched pathways and biological processes linked to the proteins within the established PPI network(Shang et al., 2023). Our emphasis centered on pathways exhibiting notably significant P-values, with a particular focus on those intricately linked to vascular endothelial function.

2.9. Meta-analysis

To ensure robust causal relationships, we conducted a meta-analysis aggregating diverse outcomes derived from MR analyses of SGLT2 inhibition on MI-related conditions. The random-effects model employed in this meta-analysis considered statistical significance at P < 0.05. Heterogeneity was assessed with a significance threshold set at 0.05, indicating potential heterogeneity when P < 0.05.

3. Results

3.1. Effect of SGLT2 inhibition on MI

Six SNPs were identified as IVs of SGLT2 inhibition ([Supplementary Table 2](#)). Our study revealed a significant association between SGLT2 inhibition and a decreased risk of MI. Using the IVW method, the odds ratio (OR) for one-standard-deviation (SD) decrease in HbA1c resulting from SGLT2 inhibition was estimated to be 0.462 (95%CI [0.222, 0.958], P = 0.038) ([Fig. 2A, Supplementary Table 3](#)). Various statistical approaches consistently revealed concordant estimate directions for the relationship between SGLT2 inhibition and MI, affirming the robustness of our primary findings. The coherence of these results was visually

represented through scatter plots and forest plots ([Fig. 2B, Supplementary Fig. 1A](#)). No evidence of pleiotropy was detected (Egger intercept = 0.008, P = 0.740; global test p-value = 0.881). Remarkably low heterogeneity was observed, with P-values of 0.887 and 0.810 for the Q statistic in the IVW and MR Egger analysis, respectively. Moreover, the I² statistic was below 50% in both methods, consistently affirming the lack of significant heterogeneity. The detailed data is presented in [Supplementary Table 3](#). The results from the leave-one-out analysis indicate that no individual SNP significantly influences the effect of SGLT2 inhibition on MI ([Supplementary Fig. 1B](#)).

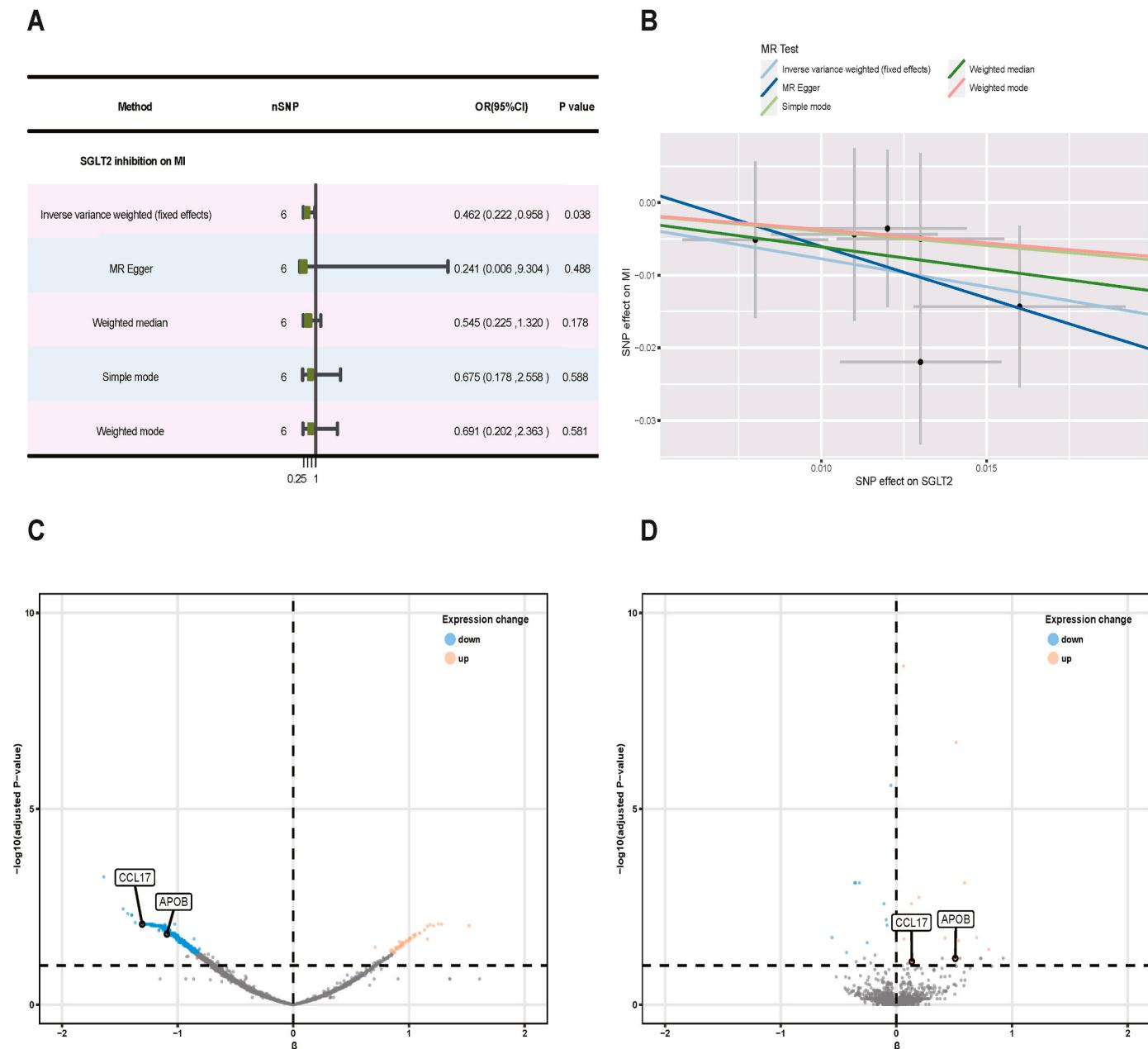


Fig. 2. Association of genetically predicted SGLT2 inhibition with MI using different MR methods and the identify of mediators. **A.** Forest plots of Mendelian randomization analysis of the causal effects of SGLT2 Inhibition on MI. **B.** Scatter plot of the causal relationship between SGLT2 inhibition and MI. The slope of each line represents the causal relationship of each method. **C.** Volcano plot illustrating the effect of SGLT2 inhibition on each circulating protein from the MR analysis using the inverse-variance-weighted method and wald ratio method. **D.** Volcano plot illustrating the effect of each circulating protein on SGLT2 inhibition from the MR analyses using the inverse-variance-weighted method and wald ratio method. IVW, inverse-variance-weighted method; OR, odds ratio; CI, confidence interval; SNP, single-nucleotide polymorphism.

3.2. Identification of APOB and CCL17 through MR analysis

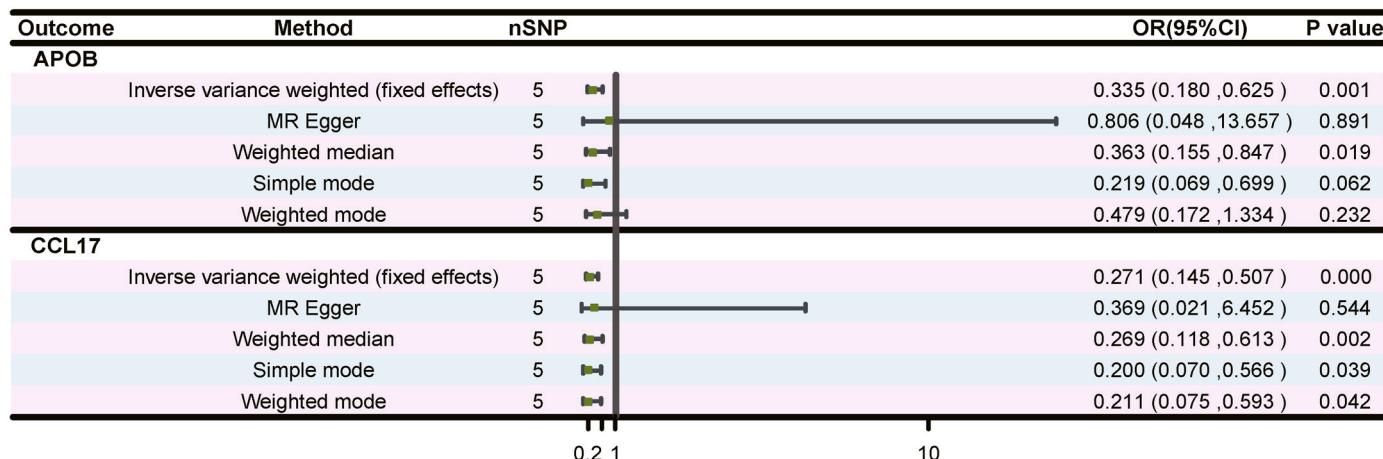
Initially, a two-sample MR study was undertaken, with SGLT2 inhibition serving as the exposure and assessing 4907 plasma proteins as the outcome. Additionally, the study involved using 4907 plasma proteins as the exposure and evaluating MI as the outcome. After FDR correction, we applied the IVW method with the Wald ratio, uncovering significant effects of SGLT2 inhibition on plasma proteins (Fig. 2C-Supplementary Table 4), as well as significant effects of plasma proteins on MI (Fig. 2D-Supplementary Table 5). Furthermore, we scrutinized pleiotropy and heterogeneity to gauge the robustness of the MR findings. No

evidence of pleiotropy was observed (all Egger intercept p-values >0.05, global test p-values >0.05). Additionally, no significant heterogeneity was detected (p-value of Q statistic >0.05; $I^2 < 50\%$) among the identified proteins (Supplementary Tables 4 and 5). Via two MR analyses, we identified two molecules APOB and CCL17.

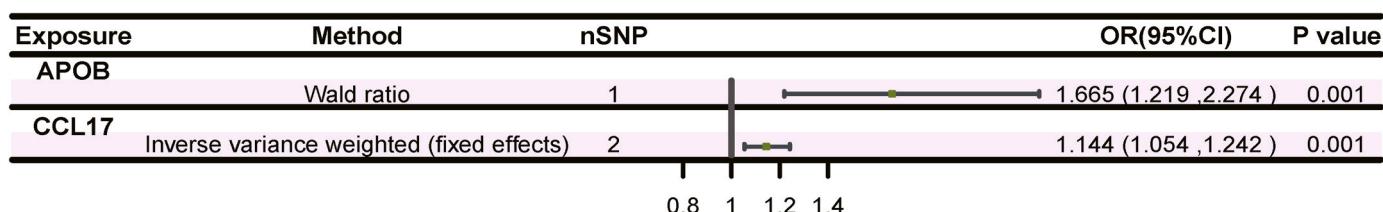
3.3. Analysis for SGLT2 inhibition to APOB and CCL17 (step1 MR)

A two-sample MR study was carried out to investigate the association between SGLT2 inhibition and APOB and CCL17. MR analysis showed that SGLT2 inhibition is negatively associated with APOB (OR = 0.335,

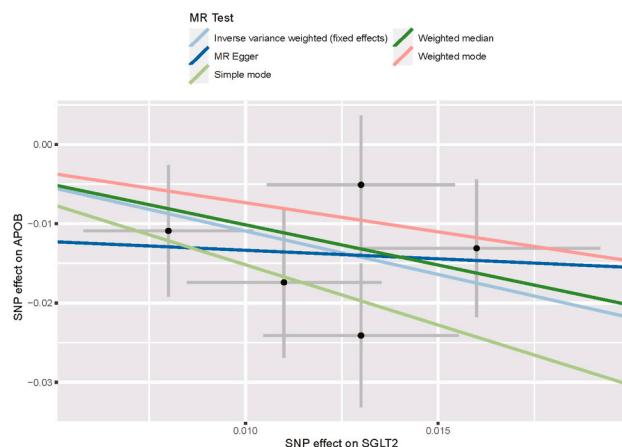
A



B



C



D

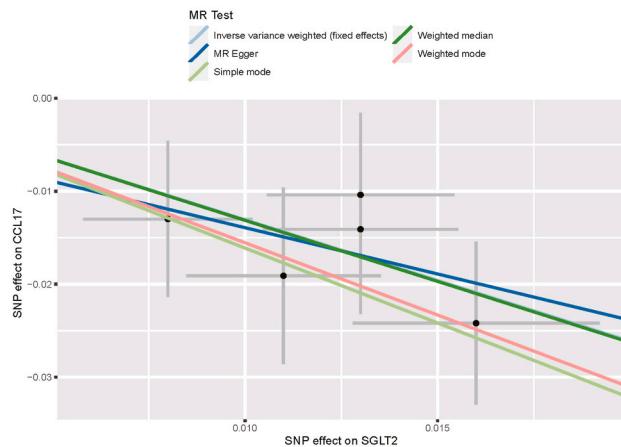


Fig. 3. A Two-step MR analysis. **A.** Forest plots of Mendelian randomization analysis of the causal effects of SGLT2 Inhibition on APOB and CCL17. **B.** Forest plots of Mendelian randomization analysis of the causal effects of APOB and CCL17 on SGLT2 Inhibition. **C.** Scatter plot of the causal relationship between SGLT2 inhibition and APOB. The slope of each line represents the causal relationship of each method. **D.** Scatter plot of the causal relationship between SGLT2 inhibition and CCL17. The slope of each line represents the causal relationship of each method.

IVW, inverse-variance-weighted method; OR, odds ratio; CI, confidence interval; SNP, single-nucleotide polymorphism.

95%CI [0.180, 0.645], $P = 0.001$) and CCL17 (OR = 0.271, 95%CI [0.145, 0.507], $P = 0.000$) of the IVW method. Alternative statistical models consistently indicated analogous estimate directions for the impact of SGLT2 inhibition on APOB and CCL17 (Fig. 3A 3C and 4D, Supplementary Table 7). The forest plot of the relationship between SGLT2 inhibition and APOB or CCL17 is illustrated in Supplementary Figs. 2A and 3A (see Fig. 4).

No evidence of pleiotropy was found for APOB (Egger intercept = -0.011, $P = 0.578$; global test P -value = 0.621) and CCL17 (Egger intercept = -0.004, $P = 0.841$; global test P -value = 0.903). No significant heterogeneity was observed, as indicated by the p -values of 0.573 and 0.471 for the Q statistic for APOB, 0.889 and 0.780 for the Q statistic for CCL17 in the IVW and MR Egger analysis, respectively. Furthermore, the I^2 statistic was below 50% in both methods, providing additional support for the lack of considerable heterogeneity in SGLT2 inhibition on APOB and CCL17 (Supplementary Table 6). Leave-one-out analysis results suggest that the impact of any single SNP on the effects of SGLT2 inhibition on APOB and CCL17 is not statistically significant (Supplementary Figs. 2B and 3B).

3.4. Analysis for APOB and CCL17 to MI (step2 MR)

In Step 2 MR, we utilized pQTLs associated with APOB or CCL17 as the exposure and MI as the outcome. One SNP significantly associated with APOB and two SNPs linked to CCL17 were identified employing a threshold of $P < 5 \times 10^{-8}$, and excluded SNPs with potential linkage disequilibrium ($R^2 > 0.001$, clump distance <10,000 kb) (Supplementary Table 7). MR findings demonstrated that a one SD elevation in genetically projected APOB levels was linked to an OR of MI (OR = 1.665, 95%CI [1.219, 2.274], $P = 0.001$) of the Wald ratio method and one SD increase in genetically predicted CCL17 levels was associated with increased OR of MI (OR = 1.144, 95%CI [1.054, 1.242], $P = 0.001$) of the IVW method (Fig. 3B–Supplementary Table 8).

There was no heterogeneity in evaluating the impact of plasma CCL17 level on MI (Supplementary Table 8).

3.5. Mediation MR analysis linking SGLT2 inhibition with MI via APOB and CCL17

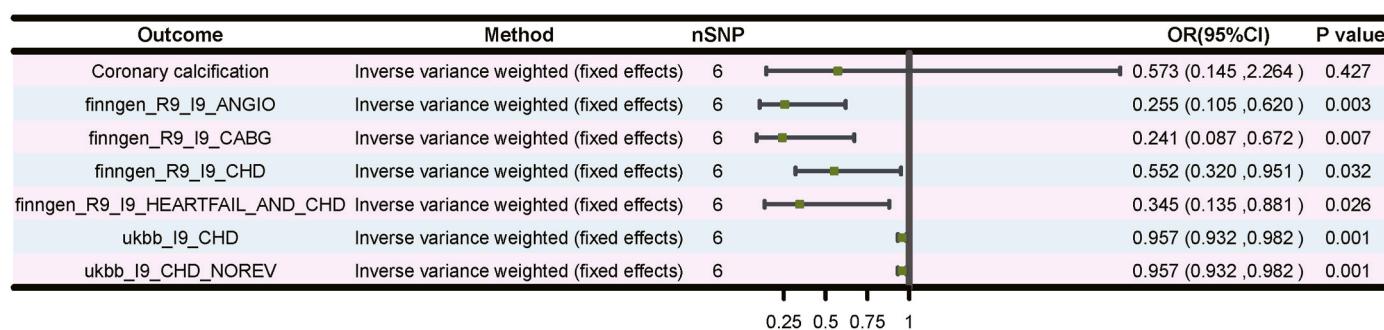
Applying a two-step MR approach with the "product of coefficient" method, we explored the mediation of plasma APOB and CCL17 levels in the association between SGLT2 inhibition and MI. The mediation effects proportion was computed by dividing the estimated effect size of APOB or CCL17 mediated effects by the total effect size of SGLT2 inhibition on MI. The results demonstrated that plasma APOB levels played a major mediating role in the improvement of MI by SGLT2 inhibition management (proportion mediated = 72%, 95%CI [20%, 100%], $P = 0.022$) and CCL17 levels played a partial mediating role (proportion mediated = 17%, 95%CI [7%, 43%], $P = 0.013$) (Table 1).

3.6. MR analysis and meta-analysis for SGLT2 inhibition and MI-related diseases

Conducted through a two-sample MR approach, our study delved into the link between SGLT2 inhibition and coronary heart diseases (CHD) across diverse datasets. The findings indicated that SGLT2 inhibition lacked a causal association with coronary calcification and displayed a negative relationship with coronary heart diseases. Furthermore, we assessed pleiotropy and heterogeneity to gauge the stability of the MR findings. No evidence of pleiotropy was shown (all Egger intercept P -values > 0.05 , global test P -values > 0.05). There was also no significant heterogeneity observed (P -value of Q statistic > 0.05 ; $I^2 < 50\%$) within the identified proteins (Supplementary Table 9).

Meta-analysis was performed to verify the effects of SGLT2 inhibition and CHD. The heterogeneity was detected ($I^2 > 50\%$) and a random effect model was utilized. Meta result showed the significant association between SGLT2 inhibition and CHD (OR = 0.56, 95%CI [0.35, 0.88], $P = 0.01$)

A



B

Study

Omitting Coronary calcification–Inverse variance weighted (fixed effects)
Omitting finngen_R9_I9_ANGIO–Inverse variance weighted (fixed effects)
Omitting finngen_R9_I9_CABG–Inverse variance weighted (fixed effects)
Omitting finngen_R9_I9_CHD–Inverse variance weighted (fixed effects)
Omitting finngen_R9_I9_HEARTFAIL_AND_CHD–Inverse variance weighted (fixed effects)
Omitting ukbb_I9_CHD–Inverse variance weighted (fixed effects)
Omitting ukbb_I9_CHD_NOREV–Inverse variance weighted (fixed effects)

Random effects model

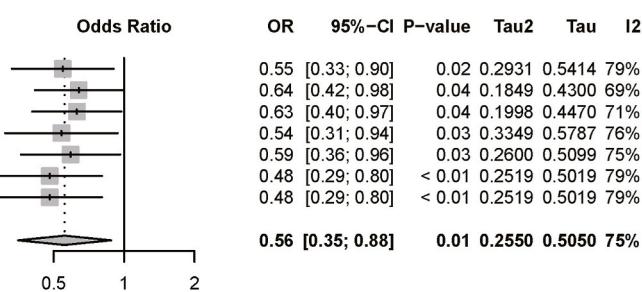


Fig. 4. MR analysis and meta analysis for SGLT2 inhibition on CHD. A. Forest plots of Mendelian randomization analysis of the causal effects of SGLT2 Inhibition on CHD from different datasets. B. Forest plots of Meta analysis of the effects of SGLT2 Inhibition on CHD from different datasets. IVW, inverse-variance-weighted method; OR, odds ratio; CI, confidence interval; SNP, single-nucleotide polymorphism.

Table 1

The mediation effect of SGLT2 inhibition on MI through APOB and CCL17.

Mediator	Total effect	Direct effect A	Direct effect B	Mediation effect	P	Mediated proportion (%) (95% CI)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)		
APOB	-0.773 (-1.503, -0.043)	-1.093 (-1.715, -0.471)	0.510 (0.198, 0.821)	-0.557 (-1.098, -0.155)	0.022	72 (20, 100)
CCL17	-0.773 (-1.503, -0.043)	-1.307 (-1.934, -0.680)	0.135 (0.053, 0.216)	-0.176 (-0.332, -0.056)	0.013	17 (7, 43)

"Total effect" indicates the effect of SGLT2 inhibition on MI, "Direct effect A" indicates the effect of SGLT2 inhibition on APOB/CCL17, "Direct effect B" indicates the effect of APOB/CCL17 on MI and "mediation effect" indicates the effect of SGLT2 inhibition on MI through APOB/CCL17. Total effect, direct effect A and direct effect B were derived by IVW or wald ratio; mediation effect was derived by using the product of coefficients method. All statistical tests were two-sided. P < 0.025 was considered significant.

3.7. PPI and enrichment analysis

To explore the mechanism of APOB or CCL17 and MI, we conducted a protein-protein interaction analysis between APOB or CCL17 and proteins associated with MI obtained from the DisGeNET website. We identified 19 key proteins that directly interact with APOB (Fig. 5A) and 20 proteins that interact with CCL17 (Fig. 5B). These proteins majorly participate in inflammation, lipid, and glucose metabolism. Moreover, our enrichment analysis demonstrated that APOB or CCL17 causing MI mainly was associated with inflammation and endothelial function (Fig. 5C). Therefore, APOB and CCL17 exert promoting effects on MI through inflammation, endothelial function impairment, lipid and glucose metabolism abnormality.

4. Discussion

In this investigation, MR analysis uncovered a causality between SGLT2 inhibition and MI. Through a two-step and mediation MR analysis, we delineated the mediating effects of APOB and CCL17 in the association between SGLT2 inhibition and MI. Additionally, MR analysis and meta-analysis highlighted the impact of SGLT2 inhibition on CHD.

APOB, as a constituent of lipoproteins and apolipoproteins, plays a pivotal role in binding to the lipoprotein surface, thereby exerting significant influence over the properties, transport, and metabolism of lipoproteins(Olofsson and Borèn, 2005; von Zychlinski et al., 2014). APOB stands as an integral component of Very Low-Density Lipoproteins (VLDLs) and their metabolites, Intermediate-Density Lipoproteins (IDLs), Low-Density Lipoproteins (LDLs), along with chylomicrons and their remnants(Olofsson and Borèn, 2005). APOB manifests in two distinct forms: the full-length apoB100, spanning 4536 amino acids, and the truncated apoB48, encompassing the N-terminal 2152 amino acids (Fisher and Ginsberg, 2002; Chan, 1992). Numerous studies have indicated that APOB may serve as a potential marker for cardiovascular diseases(Sniderman et al., 2019; Behbodikhah et al., 2021). In a MONET study, a notable reduction in APOB was found to be strongly and independently associated with decreases in inflammatory markers and insulin resistance, particularly in overweight/obese women undergoing a hypocaloric diet(Faraj et al., 2010). Dyslipidemia, including elevated APOB levels, demonstrated associations with inflammation and organ involvement in both systemic lupus erythematosus and atherosclerosis patients(Huang et al., 2023; Wang et al., 2020). A prospective observational analysis, involving participants from the population-based UK Biobank and two extensive international clinical trials (FOURIER and IMPROVE-IT), demonstrated an association between APOB and MI (Marston et al., 2022).

CCL17, a chemokine preferentially expressed in mouse and human CCR2+ macrophages, is intricately involved in eliciting or intensifying a wide range of immune responses. These encompass reactions from contact hypersensitivity and allograft rejection to inflammatory bowel disease, atopic dermatitis, atherosclerosis, and various inflammatory disorders(Alferink et al., 2003; Heiseke et al., 2012). CCL17 emerges as a novel therapeutic target in age-related and Angiotensin I-induced pathological cardiac hypertrophy and heart failure(Zhang et al., 2022, 2023; Lehallier et al., 2019). Another study showed that CCL17 deletion

could attenuate heart remodeling following reperfused MI and Angiotensin II/phenylephrine infusion(Feng et al., 2022). Moreover, CCL17 participates in endothelial dysfunction(Hueso et al., 2023).

MI is often accompanied by cell demise and acute/chronic inflammatory responses(Wang et al., 2018). Diabetes and hyperlipidemia are the common risk factors for MI(Wereski et al., 2022). These factors could promote the progression of myocardial fibrosis, inflammation, and endothelial impairment. SGLT2 inhibition plays a vital role in improving MI(Jiang et al., 2022; Li et al., 2021; Lim et al., 2019), however, there is currently a lack of genetic evidence exploring the effects of SGLT2 inhibitors on MI. This study employed MR to investigate the role of APOB and CCL17 in mediating the protective effects of SGLT2 inhibitors on MI. Meanwhile, this study utilized PPI and enrichment analyses to explore the mediating mechanism of APOB and CCL17. The results indicate a strong correlation between APOB and CCL17 and lipid metabolism, inflammation, and endothelial function. Early studies have indicated that DAPA alleviates high glucose-induced vascular endothelial dysfunction by inhibiting endothelial cell apoptosis(Huttunen et al., 2022; Faridvand et al., 2022). Furthermore, a review summarized the mechanism of action of SGLT2 inhibitors on cardiovascular diseases, indicating that SGLT2 inhibitors can improve lipid metabolism, reduce the biomarkers of myocardial inflammation, promote the endothelial functions, and maintain blood glucose homeostasis(Salvatore et al., 2022). In summary, this study implied that SGLT2 inhibitors may improve MI via inhibiting APOB and CCL17 associated with regulating inflammation, lipid metabolism, glucose homeostasis and endothelial function.

Acknowledging certain limitations is essential. Firstly, the instrumental variable for SGLT2 inhibition relies on the targeted reduction of HbA1c levels and SLC5A2 gene expression, rather than capturing the direct effects of SGLT2 inhibitors, potentially diverging from their actual mechanisms. Additionally, variables such as the dosage and duration of SGLT2 inhibitors may influence their impact on MI. Hence, these factors should be considered when interpreting our MR results. Furthermore, investigating whether APOB or CCL17 mediates the influence of SGLT2 inhibition on MI in non-European populations warrants further exploration. Finally, despite conducting diverse sensitivity analyses and applying rigorous criteria, pleiotropy in the MR setting remains a challenging aspect.

5. Conclusion

In conclusion, we integrated a two-step MR approach, sensitivity analyses, and mediation analysis to identify APOB or CCL17 as a noteworthy mediator of the effect of SGLT2 inhibition on MI. These findings provide novel insights into how SGLT2 inhibition influences MI and offer a new perspective on MI treatment exploration.

Funding

None.

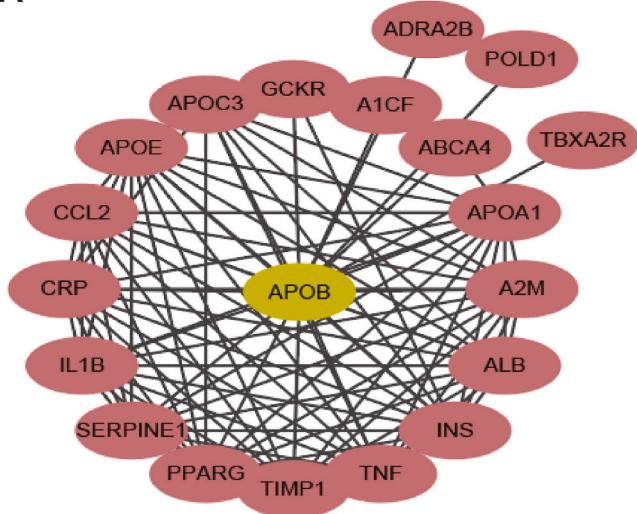
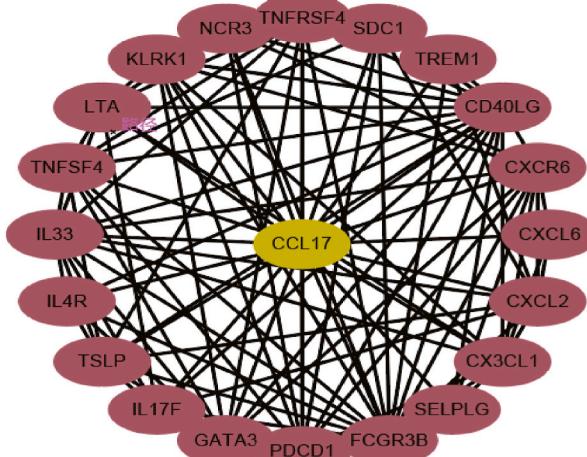
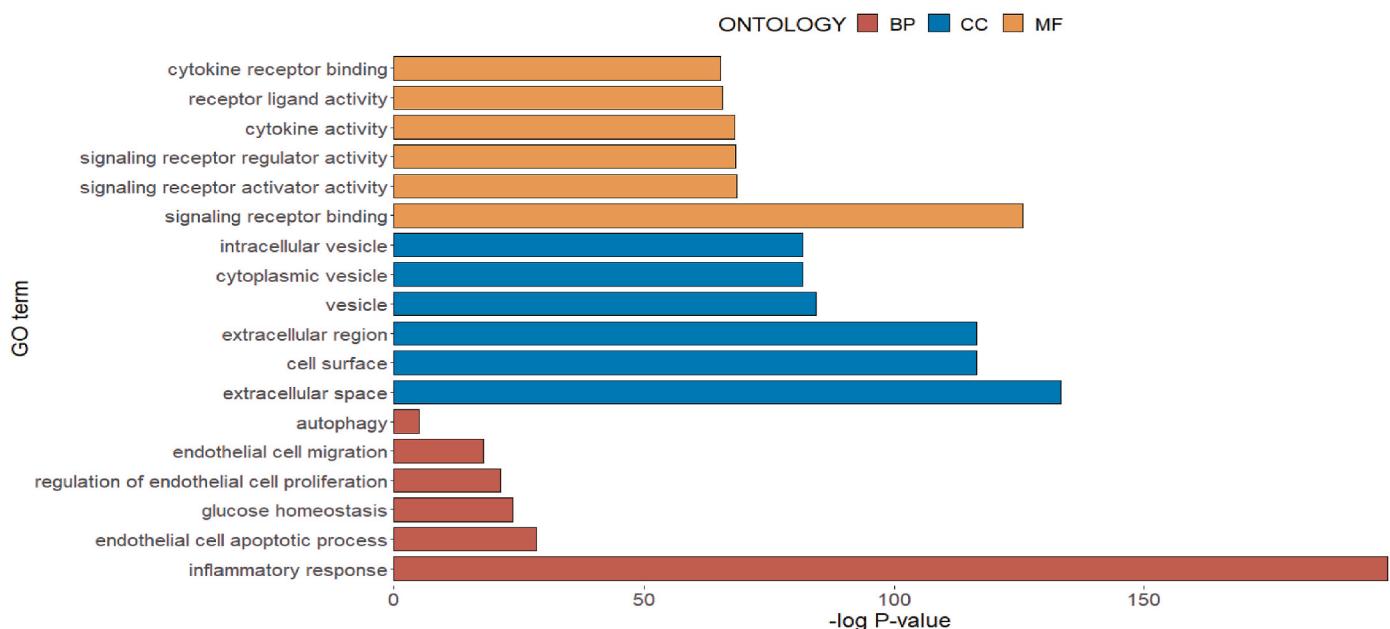
A**B****C**

Fig. 5. PPI network and enrichment analysis for the mechanism of APOB or CCL17 on MI. **A.** PPI for APOB and proteins related to MI. **B.** PPI for CCL17 and proteins related to MI. **C.** Enrichment analysis for APOB and proteins related to MI APOB, Apolipoprotein B; ADRA2B, Adrenoceptor Alpha 2B; POLD1, DNA Polymerase Delta 1; TBXA2R, Thromboxane A2 Receptor; GCSKR, Glucokinase Regulator; A1CF, APOBEC1 Complementation Factor; ABCA4, ATP Binding Cassette Subfamily A Member 4; APOA1, Apolipoprotein A1; A2M, Alpha-2-Macroglobulin; ALB, Albumin; INS, Insulin; TNF, Tumor Necrosis Factor; TIMP1, TIMP Metallopeptidase Inhibitor 1; PPARG, Peroxisome Proliferator Activated Receptor Gamma; SERPINE1, Serpin Family E Member 1; IL1B, Interleukin 1 Beta; CRP, C-Reactive Protein; CCL2, C-C Motif Chemokine Ligand 2; APOE, Apolipoprotein E; APOC3, Apolipoprotein C3; TNFRSF4, TNF Receptor Superfamily Member 4; SDC1, Syndecan 1; TREM1, Triggering Receptor Expressed On Myeloid Cells 1; CD40LG, CD40 Ligand; CXCR6, C-X-C Motif Chemokine Receptor 6; CXCL6, C-X-C Motif Chemokine Ligand 6; CXCL2, C-X-C Motif Chemokine Ligand 2; CX3CL1, C-X3-C Motif Chemokine Ligand 1; SELPLG, Selectin P Ligand; FCGR3B, Fc Gamma Receptor IIIb; PDCD1, Programmed Cell Death 1; GATA3, GATA Binding Protein 3; IL17F, Interleukin 17F; TSLP, Thymic Stromal Lymphopoietin; IL4R, Interleukin 4 Receptor; IL33, Interleukin 33; TNFSM4, TNF Superfamily Member 4; LTA, Lymphotoxin Alpha; KLRK1, Killer Cell Lectin Like Receptor K1; NCR3, Natural Cytotoxicity Triggering Receptor 3; CCL17, C-C Motif Chemokine Ligand 17.

Availability of data and materials

GTEX Portal v.8 (<https://gtexportal.org/home/datasets/>).
eQTGen phase 1 (<https://www.eqtgen.org/cis-eqtls.html>).
UK biobank (<http://biobank.ndph.ox.ac.uk/showcase/>).
deCODE study (<https://www.decode.com/summarydata/>).
HERMES consortium (<https://www.hermesconsortium.org/>).

1000 Genomes Project (<https://www.internationalgenome.org/data>).

R v.4.3.1 (<https://www.r-project.org/>).
TwoSampleMR v.0.5.6 (<https://mrcieu.github.io/TwoSampleMR/>).
snappy v.1.0 (<https://gitlab.com/richards-lab/vince.forgetta/snappy>).
coloc v.5.1.0 (<https://data/chr1swallace.github.io/coloc/>).

PLINK v.1.9 (<http://pngu.mgh.harvard.edu/purcell/plink/>).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Lili Shi: Writing – original draft. **Gen Li:** Writing – review & editing, Writing – original draft. **Ningxin Hou:** Writing – review & editing, Investigation. **Ling Tu:** Writing – review & editing, Supervision. **Jun Li:** Writing – review & editing, Supervision. **Jinlan Luo:** Writing – review & editing. **Shuiqing Hu:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank all the investigators of MRC-ieu, deCODE study, UK Biobank and FinnGen study for providing the data publicly.

Abbreviations

SGLT2	Sodium-glucose cotransporter 2
MI	Myocardial infarction
HbA1c	Glycated hemoglobin
GWAS	Genome-wide association study
MR	Mendelian randomization
IVW	Inverse variant weight
MR-PRESSO	MR Pleiotropy RESidual Sum Outlier
cis-pQTL	Cis-acting protein quantitative loci
GP	Ganglionated plexi
IVS	Instrumental variables
SNP	Single-nucleotide polymorphism
OR	Odds ratio
CVD	Cardiovascular disease
RCTs	Randomized clinical trials
GTEx	Genotype-Tissue Expression
APOB	Apolipoprotein B
CCL17	Chemokine C-C motif ligand 17
PPI	Protein-protein interaction

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejphar.2024.176619>.

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