Research article

Genetic proxy of lipid-lowering drugs and calcific aortic valve stenosis: A Mendelian randomization study

Yucheng Hou [a,1], Jingwei Zhao [b,1], Wanchuang Xu [a,c], Lei Chen [a], Jingyue Yang [a,c], Ziheng Wang [d,e,f,*], Ke Si [a,**]

[a] *Department of Cardiovascular Surgery, The First Affiliated Hospital of Soochow University, Suzhou, China*
[b] *Department of General Surgery, Xinhua Hospital, Affiliated to Shanghai Jiao Tong University School of Medicine & Shanghai Key Laboratory of Biliary Tract Disease Research, Shanghai, China*
[c] *Suzhou Medical College, Soochow University, Suzhou, China*
[d] *MOE Frontier Science Centre for Precision Oncology, University of Macau, Macau SAR, China*
[e] *The School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia*
[f] *Suzhou Industrial Park Monash Research Institute of Science and Technology, Suzhou, China*

ARTICLE INFO

ABSTRACT

Background: Lipid metabolism plays an important role in the pathogenesis and development of calcific aortic valve stenosis. Our aim was to evaluate the causal effect of lipid-lowering drugs, such as low-density lipoprotein cholesterol (LDL-C) lowering and triglyceride lowering drugs, on the outcome of aortic valve stenosis using a two-sample Mendelian randomization (MR) study.
Methods: We used two genetic tools to represent the exposure of lipid-lowering drugs, including expression quantitative trait loci for the expression of drug target genes, and genetic variants within or near drug target genes that are associated with LDL-C and triglyceride concentrations from Genome-Wide Association Studies (GWAS). Effect estimates were calculated using summary-data-based MR (SMR) and inverse-variance-weighted MR (IVW-MR) analysis.
Results: Based on the results of SMR and IVW-MR analysis, LDL-C-lowering PCSK9 inhibitors have potential in reducing the risk of aortic valve stenosis (for SMR, OR: 1.044; 95%CI: 1.002–1.404; $P = 0.047$; for IVW-MR, OR: 1.647, 95%CI: 1.316–2.062, $P < 0.001$). However, no significant association was observed between triglyceride target gene expression, as well as triglyceride-lowering drugs, and aortic valve stenosis.
Conclusion: This two-sample drug-targeted MR study suggests a potential causal relationship between PCSK9 inhibitors and the reduction of calcific aortic valve stenosis risk.

1. Introduction

Calcific aortic valve stenosis (CAVS) represents the most prevalent type of heart valve disease [1]. Clinically, it often presents with three common symptoms: dyspnea, angina pectoris, and syncope. As calcific deposits worsen on the valve, CAVS can potentially trigger

---

* Corresponding author.MOE Frontier Science Centre for Precision Oncology, University of Macau, SAR Macau, China.
** Corresponding author. Department of Cardiovascular Surgery, The First Affiliated Hospital of Soochow University, No.899, Pinghai Road, Suzhou, China.
E-mail addresses: wang.ziheng@connect.um.edu.mo (Z. Wang), sike@suda.edu.cn (K. Si).
[1] Yucheng Hou and Jingwei Zhao have contributed equally to this work.

arrhythmias, heart failure, and even sudden death [2]. This ailment poses a considerable burden, especially among the elderly. Over the past three decades, aligned with the aging trend, the incidence and prevalence of CAVS have increased by 3.51 and 4.43 times, respectively [3]. Once valve degeneration sets in, a substantial proportion of patients progress to severe aortic valve stenosis (AVS) [4]. The two-year mortality rate for symptomatic severe CAVS can even reach up to 50 % [5]. Unfortunately, there is a lack of effective drugs that can prevent the CAVS process. Most drugs are only used for the treatment of end-stage heart failure caused by CAVS, which incurs an annual economic burden of approximately 30,000 dollar per patient [6]. Aortic valve replacement, as the most commonly used treatment for CAVS, whether through open surgery or transcatheter intervention, has a high treatment cost of around 60,000 dollars [7]. These statistics highlight the enormous burden of CAVS on society and healthcare systems, emphasizing the imperative need for devising novel and effective therapeutic strategies.

Exploring the underlying mechanisms driving the development of CAVS holds the key to identifying drug targets capable of arresting its procession. CAVS is characterized by fibrocalcific remodeling and thickening of the aortic valve leaflets, which subsequently leads to left ventricular outflow obstruction. This process involves multiple pathophysiological mechanisms, with lipid deposition being a critical step at the onset of CAVS. Under the influence of blood flow shear stress and tensile stress, endothelial cells within the valve undergo damage, leading to infiltration of low-density lipoprotein cholesterol (LDL-C). TG-rich lipoproteins have been thought to increase the risk of CAVS in recent years although the exact mechanism is unclear [8]. Abnormally increased reactive oxygen species cause oxidative stress of lipids. Oxidized lipids activate cellular immunity and inflammation by promoting immune cell adhesion [9,10]. Subsequently, cell differentiation processes, such as epithelial-mesenchymal transition, myofibroblast differentiation, and osteoblast differentiation, proceed sequentially [4]. Finally, these interwoven mechanisms culminate in fibrous proliferation and calcification deposition within the valve tissue, thereby exacerbating CAVS over time.

Despite LDL-C being key factors in the initiation of CAVS, the outcomes of clinical trials aiming to reduce LDL-C levels have been less than satisfactory. Studies have indicated that statin-based treatments, such as atorvastatin or rosuvastatin, neither prevent the progression of CAVS nor facilitate its reversal [11,12]. Furthermore, combined intensive lipid-lowering therapy with simvastatin and ezetimibe has also failed to improve critical outcome of CAVS [13]. The array of lipid-lowering drugs encompassed fibric acid derivatives, bile acid sequestrants, Mipomersen, and several others. Conducting large-scale clinical research on lipid-lowering therapy for CAVS requires significant financial support but also needs to consider the challenges of screening early CAVS patients and accounting for various confounding factors such as family genetics, lifestyle habits, external environment, and comorbidities. These challenges make conducting clinical trials on lipid-lowering drugs for CAVS patients into predicament.

Mendelian randomization (MR) study is a novel approach that can be used to analyze the causal relationship between exposure factors and outcome events. Given that genes on non-homologous chromosomes undergo random segregation during meiosis in gametes, the transmission of genotypes is inherently stochastic. Genetic variations tied to exposure arise prior to birth, and by leveraging these as instrumental variables, the assessment of causality between exposure and outcome becomes insulated from common confounding elements. This approach also offers the advantage of circumventing reverse causality issues.

In this research, we conducted a two-sample drug-targeted MR analysis. We harnessed publicly available expression quantitative trait loci (eQTL) and GWAS datasets to identify appropriate genetic tools for target genes of lipid-lowering drugs. By integrating genetic proxies of various lipid-lowering agents with GWAS data related to CAVS through two different MR methods, we aim to decipher the causal association between lipid-lowering drugs and CAVS risk.

2. Methods

2.1. Study design

This two-sample MR study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) guidelines [14]. The data utilized in this research originated from publicly available summary-level datasets obtained from GWAS and eQTLs. Further details regarding these datasets were presented (see Table S1). The relevant Institutional Review Boards approved these original studies, and participants provided informed consent. Additional approvals and informed consent were deemed unnecessary.

2.2. Genetic variant selection

Based on current dyslipidemia management guidelines, a comprehensive selection of lipid-lowering drugs and innovative therapeutics was made, including statins, ezetimibe, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, bile acid sequestrants, mipomersen, fibrates, angiopoietin-like 3 (ANGPTL3) inhibitors, and antisense oligonucleotide targeting apolipoprotein C-III (APOC3) mRNA therapies [15,16]. To pinpoint the genes associated with the pharmacological targets of these drugs, the DrugBank database (https://go.drugbank.com/) and pertinent relevant reviews were consulted [17]. For a more granular classification, these target genes were segregated into LDL-C-lowering target genes (such as LDLR, HMGCR, NPC1L1, PCSK9, APOB, and ABCG5/ABCG8) and TG-lowering target genes (like LPL, PPARA, ANGPTL3, and APOC3).

We used available eQTLs linked to drug target genes as proxies to represent exposure to each drug target. These genetic variants served as proxies for exposure to lipid-lowing drugs. The data for these variants were sourced from the eQTLGen Consortium (accessible at https://www.eqtlgen.org/) [18] and the Genotype-Tissue Expression (GTEx) Consortium V8 (accessible at https://gtexportal.org/). Our analysis focused on identifying shared gene variants, specifically single nucleotide polymorphisms (SNPs) that have a minor allele frequency (MAF) higher than 1 % and a significant association ($P < 5E\text{-}08$) with gene expression in

Table 1
Information of genetic instruments.

Exposure		eQTLs	lipid level
LDL-C	LDLR	18 SNPs *cis*-eQTLs (MAF >1 %) in blood ($P <$ 5E-08), top SNP: rs8110515	10 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with LDL-C ($P <$ 5E−08), located within ±100 kb windows from LDLR region
	HMGCR	921 SNPs *cis*-eQTLs (MAF >1 %) in blood ($P <$ 5E-08), top SNP: rs6453133	7 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with LDL-C ($P <$ 5E−08), located within ±100 kb windows from HMGCR region
	NPC1L1	11 SNPs *cis*-eQTLs (MAF >1 %) in adipose subcutaneous tissue ($P <$ 5E-08), top SNP: rs41279633	3 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with LDL-C ($P <$ 5E−08), located within ±100 kb windows from NPC1L1 region
	PCSK9	24 SNPs *cis*-eQTLs (MAF >1 %) in blood ($P <$ 5E-08), top SNP: rs472495	10 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with LDL-C (P < 5E−08), located within ±100 kb windows from PCSK9 region
	APOB	161 SNPs *cis*-eQTLs (MAF >1 %) in adipose subcutaneous tissue ($P <$ 5E-08), top SNP: rs4665179	19 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with LDL-C ($P <$ 5E−08), located within ±100 kb windows from APOB region
	ABCG5/ ABCG8	ABCG8: 14 SNPs *cis*-eQTLs (MAF >1 %) in adipose subcutaneous tissue ($P <$ 5E-08), top SNP: rs78451356 (ABCG5: 0 SNP)	4 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with LDL-C ($P <$ 5E−08), located within ±100 kb windows from ABCG5/ABCG8 region
TG	LPL	1071 SNPs *cis*-eQTLs (MAF >1 %) in blood ($P <$ 5E-08), top SNP: rs3735964	22 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with TG ($P <$ 5E−08), located within ±100 kb windows from LPL region
	PPARA	220 SNPs *cis*-eQTLs (MAF >1 %) in blood ($P <$ 5E-08), top SNP: rs129600	0 SNP
	ANGPTL3	0 SNP	4 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with TG ($P <$ 5E−08), located within ±100 kb windows from ANGPTL3 region
	APOC3	0 SNP	10 common SNPs (MAF >1 %) in low linkage disequilibrium (r2 < 0.30), associated with TG ($P <$ 5E−08), located within ±100 kb windows from APOC3 region

eQTLs, expression quantitative trait loci; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; MAF, minor allele frequency; PCSK9, pro-protein convertase subtilisin/kexin type 9; SNP, single-nucleotide polymorphism.

bloodstream (including LDLR, HMGCR, PCSK9, LPL, or PPARA) and adipose subcutaneous tissue (such as NPC1L1, APOB, or ABCG8) (see Table 1). This emphasis arose due to the limited availability of significant gene variants in blood or other tissues for these particular genes. Notably, ANGPTL3 and APOC3 do not have any gene variants available in blood or other tissues that surpass the significance threshold. For this study, we only considered *cis*-eQTLs, which are gene variants located within a 1 Mb region on both sides of the target gene.

Additionally, we proposed an alternative methodology to validate the observed associations using gene variants in available eQTLs. This approach involved selecting SNPs within a 100 kb region around the target gene for each lipid-lowering drug that demonstrated a significant association ($P <$ 5E-08) with LDL-C or TG levels at a genome-wide level (outlined in Table 1). These selected SNPs served as proxies for representing the exposure to lipid-lowering drugs. We utilized the GWAS summary data of LDL-C and TG levels from the Global Lipids Genetics Consortium (GLGC), which included sample sizes of 173,082 and 177,861, respectively [19]. Only common SNPs (MAF >1 %) were included in this analysis.

2.3. Outcome sources

We obtained the GWAS summary-level data for CAVS from the FinnGen R9 GWAS (accessible at https://r9.finngen.fi/), which is a comprehensive dataset comprising 9153 CAVS cases and 368,124 control subjects of European ancestry [20]. To ensure robust statistical analysis, the model incorporated several covariates, namely sex, age, ten genetic principal components, and genotyping batches. The FinnGen database has collected genomic and health information from 500,000 Finnish Biosample participants, offering profound insights into medical and therapeutic domains while serving as an invaluable resource for future research. This extensive database offers a wealth of opportunities for further research and discovery. In our positive control analysis, the GWAS for coronary heart disease used as outcome was obtained from the IEU open GWAS project (https://gwas.mrcieu.ac.uk/datasets/ieu-a-7/) [21].

2.4. Mendelian randomization analysis

To generate effect estimates, we employed the summary-data-based MR (SMR) method, using eQTLs as instrumental variables [22]. This approach examines the association between the expression level of a gene and the CAVS risk by leveraging summary-level data derived from eQTLs and GWAS data. We used SMR software, version 1.03, for allele harmonization and subsequent analysis. Furthermore, we applied the inverse-variance-weighted MR (IVW-MR) approach to combine effect estimates, using genetic variants linked to LDL-C or TG levels as instrumental variables [23]. TwoSampleMR package in R software, version 4.3.2, was employed regarding allele harmonization and analysis. To assess the strength of SNPs as tools, the F-statistic was used, and only SNPs with an

F-statistic greater than ten were included to minimize weak tool bias, as Burgess and Thompson recommended [24].

Positive control analysis was performed to validate both genetic tools. Specifically, the association between the exposure of interest and lipid level was used as a positive control study for tools derived from eQTLs. For tools derived from lipid level GWAS, the association between the exposure of interest and coronary heart disease was studied as a positive control, as coronary heart disease is the primary indication for lipid-lowering drugs.

2.5. Sensitivity analysis

Within the SMR approach, the Heterogeneity in Instrument Dependence (HEIDI) test was employed to ascertain whether the observed associations between gene expression and outcomes resulted from a chained situation [22]. This test was performed using the SMR software, and the p-value was less than 0.05 suggesting that the association could be due to interlocking [25]. In addition, to assess the potential for horizontal pleiotropy, nearby genes significantly associated with genetic tool variants (within a 1 Mb window) were identified and subjected to SMR analysis to assess whether their expression correlated with the outcome. These rigorous analytical steps assisted in guaranteeing the validity and dependability of the findings derived from MR analysis.

In the IVW-MR approach, we employed the Cochran' Q test to evaluate heterogeneity, with $P < 0.05$ indicating the presence of heterogeneity [26]. To assess the potential horizontal pleiotropy of the SNPs used as instrumental variants, we conducted MR-Egger regression and Mendelian Randomization Pleiotropy Residual Sum and Outliers (MR-PRESSO) analyses. In MR Egger regression, the intercept term serves as an important indicator of directional horizontal pleiotropy, with $P < 0.05$ suggesting evidence of horizontal pleiotropy [27]. The MR-PRESSO analysis identifies horizontal pleiotropic outliers and provides an adjusted estimate, with a global test yielding a p-value less than 0.05, indicating the presence of horizontal pleiotropic outliers [28].

2.6. Statistical analysis

To ensure the accuracy and reliability of our research results, we applied the Bonferroni correction method to adjust the threshold for significance level. Specifically, we believe that $P < 0.05/n$ is strong evidence, and $0.05/n \le P < 0.05$ is suggestive evidence (eight exposures for SMR, n = 8; nine exposures for IVW-MR, n = 9). This correction helps to control the risk of false positive results. We strictly adhere to these statistical principles in all analyses.
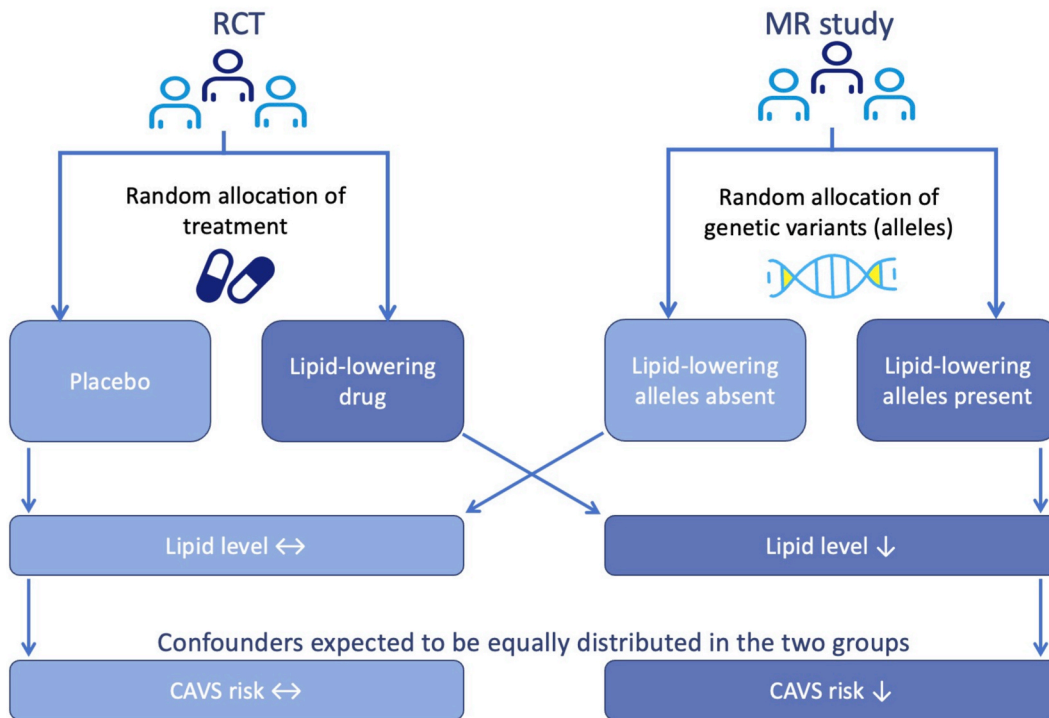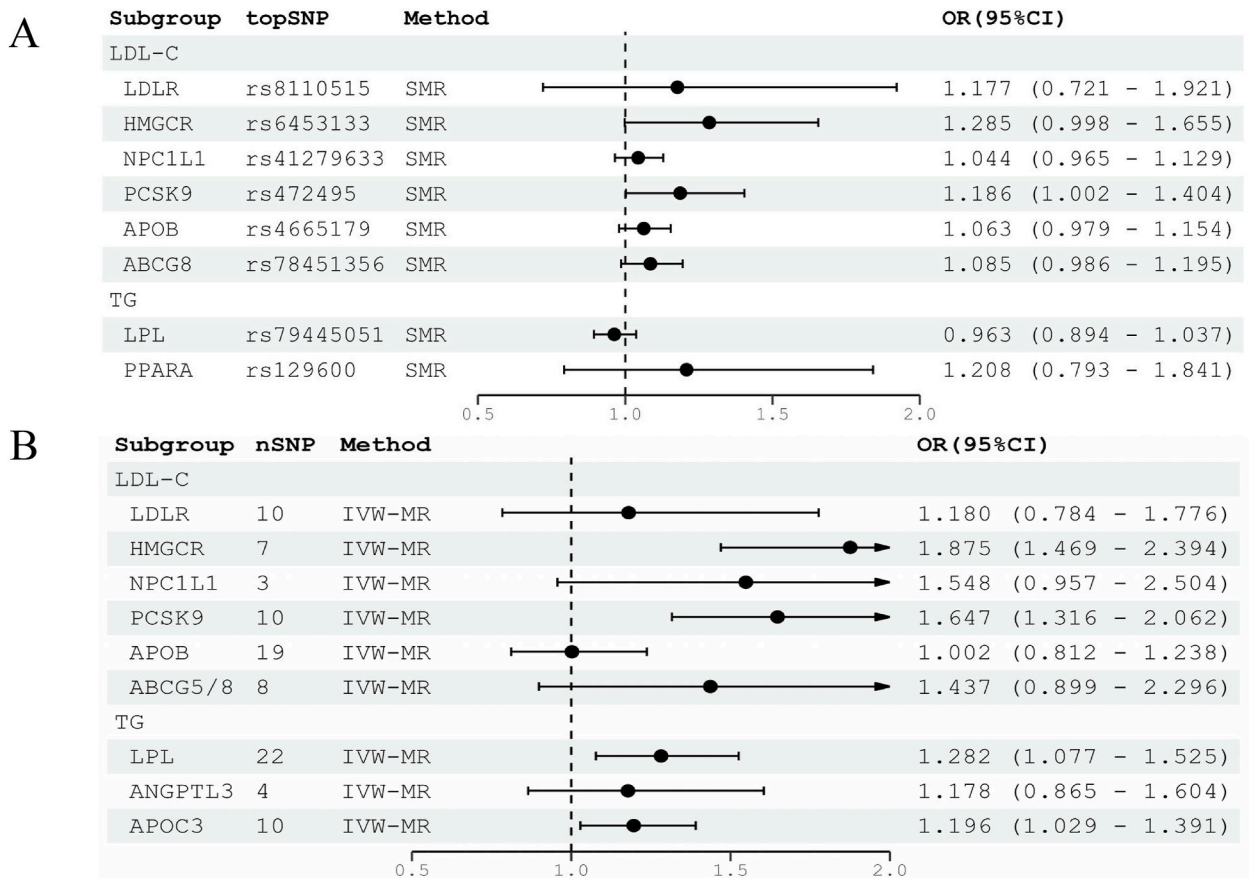

Fig. 1. Mendelian randomization estimates for the associations between lipid-lowering drugs and calcific aortic valve stenosis. (A) SMR association between expression of each gene and aortic valve stenosis; (B) IVW-MR association between LDL-C mediated or TG-mediated by targeted gene and the outcome. OR indicates odds ratio. 95%CI indicates 95 % confidence interval. SMR, summary-data-based Mendelian randomization; CAVS, calcific aortic valve stenosis; IVW-MR, inverse-variance-weighted Mendelian randomization; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.

A

Subgroup	topSNP	Method		OR(95%CI)
LDL-C				
LDLR	rs8110515	SMR		1.177 (0.721 - 1.921)
HMGCR	rs6453133	SMR		1.285 (0.998 - 1.655)
NPC1L1	rs41279633	SMR		1.044 (0.965 - 1.129)
PCSK9	rs472495	SMR		1.186 (1.002 - 1.404)
APOB	rs4665179	SMR		1.063 (0.979 - 1.154)
ABCG8	rs78451356	SMR		1.085 (0.986 - 1.195)
TG				
LPL	rs79445051	SMR		0.963 (0.894 - 1.037)
PPARA	rs129600	SMR		1.208 (0.793 - 1.841)

B

Subgroup	nSNP	Method		OR(95%CI)
LDL-C				
LDLR	10	IVW-MR		1.180 (0.784 - 1.776)
HMGCR	7	IVW-MR		1.875 (1.469 - 2.394)
NPC1L1	3	IVW-MR		1.548 (0.957 - 2.504)
PCSK9	10	IVW-MR		1.647 (1.316 - 2.062)
APOB	19	IVW-MR		1.002 (0.812 - 1.238)
ABCG5/8	8	IVW-MR		1.437 (0.899 - 2.296)
TG				
LPL	22	IVW-MR		1.282 (1.077 - 1.525)
ANGPTL3	4	IVW-MR		1.178 (0.865 - 1.604)
APOC3	10	IVW-MR		1.196 (1.029 - 1.391)

Fig. 2. Mendelian randomization studies are considered a "natural" randomized controlled trial study. Because genetic variations are randomly allocated at conception, using genetic variants as a substitute for lipid-lowering drugs can minimize confounding and reverse causality. RCT, randomized controlled trial; MR, Mendelian randomization; CAVS, calcific aortic valve stenosis.

3. Results

3.1. Genetic instrument selection

We chose the most significant *cis*-eQTL SNPs as the genetic instruments for each drug target gene (refer to Table 1). Notably, no SNPs were identified for the genes ANGPTL3 and APOC3. From the GLGC GWAS summary data on LDL-C levels, SNPs within or nearby genes LDLR, HMGCR, NPC1L1, PCSK9, APOB, and ABCG5/8 were selected, with counts of 10, 7, 3, 10, 19, and 4, respectively (refer to Table 1 and Table S2). Similarly, for the TG GWAS data, we chose SNPs within or nearby genes LPL, ANGPTL3, and APOC3, with 22, 4, and 10 SNPs, respectively. No SNPs were found to be related to the gene PPARA. In positive control studies, significant associations were observed between exposure to each drug and LDL-C or TG level when utilizing the proposed tools for eQTLs (refer to Table S3; all P values < 0.001). Associations between exposure to each drug and coronary heart disease were also detected when using the proposed tools for GWAS data of LDL-C or TG levels (refer to Table S4; all P values < 0.001), thereby supporting the validity of the selected genetic instruments (see Fig. 1).

3.2. Primary analysis

The primary analysis, as presented in Fig. 2(A) and Table S5, demonstrates a causal relationship between increased PCSK9 gene expression in blood and an increased risk of CAVS (OR: 1.044; 95%CI: 1.002–1.404; $P = 0.047$), based on the results of the SMR analysis. These findings hint at the potential of PCSK9 inhibitors in reducing CAVS risk. However, no significant associations were observed between diminished expression of TG target genes and CAVS outcomes.

Furthermore, as shown in Fig. 2(B) and Table S6, further analysis using IVW-MR revealed a strong association between PCSK9-mediated LDL-C level and the risk of CAVS (OR: 1.647, 95%CI: 1.316–2.062, $P < 0.001$). These findings strengthen the argument that PCSK9 inhibitors may have a protective effect against CAVS. Likewise, strong evidence was observed linking HCGMR-mediated LDL-C (OR:1.875; 95%CI: 1.469–2.394; $P < 0.001$) and LPL-mediated TG (OR: 1.282; 95%CI: 1.077–1.525; $P = 0.005$) to the risk of

CAVS. Moreover, suggestive evidence was observed linking APOC3-mediated TG to the risk of CAVS (OR: 1.196; 95%CI: 1.029–1.391; $P = 0.020$). However, the IVW-MR analysis did not provide any evidence to support an association between CAVS risk and NPC1L1-mediated LDL-C, LDLR-mediated LDL-C, APOB-mediated LDL-C, ABCG5/8-mediated LDL-C, as well as ANGPTL3-mediated TG.

3.3. Sensitivity analysis

Regarding sensitivity analysis, the results from the HEIDI test indicate that none of the observed associations in the SMR analysis can be attributed to linkage ($P > 0.05$), as detailed in Table S5. Additionally, findings from the Cochran's Q test within the IVW-MR analysis revealed the presence of heterogeneity in SNPs specifically in the analysis of drug targets LDLR and APOB ($P < 0.05$). Conversely, no such heterogeneity was detected in SNPs related to the remaining targets ($P > 0.05$; refer to Table S6). Furthermore, both MR-Egger regression and MR-PRESSO analyses collectively indicate the absence of significant pleiotropy (at least one $P > 0.05$; see Table S4), except for SNPs related to APOB (where P values for both MR-Egger intercept and MR-PRESSO Global test are less than 0.05).

4. Discussion

This paper utilizes two distinct MR analysis methods to investigate the existence of a causal relationship between several lipid-lowering drug targets and CAVS. The findings of the SMR analysis for gene PCSK9 expression and its relationship with CAVS point towards a potential causal link. When using SNPs related to LDL-C levels in the PCSK9 gene region as instrumental variables, the IVW-MR results indicate that the causal relationship between the two is significant after adjustment. Both MR methods suggest that PCSK9 is a risk factor for CAVS, highlighting the potential protective role of PCSK9 inhibitors in CAVS treatment. The other lipid-lowering drug targets did not show statistically significant results in both MR analyses.

PCSK9, an enzyme secreted by the liver, forms a complex with LDL-C and LDL-receptor (LDL-R), mediating the endocytosis and degradation of LDL-R within lysosomes. This process leads to a reduction in recycled LDL-R, affecting the clearance of LDL-C and increasing LDL-C levels. When PCSK9 is inhibited, LDL-R binds to LDL-C on the surface of hepatocytes and transfers it into lysosomes within the cell, causing degradation of LDL-C in the plasma and recycling of LDL-C back to the cell surface to bind new LDL-C [29]. Prospective cross-sectional studies have indicated that high levels of PCSK9 in the plasma are associated with CAVS, although not with severity [30]. In vitro experiments have observed a significant elevation in PCSK9 expression levels in human calcific aortic valve tissue [31]. Further animal experiments have shown that knocking out PCSK9 significantly reduces the incidence of aortic valve calcification in mice [32]. Similarly, loss-of-function mutations of PCSK9 R46L in humans have also been found to mitigate the risk of CAVS [33]. Consequently, therapy with PCSK9 inhibitors has emerged as a promising avenue for treating CAVS.

The rationale for treating CAVS with PCSK9 inhibitors is often attributed to the reduced lipid levels such as LDL-C and lipoprotein a [Lp(a)]. PCSK9 inhibitors can reduce circulating LDL-C concentrations by 50 %–60 % and Lp(a) by 20 %–30 % [34]. In some current studies, it is believed that loss-of-function mutations of PCSK9 are only related to LDL-C and not Lp(a) [35]. As such, this was considered when selecting instrumental variables in our study. The FOURIER study comprehensively assessed the impact of PCSK9 inhibitor treatment on individuals with high-risk aortic stenosis [36]. The findings revealed that patients who underwent PCSK9 inhibitor therapy exhibited a reduced risk of developing aortic stenosis or undergoing aortic valve replacement. However, it is worth noting that the study's conclusions are drawn from a relatively small sample size. Other clinical trials, such as NCT03051360 and NCT04968509, evaluating the use of PCSK9 inhibitors in treating CAVS, have yet to report their findings. Therefore, there is still a lack of solid evidence supporting the use of PCSK9 inhibitors as a treatment option for CAVS. By using genetic proxies to inhibit the action of PCSK9 on LDL-C, our two MR analyses utilizing a large sample size and simulated randomized controlled methods provide evidence for a causal relationship between PCSK9 and CAVS, suggesting that PCSK9 inhibitors have significant clinical implications as a therapeutic agent for CAVS.

As shown in Fig. 2, the novelty of this research lies in the adoption of a relatively innovative drug-targeted MR analysis method, mimicking the effect of different kinds of inhibitors on lipid levels. This approach offers several advantages:

1 It considerably reduces the costs associated with large-scale clinical trials.
2 It minimizes confounding biases and reverses causality commonly encountered in traditional trials.
3 We can cross-validate our findings using two distinct genetic proxies and enhance confidence in the observed associations.
4 We applied multiple sensitivity analyses to account for potential horizontal confounding.

However, there are limitations to our study:

1 Due to aggregated data, we could not stratify CAVS severity based on PCSK9, limiting our ability to discern dose-dependent relationships and impacts on CAVS progression.
2 After strict FDR correction, the SMR results for PCSK9 and CAVS lost statistical significance, although IVW-MR results remained notable. This does not exclude potential false positives.
3 Genetic variants, serving as exposure proxies, reflect lifelong drug effects, contrasting with short-term pharmacological interventions.

5. .Conclusions

In summary, the genetic proxy approach provides compelling evidence of a potential causal relationship between the use of lipid-lowering drugs and reduced risk of CAVS. Our research findings indicate that PCSK9 inhibitors is the only lipid-lowering drug that is associated with a reduced risk of CAVS using both MR analysis methods.

Data

The original data used in this study are included in Table S1.

Data availability statement

The datasets presented in this study come from the IEU Open GWAS (https://gwas.mrcieu.ac.uk/), FinnGen R9 GWAS (https://r9.finngen.fi/), eQTLGen Consortium (https://www.eqtlgen.org/), and GTEx Consortium V8 (https://gtexportal.org/) are available for free download. Further inquiries can be directed to the corresponding authors. Other data can be accessed via correspondence authors.

CRediT authorship contribution statement

Yucheng Hou: Writing – original draft, Validation, Software, Investigation, Data curation. **Jingwei Zhao:** Writing – original draft, Validation, Software, Investigation, Data curation. **Wanchuang Xu:** Writing – original draft, Investigation. **Lei Chen:** Writing – original draft, Investigation. **Jingyue Yang:** Writing – original draft, Investigation. **Ziheng Wang:** Writing – review & editing, Writing – original draft, Software, Conceptualization. **Ke Si:** Writing – review & editing, Visualization, Validation, Software, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:The author Ziheng Wang is the Associated Editor of Heliyon. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34089.

References

[1] R.L. Osnabrugge, et al., Aortic stenosis in the elderly: disease prevalence and number of candidates for transcatheter aortic valve replacement: a meta-analysis and modeling study, J. Am. Coll. Cardiol. 62 (11) (2013) 1002–1012.
[2] K.E. Yutzey, et al., Calcific aortic valve disease: a consensus summary from the Alliance of investigators on calcific aortic valve disease, Arterioscler. Thromb. Vasc. Biol. 34 (11) (2014) 2387–2393.
[3] B. Yi, et al., Changing epidemiology of calcific aortic valve disease: 30-year trends of incidence, prevalence, and deaths across 204 countries and territories, Aging (Albany NY) 13 (9) (2021) 12710–12732.
[4] P.R. Goody, et al., Aortic valve stenosis: from basic mechanisms to novel therapeutic targets, Arterioscler. Thromb. Vasc. Biol. 40 (4) (2020) 885–900.
[5] C.M. Otto, B. Prendergast, Aortic-valve stenosis–from patients at risk to severe valve obstruction, N. Engl. J. Med. 371 (8) (2014) 744–756.
[6] M.A. Clark, et al., Five-year clinical and economic outcomes among patients with medically managed severe aortic stenosis: results from a Medicare claims analysis, Circ Cardiovasc Qual Outcomes 5 (5) (2012) 697–704.
[7] S.J. Baron, et al., Cost-effectiveness of transcatheter versus surgical aortic valve replacement in patients with severe aortic stenosis at intermediate risk, Circulation 139 (7) (2019) 877–888.
[8] H.N. Ginsberg, et al., Triglyceride-rich lipoproteins and their remnants: metabolic insights, role in atherosclerotic cardiovascular disease, and emerging therapeutic strategies-a consensus statement from the European Atherosclerosis Society, Eur. Heart J. 42 (47) (2021) 4791–4806.

[9] S. Kraler, V. Garg, A. Akhmedov, Calcific aortic valve disease: novel insights into nitric oxide signalling, Eur. Heart J. 43 (17) (2022) 1665–1667.

[10] P. Mathieu, R. Bouchareb, M.C. Boulanger, Innate and adaptive immunity in calcific aortic valve disease, J Immunol Res 2015 (2015) 851945.

[11] S.J. Cowell, et al., A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis, N. Engl. J. Med. 352 (23) (2005) 2389–2397.

[12] K.L. Chan, et al., Effect of Lipid lowering with rosuvastatin on progression of aortic stenosis: results of the aortic stenosis progression observation: measuring effects of rosuvastatin (ASTRONOMER) trial, Circulation 121 (2) (2010) 306–314.

[13] A.B. Rossebo, et al., Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis, N. Engl. J. Med. 359 (13) (2008) 1343–1356.

[14] V.W. Skrivankova, et al., Strengthening the reporting of observational studies in epidemiology using mendelian randomisation (STROBE-MR): explanation and elaboration, BMJ 375 (2021) n2233.

[15] S. Aygun, L. Tokgozoglu, Comparison of current international guidelines for the management of dyslipidemia, J. Clin. Med. 11 (23) (2022).

[16] E.J. Rhee, et al., 2018 Guidelines for the management of dyslipidemia, Korean J Intern Med 34 (4) (2019) 723–771.

[17] D.S. Wishart, et al., DrugBank 5.0: a major update to the DrugBank database for 2018, Nucleic Acids Res. 46 (D1) (2018) D1074–D1082.

[18] U. Vosa, et al., Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression, Nat. Genet. 53 (9) (2021) 1300–1310.

[19] C.J. Willer, et al., Discovery and refinement of loci associated with lipid levels, Nat. Genet. 45 (11) (2013) 1274–1283.

[20] M.I. Kurki, et al., FinnGen provides genetic insights from a well-phenotyped isolated population, Nature 613 (7944) (2023) 508–518.

[21] G. Hemani, et al., The MR-Base platform supports systematic causal inference across the human phenome, Elife 7 (2018).

[22] Z. Zhu, et al., Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets, Nat. Genet. 48 (5) (2016) 481–487.

[23] J.P. Higgins, et al., Measuring inconsistency in meta-analyses, BMJ 327 (7414) (2003) 557–560.

[24] S. Burgess, S.G. Thompson, C.C.G. Collaboration, Avoiding bias from weak instruments in Mendelian randomization studies, Int. J. Epidemiol. 40 (3) (2011) 755–764.

[25] S. Chauquet, et al., Association of antihypertensive drug target genes with psychiatric disorders: a mendelian randomization study, JAMA Psychiatr. 78 (6) (2021) 623–631.

[26] J. Zhao, et al., Genome-wide Mendelian randomization identifies putatively causal gut microbiota for multiple peptic ulcer diseases, Front. Immunol. 14 (2023) 1260780.

[27] S. Burgess, S.G. Thompson, Erratum to: interpreting findings from Mendelian randomization using the MR-Egger method, Eur. J. Epidemiol. 32 (5) (2017) 391–392.

[28] M. Verbanck, et al., Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases, Nat. Genet. 50 (5) (2018) 693–698.

[29] M.D. Shapiro, H. Tavori, S. Fazio, PCSK9: from basic science discoveries to clinical trials, Circ. Res. 122 (10) (2018) 1420–1438.

[30] W.G. Wang, et al., Proprotein convertase subtilisin/kexin type 9 levels and aortic valve calcification: a prospective, cross sectional study, J. Int. Med. Res. 44 (4) (2016) 865–874.

[31] N. Perrot, et al., Genetic and in vitro inhibition of PCSK9 and calcific aortic valve stenosis, JACC Basic Transl Sci 5 (7) (2020) 649–661.

[32] P. Poggio, et al., PCSK9 involvement in aortic valve calcification, J. Am. Coll. Cardiol. 72 (24) (2018) 3225–3227.

[33] A. Langsted, et al., PCSK9 R46L loss-of-function mutation reduces lipoprotein(a), LDL cholesterol, and risk of aortic valve stenosis, J. Clin. Endocrinol. Metab. 101 (9) (2016) 3281–3287.

[34] M.S. Sabatine, et al., Evolocumab and clinical outcomes in patients with cardiovascular disease, N. Engl. J. Med. 376 (18) (2017) 1713–1722.

[35] J.A. Leopold, PCSK9 and calcific aortic valve stenosis: moving beyond lipids, JACC Basic Transl Sci 5 (7) (2020) 662–664.

[36] B.A. Bergmark, et al., An exploratory analysis of proprotein convertase subtilisin/kexin type 9 inhibition and aortic stenosis in the FOURIER trial, JAMA Cardiol 5 (6) (2020) 709–713.