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SGLT2 inhibition, blood lipids, and cardiovascular disease: A Mendelian randomization study

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Abstract

Aims   We aim to investigate the causal effect of blood lipids mediating sodium-glucose cotransporter 2 (SGLT2) inhibition in cardiovascular disease (CVD) using Mendelian randomization (MR).

Methods and results   A two-sample two-step MR study was conducted to evaluate the association of SGLT2 inhibition with CVDs and the mediation effects of blood lipids linking SGLT2 inhibition with CVDs. Genetic instruments for SGLT2 inhibition were identified as genetic variants, which were associated with the expression of the SLC5A2 gene and glycated haemoglobin level (HbA1c). SGLT2 inhibition was associated with reduced risk of heart failure (HF) (OR 0.44 [95% CI 0.32–0.61]; $P = 6.0 \times 10^{-7}$), atrial fibrillation (AF) (0.47 [0.37–0.61]; $P = 1.81 \times 10^{-8}$), coronary artery disease (CAD) (0.47 [0.30–0.73]; $P = 7.46 \times 10^{-4}$), myocardial infarction (MI) (0.30 [0.15–0.61]; $P = 7.44 \times 10^{-4}$), any stroke (AS) (0.28 [0.18–0.42]; $P = 1.14 \times 10^{-9}$), and ischaemic stroke (IS) (0.27 [0.17–0.44]; $P = 1.97 \times 10^{-7}$). Our results indicated that the proportion mediated of the mediating effect of total cholesterol was 1.7% (OR 0.99 [95% CI 0.98, 0.99], $P = 0.004$), 4.7% (0.96 [0.95, 0.98], $P = 0.002$), and 2.7% (0.97 [0.95, 0.98], $P = 0.002$) in the association between SGLT2 inhibition and the risk of HF, CAD, and MI, respectively. For low-density lipoprotein cholesterol, the proportion mediated of the mediating effect was 2.2% for HF (OR 0.98 [95% CI 0.98, 0.99], $P = 0.003$), 8.6% for CAD (0.93 [0.91, 0.95], $P = 5.74 \times 10^{-4}$), and 5.0% for MI (0.95 [0.94, 0.96], $P = 6.97 \times 10^{-4}$). For non-high-density lipoprotein cholesterol, the proportion mediated of the mediating effect was 3.4% for HF (OR 0.98 [95% CI 0.97, 0.98], $P = 4.42 \times 10^{-6}$), 11.8% for CAD (0.92 [0.90, 0.93], $P = 7.23 \times 10^{-8}$), 5.7% for MI (0.94 [0.92, 0.95], $P = 8.17 \times 10^{-7}$), 1.5% for AS (0.98 [0.98, 0.99], $P = 0.001$), and 1.4% for IS (0.98 [0.98, 0.99], $P = 0.004$).

Conclusions   Our study showed the association of SGLT2 inhibition with the reduced risk of CVDs and blood lipids might mediate this association.

Introduction

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are a class of oral antidiabetic drugs that reduce serum glucose concentrations by inhibiting glucose reabsorption in the proximal tubule and enhancing urinary glucose excretion.[1] Several large clinical trials have shown their benefit in improving cardiovascular and renal outcomes.[2–5] In addition, SGLT2 inhibitors were believed to have extra beneficial metabolic effects beyond glycaemic control,[6] which might play an important role in improving cardiovascular outcomes, but the underlying mechanism remains unclear.

SGLT2 inhibitors were reported to have remarkable effects on circulating metabolites, particularly blood lipids.[7] SGLT2 inhibitors have been reported to be inactive 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr), a key rate-limiting enzyme in de novo synthesis of cholesterol.[8] In addition, some evidence suggested that SGLT2 inhibition reduced the concentration of total cholesterol (TCH), low-density lipoprotein-cholesterol (LDL-C), and triglyceride.[9] However, others failed to observe a significant change in the serum lipid profile,[10] or even found the opposite change.[11] The discrepancy in these studies may be partly due to the limited sample size, the presence of residual confounding, or short time follow-up.

Mendelian randomization (MR) studies, which could support and guide new randomized controlled trials (RCT) designs, help to reach a better comprehension of data from observational studies.[12] It uses genetic variants for a specific drug target as instrumental variables (IVs) to investigate the causal effect of the drug on a disease based on the random assignment of genetic variants at conception. Thus, MR using genetic variants as instrumental variables for drug effects serves as a potentially efficient approach to investigating the repurposing potential of relevant drugs.[13]

A large number of studies have suggested an association between blood lipids and the occurrence of cardiovascular diseases (CVD).[14–17] Given the underexplored metabolic mechanism of SGLT2 inhibition in protecting against CVDs and the important role of blood lipids in the pathogenesis of CVDs, we hypothesized that blood lipids might mediate the effect of SGLT2 inhibition on CVDs. In the present study, we first conducted a two-sample MR to investigate the causal association between SGLT2 inhibition and CVDs. Second, we performed a two-step MR study to identify the potential metabolic pathway from SGLT2 inhibition to CVDs through blood lipids. We identified the causal effect of SGLT2 inhibition on blood lipids, which would provide insight into exploring the mechanism of SGLT2 inhibition in reducing the risk of CVDs.

Methods

Study design and data source

A two-sample MR design was performed in the current study (Figure 1A). To maintain the validity of the causal estimation, three MR assumptions are essential, which are (1) a robust association between IVs and the exposure (relevance), (2) independence of IVs from confounders (exchangeability), and (3) no direct effects of IVs on CVD risk other than through the drug targets (exclusion restriction). This study was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) guidelines.[18]

Instrument selection for sodium-glucose cotransporter 2 inhibition

The identification of genetic variants for SGLT2 inhibition involved four steps. First, genetic variants associated with the mRNA expression level of the SLC5A2 gene (the target gene for SGLT2 inhibition) were selected using data from the Genotype-Tissue Expression (GTEx)[19] and eQTLGen Consortium.[20] Second, SLC5A2 variants showing region-wide

association with glycated haemoglobin (HbA1c) levels (P value $< 1 \times 10^{-4}$) were selected using data from unrelated individuals of European ancestry without diabetes in the UK Biobank (n = 344 182). Third, genetic colocalization was used to validate whether the expression of SLC5A2 and HbA1c shared the same causal variant in the SLC5A2 region (a genetic colocalization probability >0.7 was used as evidence of colocalization). Finally, a standard clumping procedure was performed using a correlation between variants < 0.8 as a threshold to remove variants with very high correlation.[21] After multiple selection and validation steps, six genetic variants robustly associated with SGLT2 inhibition via HbA1c were selected as genetic instruments for the MR analysis. To quantify the statistical power of the single nucleotide polymorphism (SNP), we estimated the strength of the genetic predictors of each tested exposure using F-statistics (Supporting Information, Table S1).

Study outcomes

Study outcomes included six CVDs, including heart failure (HF), atrial fibrillation (AF), coronary artery disease (CAD), myocardial infarction (MI), any stroke (AS), and ischaemic stroke (IS). For the following MR analysis, we used the summary statistics derived from relevant genome-wide association studies (GWAS) for these outcomes. Summary data for HF were obtained from the Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES) consortium (47 309 cases and 930 014 controls). Summary data for AF were obtained from the Atrial Fibrillation Genetics (AFGen) Consortium (60 620 cases and 970 216 controls). Summary data for CAD (60 801 cases and 123 504 controls) and MI (60 801 cases and 123 699 controls) were obtained from the Coronary ARtery Disease Genome-wide Replication and Meta-analysis plus The Coronary Artery Disease Genetics (CARDIoGRAMplusC4D) consortium. Summary data for AS (40 585 cases of stroke and 406 111 controls) and IS (34 217 cases and 406 111 controls) were obtained from the MEGASTROKE consortium (Supporting Information, Table S2).

All participants were of European ancestry to minimize bias due to population structure and did not overlap with the participants in the UK Biobank to avoid any potential bias due to participant overlap for a weak instrument.[22]

Selection of blood lipids

We obtained five blood lipid traits from 930 672 participants of European ancestry (excluding the UK biobank participants) generated by the Global Lipids Genetics Consortium.[23] These lipid data comprised total cholesterol (TCH, n = 930 672), low-density lipoprotein cholesterol (LDL-C, n = 842 660), high-

Figure 1 Study design flowchart of the Mendelian randomization (MR) study. (A) Overview of the Mendelian randomization. Assumption I: the instrumental variables (IVs) must be strongly associated with the exposure; Assumption II: the IVs should be independent of the potential confounding factors of the exposure-outcome association; Assumption III: the IVs should not be directly linked to outcomes. (B) The framework of the two-step method of Mendelian randomization. Total effect = $\beta1$; Mediation effect = $\beta2 \times \beta3$; Direct effect = $\beta1$-$\beta2 \times \beta3$; Proportion mediated = $(\beta2 \times \beta3)/\beta1$. LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; SGLT2, sodium-glucose cotransporter 2; TCH, total cholesterol.
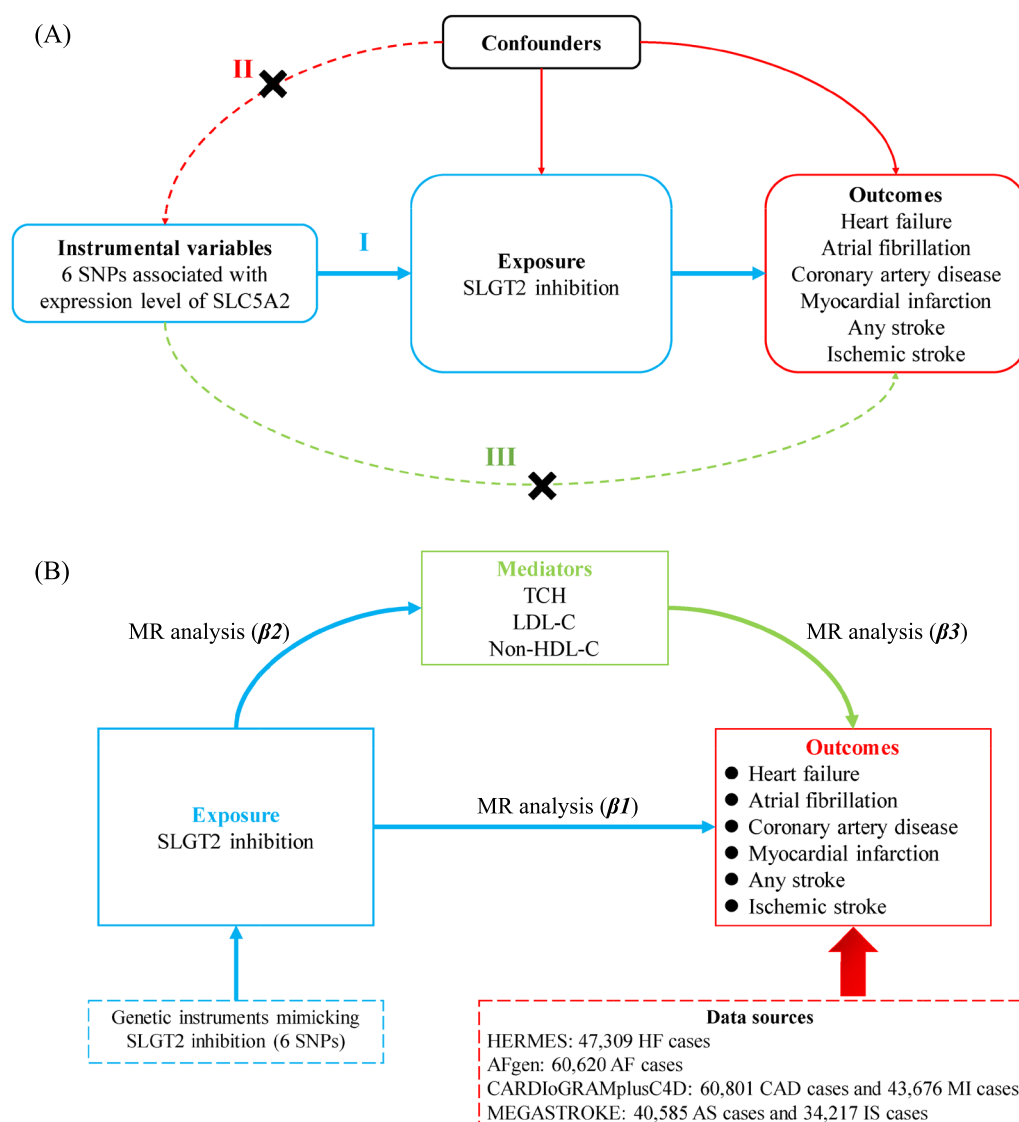
density lipoprotein cholesterol (HDL-C, n = 888 227), non-high-density lipoprotein cholesterol (non-HDL-C, n = 570 286), and triglycerides (n = 864 240). The genetic variants that showed strong associations with the above blood lipid traits ($P < 5 \times 10^{-8}$) were selected as candidate genetic predictors. Further clustering was performed to remove genetic variants that were correlated with each other (correlation among variants <0.001). After selection, 368 SNPs associated with TCH, 314 SNPs associated with LDL-C, and 281 SNPs associated with non-HDL-C were selected as genetic predictors of these three blood lipids (Supporting Information, Tables S3–S5).

Positive control analysis

To test our selection of IVs, the positive control analysis was performed with T2D as the outcome, which is the original indication for antidiabetic drugs. Summary data for T2D were obtained from the FinnGen consortium (57 698 cases and 308 252 controls).[24]

Statistical analysis

Mendelian randomization analyses of sodium-glucose cotransporter 2 inhibition and type 2 diabetes and cardiovascular disease risk

We used a two-sample MR to estimate the effect of SGLT2 inhibition on CVDs, including HF, AF, CAD, MI, AS, and IS. The summary data of the genetic associations of the six instrumental variables were extracted from the GWAS for each outcome and were harmonized to ensure that the effect of the SNP on the exposure and outcome corresponded to the same allele before conducting causal estimation. In addition, Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO)[25] was applied to identify and correct for potential horizontal pleiotropy and heterogeneity by removing the outlying SNP. The inverse variant weight (IVW) method[26] was used as the primary analysis to estimate the effect of SGLT2 inhibition on CVDs ($\beta 1$), which can provide the most accurate and powerful estimates when all genetic variants are valid instruments.

Mediation Mendelian randomization analysis linking sodium-glucose cotransporter 2 inhibition with cardiovascular diseases via blood lipids

A two-step MR was performed to estimate the mediation effect of blood lipids on the association between SGLT2 inhibition and CVDs (*Figure 1B*). First, we used IVW as the primary approach to estimate the effect of SGLT2 inhibition on five blood lipids ($\beta 2$). SGLT2 inhibition was only significantly associated with three blood lipids, including TCH, LDL-C, and non-HDL-C. Second, 368 SNPs associated with TCH, 314 associated with LDL-C, and 281 associated with non-HDL-C were used as genetic predictors for the three exposures, and CVDs were selected as the outcomes. The effect of three blood lipids on CVDs ($\beta 3$) was assessed. The product of coefficients method was used as the main method to calculate the mediation (or indirect) effect of SGLT2 inhibition on CVDs, that is, the casual effect of SGLT2 inhibition on outcomes via blood lipids ($\beta 2 \times \beta 3$). Therefore, the mediation proportion of each blood lipid in the association between SGLT2 inhibition and CVDs was calculated as the indirect effect ($\beta 2 \times \beta 3$) divided by the total effect of SGLT2 inhibition on CVDs ($\beta 2 \times \beta 3/\beta 1$). The 95% confidence intervals (CIs) of the mediation proportions were calculated using the delta method.[27]

Sensitivity analysis

To investigate the robustness of the results assessing the effects of SGLT2 inhibition on CVDs, we performed the MR-Egger, weighted median, weighted mode, and MR-PRESSO methods as sensitivity analyses. The MR-Egger method[28] examines whether there is directional pleiotropy based on its intercept term, where a value different from zero indicates the presence of directional pleiotropy and that the IVW estimate is biased. The weighted median method[29] provides a reliable estimate if at least 50% of the instruments are valid. The weighted mode method[30] provides a reliable estimate when the horizontal pleiotropy is zero in the largest cluster. The MR-PRESSO method[25] can also determine the presence of directional pleiotropy by detecting possible outliers and recalculating the estimates after removing outliers. The strength of the genetic instruments was assessed by F-statistics and indicates weak instruments when F-statistics < 10. In addition, we computed the statistical power for the MR analyses via the online web tool (https://sb452.shinyapps.io/power/) (Supporting Information, *Table S6*).[31] Cochrane's Q statistics for IVW and MR-Egger and the global test for MR-PRESSO were calculated to assess the heterogeneity between instruments.

A Bonferroni-corrected significance level of $P < 8.3 \times 10^{-3}$ (0.05/6 CVDs) was used to adjust for multiple testing. All analyses were performed using the 'TwoSampleMR' package in R software version 4.3.1.

Results

Strength of the genetic predictors for sodium-glucose cotransporter 2 inhibition and blood lipids

The predictors of SGLT2 inhibition showed good strength (F-statistic = 23.9, which is over the threshold of 10) (Supporting Information, *Table S2*). The predictors for the three blood lipids were also very strong (F-statistics for TCH = 247.7, LDL-C = 241.2, and non-HDL-C = 246.8) (Supporting Information, *Tables S3–S5*). The significant strength suggested that weak instrument bias was unlikely to be an issue in this study; thus, all of these genetic predictors were kept for the MR analysis.

Positive control analyses

Genetically predicted SGLT2 inhibition was found to be negatively associated with T2D (odds ratio [OR] 0.27 [95% CI 0.22, 0.32], $P = 5.35 \times 10^{-42}$) for per 1-SD unit (6.75 mmol/mol or 1.09%) lowering of HbA1c via SGLT2 inhibition (*Table 1* and *Figure 2*). The heterogeneity test using the Cochran's Q test for IVW showed that the Q statistics and P values were not significant (Q = 0.858, P = 0.973), which implied no evidence of heterogeneity for the effect of SGLT2 inhibition on T2D. In addition, no horizontal pleiotropy was detected using the MR-Egger method (Egger intercept = −0.009, P value of intercept = 0.588) (*Table 1*).

Table 1 MR estimates of the effect of genetically predicted SGLT2 inhibition on type 2 diabetes and cardiovascular diseases

Outcome	Methods	OR (95%)	P value	Q statistic*	P_h	Egger intercept	$P_{intercept}$
T2D	Inverse variance weighted	0.27 (0.22–0.32)	5.35×10^{-42}	0.858	0.973		
	MR-Egger	0.53 (0.05–5.34)	0.617	0.512	0.972	−0.009	0.588
	Weighted median	0.27 (0.15–0.50)	2.18×10^{-5}				
	Weighted mode	0.29 (0.15–0.56)	0.015				
	MR-PRESSO	0.27 (0.22–0.32)	3.88×10^{-5}	2.463	0.898		
Heart failure	Inverse variance weighted	0.44 (0.32–0.61)	6.00×10^{-7}	1.772	0.880		
	MR-Egger	0.21 (0.01–3.00)	0.313	1.456	0.834	0.009	0.604
	Weighted median	0.43 (0.22–0.86)	0.016				
	Weighted mode	0.38 (0.15–0.94)	0.092				
	MR-PRESSO	0.44 (0.32–0.61)	0.004	2.267	0.914		
Atrial fibrillation	Inverse variance weighted	0.47 (0.37–0.61)	1.81×10^{-8}	1.619	0.899		
	MR-Egger	0.95 (0.10–9.05)	0.967	1.235	0.872	−0.009	0.569
	Weighted median	0.44 (0.25–0.78)	0.005				
	Weighted mode	0.43 (0.19–0.94)	0.089				
	MR-PRESSO	0.47 (0.37–0.61)	0.002	2.632	0.818		
Coronary heart disease	Inverse variance weighted	0.47 (0.30–0.73)	7.46×10^{-4}	1.582	0.812		
	MR-Egger	0.58 (0.02–14.5)	0.760	1.565	0.667	−0.003	0.907
	Weighted median	0.41 (0.18–0.96)	0.039				
	Weighted mode	0.37 (0.12–1.18)	0.169				
	MR-PRESSO	0.47 (0.30–0.73)	0.028	4.989	0.599		
Myocardial infarction	Inverse variance weighted	0.30 (0.15–0.61)	7.44×10^{-4}	3.166	0.530		
	MR-Egger	1.55 (0.04–56.78)	0.827	2.343	0.504	0.021	0.431
	Weighted median	0.36 (0.13–0.96)	0.053				
	Weighted mode	0.41 (0.12–1.44)	0.247				
	MR-PRESSO	0.30 (0.15–0.61)	0.028	1.433	0.870		
Any stroke	Inverse variance weighted	0.28 (0.18–0.42)	1.14×10^{-9}	0.866	0.834		
	MR-Egger	0.53 (0.02–13.01)	0.736	0.694	0.707	−0.008	0.719
	Weighted median	0.32 (0.12–0.84)	0.021				
	Weighted mode	0.33 (0.11–1.00)	0.144				
	MR-PRESSO	0.28 (0.18–0.42)	0.009				
Ischaemic stroke	Inverse variance weighted	0.27 (0.17–0.44)	1.97×10^{-7}	1.020	0.796		
	MR-Egger	0.88 (0.03–29.52)	0.952	0.556	0.757	−0.015	0.566
	Weighted median	0.35 (0.12–0.98)	0.046				
	Weighted mode	0.35 (0.11–1.15)	0.183				
	MR-PRESSO	0.27 (0.17–0.44)	0.014	1.769	0.826		

CI, confidence interval; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; OR, odds ratio; P_h, P value for heterogeneity; $P_{intercept}$, P value for intercept of MR-Egger regression.
*Heterogeneity test in the IVW methods was through use of Cochran's Q statistic and for the MR-PRESSO method the global test.

Figure 2 The causal effect of SGLT2 inhibition on T2D and CVDs. The OR and 95% CI indicate the effect estimates of a decrease in T2D and CVDs per SD unit (6.75 mmol/mol or 1.09%) lowering of HbA1c via SGLT2 inhibition by the inverse-variance weighted method. CI, confidence interval; CVD, cardiovascular diseases; HbA1c, glycated haemoglobin; OR, odds ratio; SGLT2, sodium-glucose cotransporter 2; T2D, type 2 diabetes.

Outcome	SNPs	Cases/controls		OR (95% CI)	P value
Type 2 diabetes	6	57,698/308,252		0.27 (0.22 to 0.32)	5.35×10^{-42}
Heart failure	6	47,309/930,014		0.44 (0.32 to 0.61)	6.00×10^{-7}
Atrial fibrillation	6	60,620/970,216		0.47 (0.37 to 0.61)	1.81×10^{-8}
Coronary artery disease	5	60,801/123,504		0.47 (0.30 to 0.73)	7.46×10^{-4}
Myocardial infarction	5	43,676/128,199		0.30 (0.15 to 0.61)	7.44×10^{-4}
Any stroke	4	40,585/406,111		0.28 (0.18 to 0.42)	1.14×10^{-9}
Ischemic stroke	4	34,217/406,111		0.27 (0.17 to 0.44)	1.97×10^{-7}
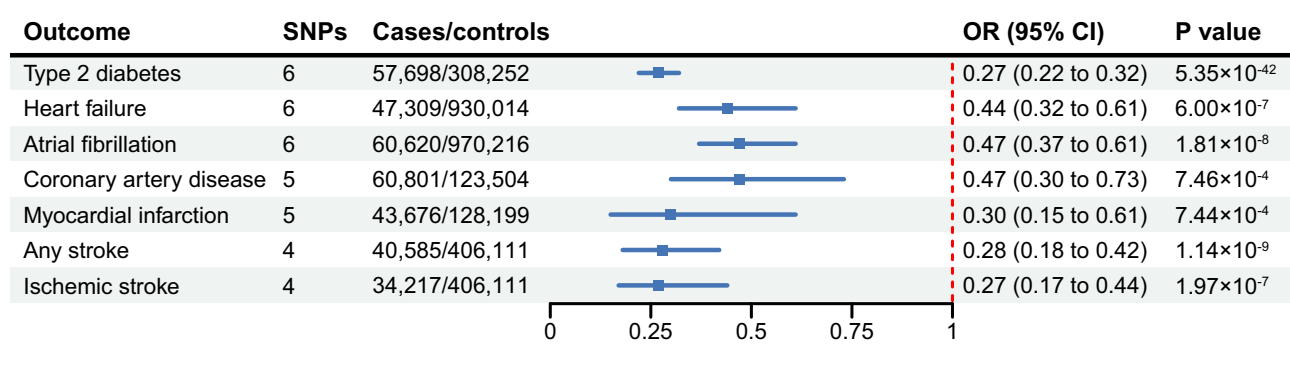
Sodium-glucose cotransporter 2 inhibition and cardiovascular disease risk

As for the primary analysis, genetically predicted SGLT2 inhibition was significantly associated with a reduced risk of HF (OR, 0.44; 95% CI, 0.32–0.61; $P = 6.0 \times 10^{-7}$), AF (OR, 0.47; 95% CI, 0.37–0.61; $P = 1.81 \times 10^{-8}$), CAD (OR, 0.47; 95% CI, 0.30–0.73; $P = 7.46 \times 10^{-4}$), MI (OR, 0.30; 95% CI, 0.15–0.61; $P = 7.44 \times 10^{-4}$), AS (OR, 0.28; 95% CI, 0.18–0.42; $P = 1.14 \times 10^{-9}$), and IS (OR, 0.27; 95% CI, 0.17–0.44; $P = 1.97 \times 10^{-7}$) (*Figure 2*). MR estimates from the weighted median were similar to those from IVW analyses for SGLT2 inhibition, though with wider CIs (*Table 2*). There was no evidence of significant heterogeneity in all CVDs using the Cochran's Q test for IVW (all P values > 0.05). or evidence of directional pleiotropy, as assessed by the P value of intercept (all P values > 0.05; *Table 2*).

Mediation Mendelian randomization of sodium-glucose cotransporter 2 inhibition, lipid metabolites, and cardiovascular disease risk

We estimated the effect of SGLT2 inhibition on the five blood lipids and observed three of them were significantly associated with SGLT2 inhibition (Bonferroni-corrected P value threshold = 0.01 [0.05/5]). We found that SGLT2 inhibition had negative association with TCH (OR, 0.91; 95% CI, 0.86–0.96; $P = 2.82 \times 10^{-4}$), LDL-C (OR, 0.88; 95% CI, 0.82–0.93; $P = 6.22 \times 10^{-5}$), and non-HDL-C (OR, 0.83; 95% CI, 0.78–0.88; $P = 2.18 \times 10^{-9}$), but little evidence to support association with HDL-C and triglycerides (*Table 2* and *Figure 3*). The Q statistics and P values were not significant (P values from 0.905 to 0.973), which implied no evidence of heterogeneity. The pleiotropy test using the MR-Egger intercept term showed that P values of the intercepts varied from 0.542 and 0.985, which meant little evidence of directional pleiotropy (*Table 2*).

We further estimated the effect of TCH, LDL-C, and non-HDL-C on risk for CVDs, including HF, AF, CAD, MI, AS, and IS (Bonferroni-corrected P value threshold = 2.78×10^{-3} [0.05/18]). For TCH and LDL-C, we observed a positive association with HF (OR for TCH 1.16 [95% CI 1.11, 1.22], $P = 1.69 \times 10^{-9}$; OR for LDL-C 1.15 [95% CI 1.09–1.21], $P = 9.26 \times 10^{-8}$), CAD (OR for TCH 1.47 [95% CI 1.37, 1.57], $P = 3.49 \times 10^{-28}$; OR for LDL-C 1.67 [95% CI 1.55, 1.81], $P = 2.40 \times 10^{-37}$), MI (OR for TCH 1.41, [95% CI 1.31, 1.52], $P = 2.18 \times 10^{-21}$; OR for LDL-C 1.60 [95% CI 1.47, 1.75], $P = 1.20 \times 10^{-27}$) (Supporting Information, Tables S7–S8 and *Figure 4*). In addition to HF, CAD, and MI, non-HDL-C was also associated with an increased risk of AS (OR, 1.10; 95% CI, 1.05–1.16; $P = 1.94 \times 10^{-4}$) and
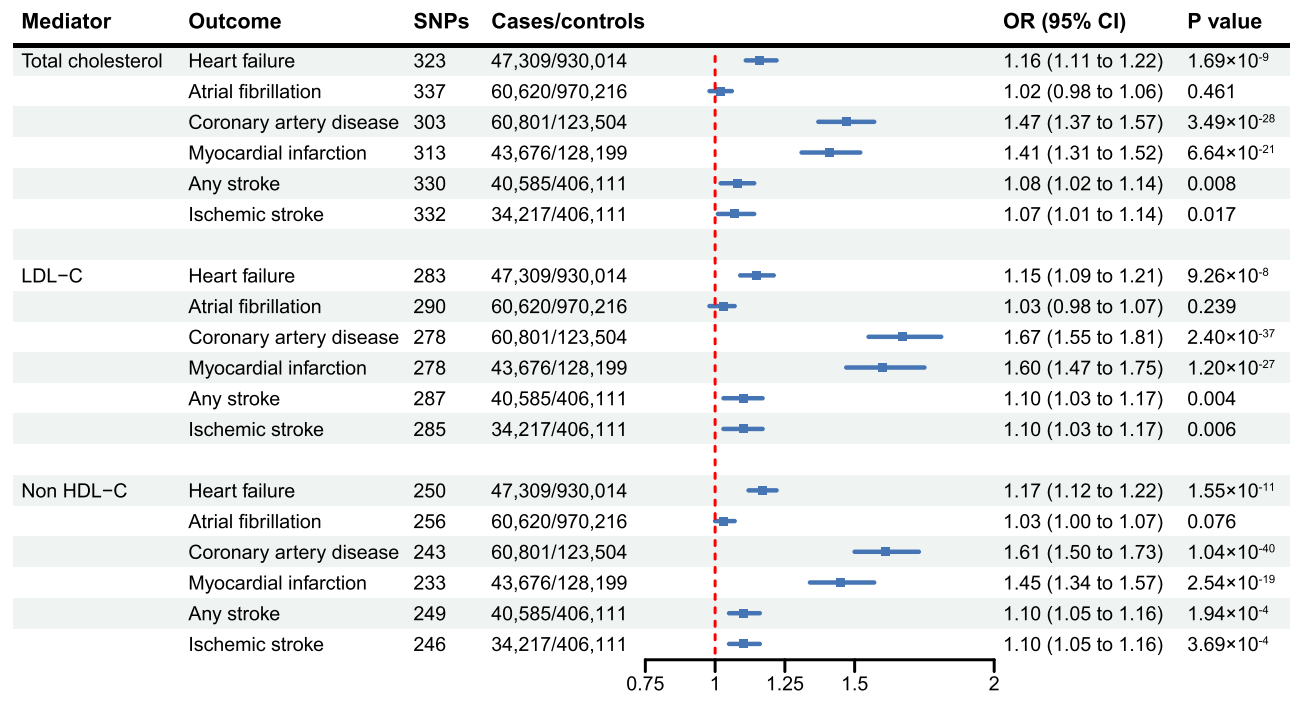
Table 2 MR estimates of the effect of genetically predicted SGLT2 inhibition on blood lipids

Mediator	Methods	OR (95% CI)	P value	Q statistic*	P_h	Egger intercept	$P_{intercept}$
Total cholesterol	Inverse variance weighted	0.91 (0.86–0.96)	2.82×10^{-4}	1.182	0.947		
	MR Egger	0.85 (0.5–1.46)	0.588	1.130	0.890	0.001	0.830
	Weighted median	0.94 (0.82–1.08)	0.351				
	Weighted mode	0.95 (0.79–1.15)	0.640				
	MR-PRESSO	0.91 (0.86–0.96)	0.015	1.801	0.942		
LDL-C	Inverse variance weighted	0.88 (0.82–0.93)	6.22×10^{-5}	1.572	0.905		
	MR Egger	1.06 (0.6–1.85)	0.860	1.128	0.890	−0.002	0.542
	Weighted median	0.90 (0.78–1.03)	0.122				
	Weighted mode	0.91 (0.76–1.08)	0.317				
	MR-PRESSO	0.88 (0.82–0.93)	0.010	2.066	0.925		
Non-HDL-C	Inverse variance weighted	0.83 (0.78–0.88)	2.18×10^{-9}	0.854	0.973		
	MR Egger	0.83 (0.41–1.67)	0.625	0.854	0.931	9.02351E-05	0.985
	Weighted median	0.81 (0.68–0.97)	0.024				
	Weighted mode	0.80 (0.63–1.01)	0.122				
	MR-PRESSO	0.83 (0.78–0.88)	0.002	1.335	0.971		
HDL	Inverse variance weighted	1.00 (0.91–1.09)	0.956	3.162	0.675		
	MR Egger	1.43 (0.83–2.47)	0.271	1.435	0.838	−0.005	0.259
	Weighted median	0.97 (0.83–1.12)	0.664				
	Weighted mode	0.96 (0.79–1.15)	0.650				
	MR-PRESSO	1.00 (0.91–1.09)	0.958	4.457	0.703		
Triglyceride	Inverse variance weighted	1.04 (0.89–1.22)	0.621	9.418	0.094		
	MR Egger	0.53 (0.30–0.92)	0.087	3.404	0.493	0.009	0.070
	Weighted median	0.93 (0.79–1.08)	0.345				
	Weighted mode	0.92 (0.78–1.09)	0.391				
	MR-PRESSO	1.04 (0.89–1.22)	0.642	14.114	0.126		

CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein-cholesterol; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; non-HDL-C, non- high-density lipoprotein cholesterol; OR, odds ratio; P_h, P value for heterogeneity; $P_{intercept}$, P value for intercept of MR-Egger regression.
*Heterogeneity test in the IVW methods was through use of Cochran's Q statistic and for the MR-PRESSO method the global test.

Figure 3 The causal effect of three blood lipids on CVDs. The OR and 95% CI indicate the effect estimates of a decrease in T2D and CVDs per SD unit (6.75 mmol/mol or 1.09%) lowering of HbA1c via SGLT2 inhibition by the inverse-variance weighted method. CI, confidence interval; CVD, cardiovascular diseases; HbA1c, glycated haemoglobin; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; OR, odds ratio; SGLT2, sodium-glucose cotransporter 2.

Mediator	Outcome	SNPs	Cases/controls	OR (95% CI)	P value
Total cholesterol	Heart failure	323	47,309/930,014	1.16 (1.11 to 1.22)	1.69×10^{-9}
	Atrial fibrillation	337	60,620/970,216	1.02 (0.98 to 1.06)	0.461
	Coronary artery disease	303	60,801/123,504	1.47 (1.37 to 1.57)	3.49×10^{-28}
	Myocardial infarction	313	43,676/128,199	1.41 (1.31 to 1.52)	6.64×10^{-21}
	Any stroke	330	40,585/406,111	1.08 (1.02 to 1.14)	0.008
	Ischemic stroke	332	34,217/406,111	1.07 (1.01 to 1.14)	0.017
LDL−C	Heart failure	283	47,309/930,014	1.15 (1.09 to 1.21)	9.26×10^{-8}
	Atrial fibrillation	290	60,620/970,216	1.03 (0.98 to 1.07)	0.239
	Coronary artery disease	278	60,801/123,504	1.67 (1.55 to 1.81)	2.40×10^{-37}
	Myocardial infarction	278	43,676/128,199	1.60 (1.47 to 1.75)	1.20×10^{-27}
	Any stroke	287	40,585/406,111	1.10 (1.03 to 1.17)	0.004
	Ischemic stroke	285	34,217/406,111	1.10 (1.03 to 1.17)	0.006
Non HDL−C	Heart failure	250	47,309/930,014	1.17 (1.12 to 1.22)	1.55×10^{-11}
	Atrial fibrillation	256	60,620/970,216	1.03 (1.00 to 1.07)	0.076
	Coronary artery disease	243	60,801/123,504	1.61 (1.50 to 1.73)	1.04×10^{-40}
	Myocardial infarction	233	43,676/128,199	1.45 (1.34 to 1.57)	2.54×10^{-19}
	Any stroke	249	40,585/406,111	1.10 (1.05 to 1.16)	1.94×10^{-4}
	Ischemic stroke	246	34,217/406,111	1.10 (1.05 to 1.16)	3.69×10^{-4}

IS (OR, 1.10; 95% CI, 1.5–1.16; $P = 3.69 \times 10^{-4}$) (Supporting Information, *Table* S9 and *Figure 4*). These results were supported by the MR-PRESSO method. Although heterogeneity existed, there was no horizontal pleiotropy (all *P* values of intercept > 0.05).

The two-step MR was performed to explore whether the association between SGLT2 inhibition and the risk of HF, CAD, MI, AS, and, IS were mediated through TCH, LDL-C, and non-HDL-C (Supporting Information, *Table* S10). Our results indicated that the proportion mediated of the mediating effect of TCH was 1.7% (OR 0.99 [95% CI 0.98, 0.99], $P = 0.004$), 4.7% (OR 0.96 [0.95, 0.98], $P = 0.002$), and 2.7% (OR 0.97 [0.95, 0.98], $P = 0.002$) in the association between SGLT2 inhibition and the risk of HF, CAD, and MI, respectively. For LDL-C, the proportion mediated of the mediating effect was 2.2% (OR 0.98 [95% CI 0.98, 0.99], $P = 0.003$), 8.6% (OR 0.93 [0.91, 0.95], $P = 5.74 \times 10^{-4}$), and 5.0% (OR 0.95 [0.94, 0.96], $P = 6.97 \times 10^{-4}$) in the association between SGLT2 inhibition and the risk of HF, CAD, and MI, respectively. For non-HDL-C, the proportion mediated of the mediating effect was 3.4% (OR for HF 0.98 [95% CI 0.97, 0.98], $P = 4.42 \times 10^{-6}$), 11.8% (OR for CAD 0.92 [0.90, 0.93], $P = 7.23 \times 10^{-8}$), 5.7% (OR for MI 0.94 [0.92, 0.95], $P = 8.17 \times 10^{-7}$), 1.5% (OR for AS 0.98 [0.98, 0.99], $P = 0.001$), and 1.4% (OR for IS 0.98 [0.98, 0.99], $P = 0.004$).

Discussion

Principal findings

In the present MR study, we identified the causal role of SGLT2 inhibition on cardiovascular disease outcomes. Furthermore, we investigated the mediating role of several blood lipids in the association between SGLT2 inhibition and CVDs. Our study showed that genetically predicted SGLT2 inhibition was associated with a lower risk of HF, AF, CAD, MI, AS, and IS. The mediation MR analysis further suggested that SGLT2 inhibition may influence CVDs via three blood lipids. TCH and LDL-C were estimated to mediate the effect of SGLT2 inhibition on HF (2% for TCH and 2% for LDL-C), CAD (5% for TCH and 9% for LDL-C), and MI (3% for TCH and 5% for LDL-C), whereas non-HDL-C was estimated to mediate the effect of SGLT2 inhibition on HF (3%), CAD (12%), MI (6%), AS (1%), and IS (1%).

The association between sodium-glucose cotransporter 2 inhibition and cardiovascular diseases

In a large clinical trial involving 7020 patients with T2D and CVDs, Zinman and colleagues[4] reported that patients treated

Figure 4 The causal evidence is summarized from the two-sample two-step Mendelian randomization analysis. LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; SGLT2, sodium-glucose cotransporter 2; TCH, total cholesterol.
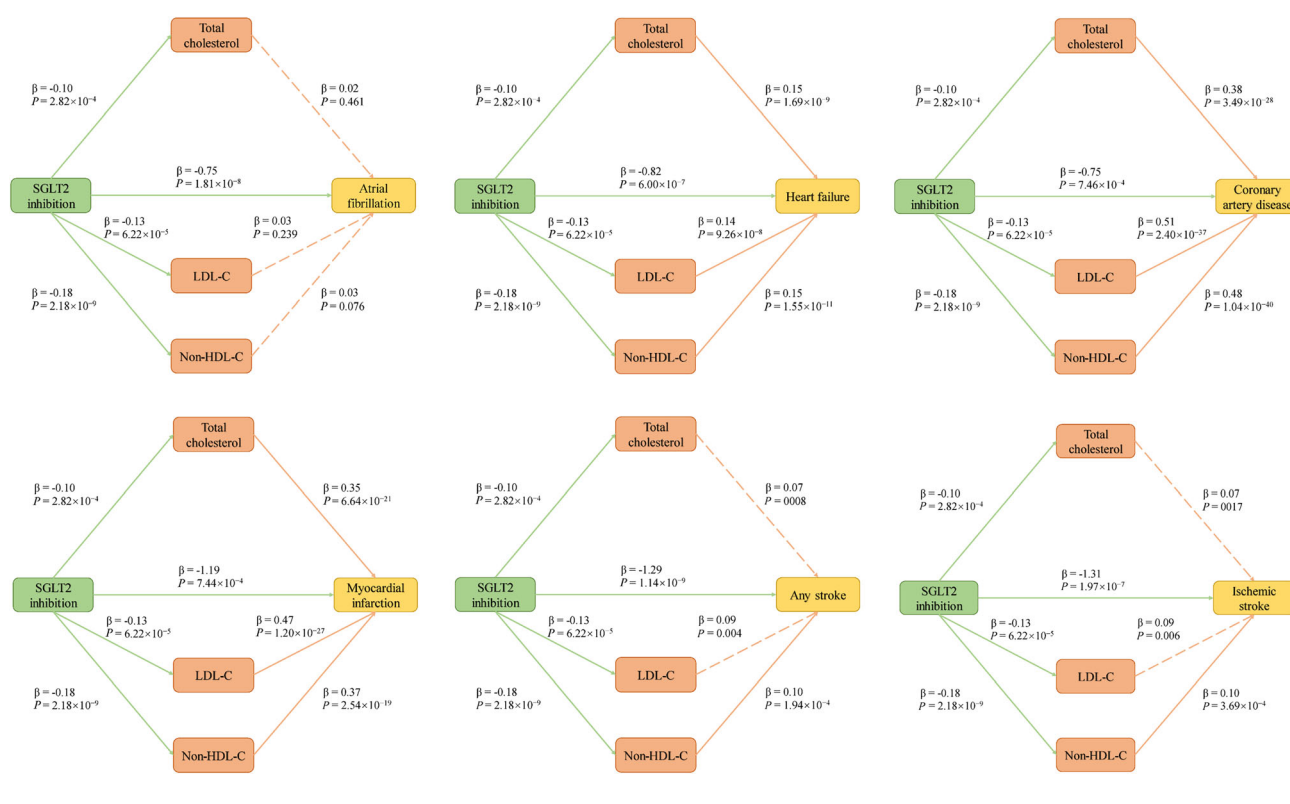
with empagliflozin had a lower rate of major adverse cardiovascular events, including death from cardiovascular causes, non-fatal myocardial infarction, and non-fatal stroke. Furthermore, in the EMPA-REG OUTCOME trial, the Canagliflozin Cardiovascular Assessment Study Program, and the Dapagliflozin Effect on Cardiovascular Events–Thrombolysis in Myocardial Infarction 58 trial, the relative risk reduction of heart failure hospitalization in patients with T2D treated with SGLT2 inhibitors was 25–35%.[3,32,33] Zelniker et al.[34] reported that although robust reductions in heart failure hospitalization were seen regardless of baseline atherosclerotic risk category or a history of HF, SGLT2 inhibitors had a moderate benefit on atherosclerotic major adverse cardiovascular events that appeared to be restricted to patients with established atherosclerotic cardiovascular disease. In addition, a very recent meta-analysis of 43 RCTs involving 79 504 patients with type 2 diabetes reported that the use of SGLT2 inhibitors was associated with a reduction in cardiovascular mortality and all-cause mortality, but patients treated with SGLT2 inhibitors did not have a significantly reduced risk of developing acute coronary syndrome or ischaemic stroke compared with controls.[35] Whether SGLT2 inhibitors result in a reduction in progression to CVDs is yet to be confirmed. Our study provided timely and strong evidence of the beneficial effect of SGLT2 inhibition on CVDs in the general population by using

a set of robust genetic instruments for SGLT2 inhibition and large GWASs for HF, AF, CAD, MI, AS, and IS. Several possible mechanisms have been proposed for the protection against CVD by SGLT2 inhibitors, including the modulation of risk factors and off-target effects on the heart and vasculature.[6,36] The former includes glucose control, weight loss, and effects on plasma lipids.[37] The latter refers to the improvement of ventricular compliance and myocardial fibrosis, inhibition of myocardial Na^+/H^+ exchange, and reduction of myocardial apoptosis.[6,38]

The mediation effect of blood lipids in the association between sodium-glucose cotransporter 2 inhibition and cardiovascular diseases

Few studies have investigated the molecular mechanisms underlying the effect of SGLT2 inhibition on CVD risk prevention. In a clinical trial involving 7020 patients with T2D and established CVDs, it was suggested that the benefit of empagliflozin on cardiovascular death and heart failure hospitalization was independent of HbA1c levels before and during therapy.[39] This indicated that there might be other metabolic pathways mediating the effects of SGLT2 inhibitors on CVDs.

The effects of SGLT2 inhibitors on blood lipids have been reported in a large number of studies.[40,41] Canagliflozin has been reported to decrease the level of Hmgcr and therefore reduce the synthesis of cholesterol.[8] It was reported that canagliflozin facilitated biliary and faecal cholesterol excretion and improved blood lipids, which may be a partial reason for improving CVD.[42] In addition, a retrospective study by Calapkulu et al.[9] showed that 6 months of dapagliflozin treatment in patients with T2D reduced levels of TCH, LDL-C, and triglyceride. However, a meta-analysis of 48 randomized controlled trials showed that SGLT2 inhibitors significantly increased TCH, LDL-C, non-HDL-C, and HDL-C, and decreased TG levels in patients with T2D.[11] Basu et al.[43] reported that SGLT2 inhibitors reduced the expression of angiopoietin-like protein 4, which is a known inhibitor of lipoprotein-lipase in white and brown adipose, skeletal muscle, and heart tissues. An increased lipoprotein-lipase activity may lead to an increase in the LDL-C levels. In addition, several studies reported no significant change in the lipid profile following SGLT2 treatment.[10,44] Bosch et al.[45] revealed that empagliflozin had no significant effect on TCH, LDL-C, and HDL-C levels in a clinical trial of 58 patients with T2D. Inconsistencies in these studies may be related to small sample size, retrospective design, potential residual confounding, or a short period of follow-up. In the present study, we investigated the causal effect of SGLT2 inhibitors on lipid metabolism by using a set of robust genetic proxies for SGLT2 inhibition as the instrument variables (IVs) and the largest blood lipid GWAS to date. We found that SGLT2 inhibition had a significant effect on three blood lipids, including TCH, LDL-C, and non-HDL-C. SGLT2 inhibition significantly decreased the concentration of TCH, LDL-C, and non-HDL-C. The results might broaden our understanding of the metabolic mechanism of SGLT2 inhibition in influencing various CVDs.

Our results confirmed that genetically predicted TCH, LDL-C, and non-HDL-C were associated with an increased risk of HF, CAD, and MI, which were consistent with previous findings from a recent MR study[46] and several cohort studies.[16,17,47] Furthermore, only non-HDL-C had a positive association with AS and IS. In a large cohort study of 2 682 045 young adults (aged 20–39 years), elevated TCH levels were significantly associated with an increased risk of ischaemic heart disease and cerebrovascular disease.[48] Atherosclerosis, a chronic inflammatory disease, has been proposed to be triggered by the accumulation of LDL-C in the arterial wall and is the major cause of CVDs.[14,49] In addition to LDL-C, non-HDL-C was also reported to be associated with atherosclerosis, which involved several atherogenic constituents.[50,51] Therefore, TCH, LDL-C, and non-HDL-C may mediate the causal effect of SGLT2 inhibition on CVDs. Our study provided genetic evidence that TCH, LDL-C, and non-HDL-C mediated the protective effect of SGLT2 inhibition on HF, CAD, and MI. In addition, non-HDL-C also mediated the effect of SGLT2 inhibition on AS and IS. However, the results did not support that TC, LDL-C, and non-HDL-C mediated the protective effect of SGLT2 inhibition on AF, which was consistent with a previous report.[52] Nevertheless, it is worth noting that the mediation association observed might not be causal and needs to be further validated through experimental studies.

Strengths and limitations

The present MR study has several strengths. First, we used the genetic target of SGLT2 inhibition as the IVs of our exposure, which fits well with the MR design of estimating the effect of a single drug target. Second, there are non-overlapping samples of GWAS for SGLT2 inhibition, blood lipid traits, and CVDs, which avoided introducing associations between instruments and confounders. In addition, the genetic variants for SGLT2 inhibition and blood lipids in this study showed strong power (all F-statistics > 23), suggesting that weak instrument bias was unlikely to be an issue in this study. Third, the sample size of blood lipid traits was >930 000 participants, while the CVD data were derived from the largest GWAS studies to date, which guaranteed the statistical power of the findings and conclusions of the study. Finally, we investigated the association between SGLT2 inhibition, blood lipids, and CVDs in the general population and provided genetic evidence for the potential mechanism of SGLT2 inhibition exerting a beneficial effect on CVD through TCH, LDL-C, and non-HDL-C.

Our study has several limitations. First, only a limited number of blood lipids were included in this study, which did not cover other lipid subfractions. Second, the genetically predicted drug effects may differ somewhat from the real-world therapeutic practice. An exposure instrumented by genetic variants is present from birth and continues throughout life. Our analyses can therefore be interpreted as assessing long-term modulatory effects of drug targets. Furthermore, because genetic effects are lifelong, our estimates may not truly reflect the effects of exposure to anti-diabetic drugs during a given period of life. Third, because we only used the genetic summary data restricted to the population of European ancestry, the generalizability of our results would be limited to populations of European ancestry. Finally, this study did not involve epigenetics and genomic imprinting, which could modify the interpretation of our results. A meta-analysis by Singh et al.[53] found that men receiving SGLT2 inhibitors had a significant reduction in major adverse cardiac outcomes while women did not. Biological sex and sex hormones influence the expression of renal SGLT2,[54] which may suggest a sex difference in response to SGLT2 inhibitors. Further study was needed to investigate the different effects of SGLT2 inhibition between males and females.

Conclusions

In conclusion, this study supports the association between genetically predicted SGLT2 inhibition, blood lipids, and CVDs. Specifically, TCH, LDL-C, and non-HDL-C mediated the protective effect of SGLT2 inhibition on HF, CAD, and MI. In addition, non-HDL-C mediated the protective effect of SGLT2 inhibition on AS and IS. These findings provide genetic evidence for the mechanisms of SGLT2 inhibition in reducing CVD risk and may inform future mechanistic and clinical studies.

Conflict of interest

The authors declare no competing interests.

Data availability statement

The GWAS summary statistics used in our study were publicly accessed from the IEU Open-GWAS database (https://gwas.mrcieu.ac.uk/), the GTEx Portal (https://www.gtexportal.org/), the eQTLGen Consortium (https://eqtlgen.org/), the FinnGen consortium (https://www.finngen.fi/en), the HERMES consortium (https://www.hermesconsortium.org/), the CARDIoGRAMplusC4D consortium (http://www.cardiogramplusc4d.org/), and the MEGASTROKE consortium (http://www.megastroke.org/). The MEGASTROKE project received funding from sources specified at http://www.megastroke.org/acknowledgments.html.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. The causal effect of SGLT2 inhibition on five blood lipids. The OR and 95% CI indicate the effect estimates of a decrease in each blood lipid per SD unit (6.75 mmol/mol or 1.09%) lowering of HbA1c via SGLT2 inhibition by the inverse-variance weighted method. CI: confidence interval; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; OR: odds ratio; SGLT2, sodium-glucose cotransporter 2.
Table S1. The genetic variants selected for the instrument variables of SGLT2 inhibition.
Table S2. Data sources in mendelian randomization analysis.
Table S3. The genetic variants selected for instrumental variables of total cholesterol.
Table S4. The genetic variants selected for instrumental variables of low-density lipoprotein cholesterol.
Table S5. The genetic variants selected for instrumental variables of non-high-density lipoprotein cholesterol.
Table S6. Statistical power for the Mendelian randomization analysis.
Table S7. MR estimates of the effect of total cholesterol on cardiovascular diseases.
Table S8. MR estimates of the effect of low-density lipoprotein cholesterol on cardiovascular diseases.
Table S9. MR estimates of the effect of non-high-density lipoprotein cholesterol on cardiovascular diseases.
Table S10. Mediated Mendelian randomization analysis.

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