Clinical Medicine & Pharmacology

https://doi.org/10.70731/91pd8g49

Causal Relationship between PCSK9 Inhibitor and Atrial Fibrillation: A Drug Target Mendelian Randomization Study

Xinlong Zhang [a,#], Zihong Yang [a,b,#], Rongjie Huang [a,c,d,*]

[a] *Department of Cardiology, the First Affiliated Hospital of Guangxi Medical University, Nanling, China*
[b] *People's Hospital of Bobai County, Yulin, China*
[c] *Guangxi Key Laboratory of Precision Medicine in Cardio-cerebrovascular Diseases Control and Prevention, Nanling, China*
[d] *Guangxi Clinical Research Center for Cardio-cerebrovascular Diseases, Nanling, China*

KEYWORDS

Drug-Target Mendelian Randomization;
PCSK9;
HMGCR;
Atrial Fibrillation

ABSTRACT

In addition to reducing cholesterol levels, proprotein convertase subtilis kexin 9 (PCSK9) inhibitors exhibit pleiotropic effects, including potential prevention of atrial fibrillation; however, their impact on this cardiac arrhythmia remains controversial. To investigate this relationship, we employed drug-target Mendelian randomization (MR) analysis, collecting single-nucleotide polymorphisms (SNPs) of PCSK9 from published genome-wide association study statistics. Our findings demonstrated that PCSK9 inhibitors significantly reduced the risk of atrial fibrillation (OR [95%CI] = 0.59 [0.45 to 0.78], p=1.38×10-4). For comparison, we also evaluated the effects of 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) inhibitors (targeting statins), using coronary heart disease risk as a positive control, and observed that HMGCR inhibitors may conversely increase the risk of atrial fibrillation. These results suggest that PCSK9 inhibitors confer a protective effect against atrial fibrillation, while HMGCR inhibitors could pose a potential risk for this condition.

INTRODUCTION

The incidence of atrial fibrillation (AF) is progressively increasing, posing a significant clinical challenge in the current medical field and imposing a substantial burden on society and families. According to the data from the 2019 Global Burden of Disease (GBD) study[1], the global prevalence of AF reached 59.7 million cases, which represents a notable rise compared to 28.3 million in 1990 and 45.6 million in 2010. Various factors such as race, ethnicity, sex, and burden of clinical comorbidities contribute to the lifetime risk of AF. The occurrence of AF significantly escalates healthcare expenses. Implementing strategies like the American Heart Association (AHA) 'Life's Essential 8'[2] can aid in reducing global AF prevalence and its subsequent health-care burden [3-8]. Primary treatment approaches for atrial fibrillation encompass anticoagulation therapy, rate control measures, and radiofrequency ablation procedures; however, these treatments present challenges due to potential adverse reactions to traditional antiarrhythmic drugs or postoperative complications like proarrhythmic effects following drug therapy or pericarditis after radiofrequency ablation [9-11]. Consequently, there is an urgent need for further research into the pathogenesis of AF to identify new drug targets and enhance existing treatment methods for more effective prevention and management of this condition. Recent studies have indicated that dyslipidemia is associated with an increased risk among patients with atrial fibrillation [12-16]. Furthermore, some studies suggested that lipid-lowering therapy may positively impact prognosis in individuals with AF[17]. These findings imply a potential link between dyslipidemia and both onset and progression of atrial fibrillation; nevertheless, additional

# These authors contributed equally to this work.
* Corresponding author. E-mail address: huangrongjie67556@163.com

Received 3 May 2025; Received in revised form 14 May 2025; Accepted 25 May 2025; Available online 31 May 2025.

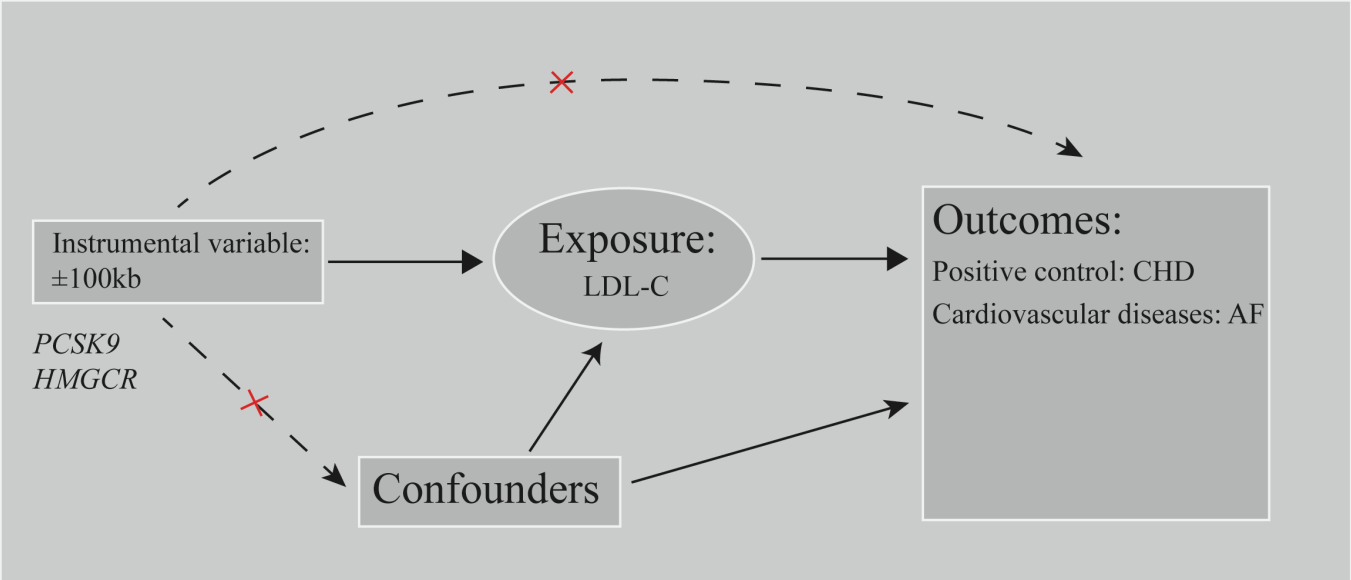

Figure 1 | Research overview and design of drug target Mendelian randomization analysis. PCSK9 and HMGCR inhibitors have been widely used to reduce the risk of coronary heart disease (CHD) . So, we selected CHD as a positive control. In order to verify the existence of causal correlation, it is necessary to meet the conditions as follows: (1) the instrumental variables are not related to the confounders (dashed line), (2) the instrumental variables are related to the exposure factor (solid line), and (3) the instrumental variables are not directly related to the outcome (dashed line). LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; CHD, coronary heart disease; AF, atrial fibrillation.

research is required to investigate the efficacy of specific lipid-lowering medications on this condition thoroughly. Scientific exploration within this domain holds promise for providing novel directions and methods for the treatment of AF.

PCSK9, a serine protease, plays a crucial role in regulating LDL-C metabolism and has emerged as a significant target for cholesterol-lowering therapy [18, 19]. While the protective effect of PCSK9 inhibitors (PCSK9i) on most cardiovascular diseases is well-established, their impact on atrial fibrillation remains uncertain[20]. In a prospective, single-center cohort study conducted by Daniele Pastori et al., it was observed that plasma levels of PCSK9 were positively associated with disease activity and damage in patients with atrial fibrillation[21]. Currently, there is no direct evidence or support from evidence-based medicine to establish the role of PCSK9 in AF. However, some studies have suggested a potential link between PCSK9 and AF[22]. Notably, apart from lipid lowering effects, PCSK9i may also exert pleiotropic effects. Compared to traditional lipid-lowering drugs like statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, HMGCRi), PCSK9 not only exhibits superior lipid-lowering efficacy but can also induce proinflammatory cytokine secretion by macrophages, hepatocytes, and various tissues [23, 24]. These findings indicate that pathways other than lipid lowering might be involved in the pathogenesis of atrial fibrillation mediated by PCSK9i.

Drug-target Mendelian randomization (MR) analyses utilize genetic variants mimicking pharmacological inhibition of drug targets as instrumental variables. Regression analysis can elucidate the long-term medication effects and strengthen causal inference regarding the potential impact of these drug gene targets on AF [18, 25]. In this study, we utilized summary statistics from recently published genome-wide association studies (GWAS) to investigate the causality between genetically predicted inhibition of PCSK9 and HMGCR with AF through drug-target MR analyses.

METHODS

Source of Instrumental Variables for PCSK9 and HMGCR

LDL-C summary data were obtained from a GWAS summary statistics of 440,546 Europeans[26]. By obtaining instrumental variables that can target PCSK9 and HMGCR to reduce LDL-C, they can be used to simulate the effects of PCSK9 inhibitors and HMGCR inhibitors (statins)[26]. Instrumental variables were selected for single nucleotide polymorphisms (SNPs) located within ±100 KB of the PCSK9 or HMGCR loci and associated with LDL-C levels (Fig.1). In order to avoid the influence of strong linkage disequilibrium (LD) on the results, the threshold of LD (kb=100, r2< 0.3) was set. Finally, 33 significant SNPS for PCSK9 and 19 significant SNPS for HMGCR were retained (supplementary table: Table S1). Analyses were repeated to ensure the stability of the results, using pooled data from another GWAS involving 201,678 persons of European descent, with the instrumental variables for PCSK9 and HMGCR again obtained as described above (supplementary table: Table S2).

Source of Outcomes

We used AF as the outcome of the drug-target MR Analysis, with coronary heart disease (CHD) as the positive control

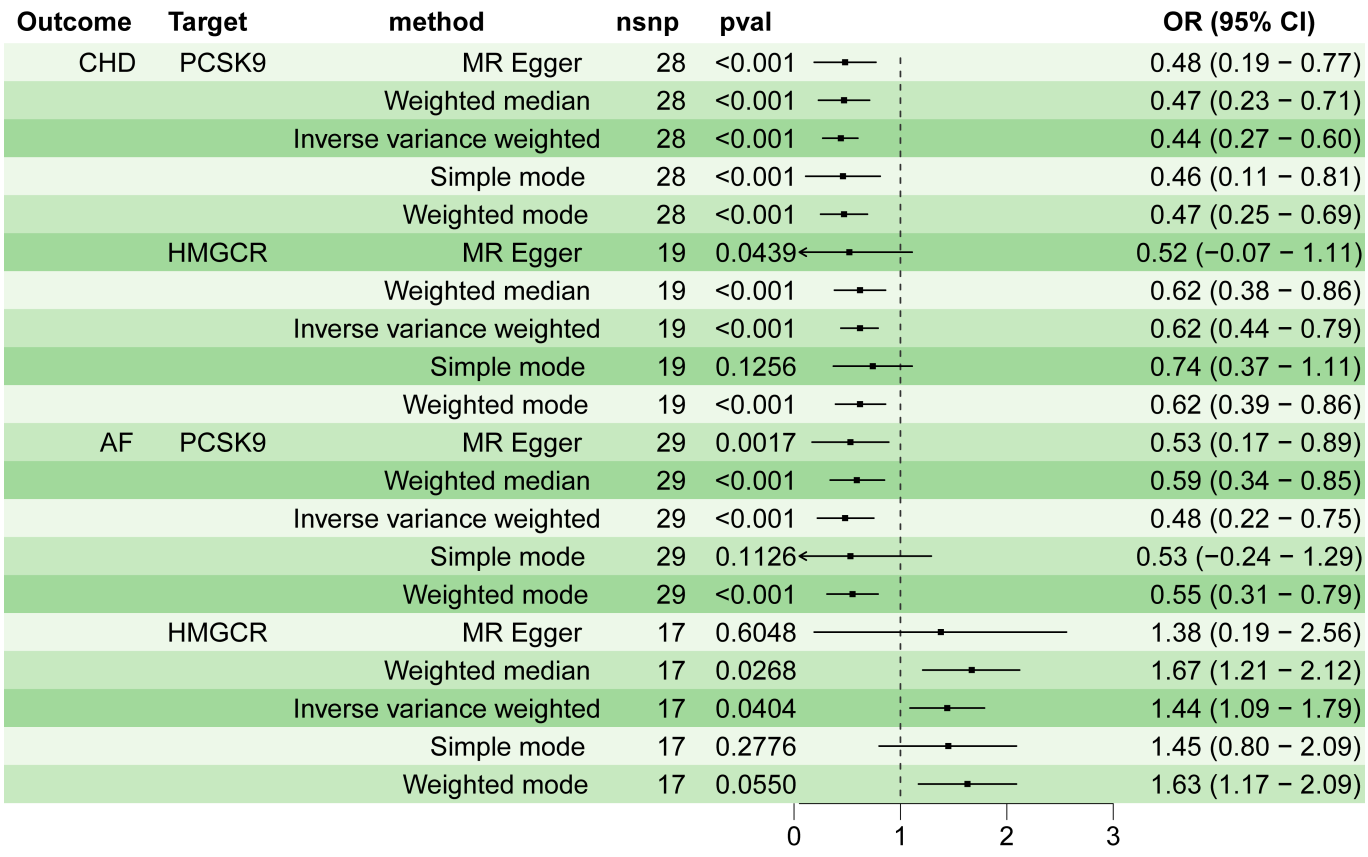

Figure 2 | The effect of PCSK9 and HMGCR inhibitor on coronary heart disease and atrial fibrillation. nsnp, number of single nucleotide polymorphisms; OR, odds ratio; CI, confdence interval; PCSK9, proprotein convertase subtilisin/kexin 9; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; CHD, coronary heart disease; AF, atrial fibrillation.

dataset. The datasets were all derived from the European population. The CHD dataset was derived from GWAS summary statistics, which contained 60,801 cases and 123,504 controls[27]. The AF data set, also from GWAS summary statistics, contained 10,516 cases and 116,926 controls.

Data Analysis

PCSK9 and HMGCR inhibitors have been widely used in the treatment of coronary heart disease. Therefore, we used pooled data from GWAS of CHD as a positive control for the results to verify the validity of instrumental variables. First, we harmonized the exposure-related drug targeting instrumental variables with the outcome data set, Then MR Egger, weighted median, inverse variance weighted (IVW), simple mode, weighted mode, and MR-PRESSO were used for analysis. Among them, IVW method is the most commonly used method[28]. Heterogeneity was tested by MR Egger and IVW methods. Cochrane's Q value was used to evaluate the heterogeneity of genetic tools, $p > 0.05$ indicates that there is no significant heterogeneity. MR Egger regression equation was used to evaluate the horizontal pleiotropy of genetic tools, $p > 0.05$ indicates that there is no horizontal pleiotropy[29].

The MR hypothesis requires that SNPS are not directly related to the outcome (Fig.1). Therefore, we use online website PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) to find and instrumental variable directly related to the properties of SNP, eliminate SNPs associated with coronary heart disease and atrial fibrillation. Sensitivity analysis was performed again after removing outliers by MR-PRESSO test. To ensure that our results were not significantly affected by a single SNP, we removed each SNP in turn using the leave-one-out method and compared the results of the IVW method with all variants. Data analysis was performed using MR-PRESSO and TwoSampleMR software packages on R version 4.2.1[29, 30].

RESULTS

Positive Control Analysis

As expected, PCSK9i significantly reduced the risk of coronary artery disease by the IVW method (OR [95%]=0.44 [0.27 to 0.60], p=7.2× 10−24). This was similar to the effect of HMGCRi (OR [95%]=0.62 [0.44 to 0.79], p=8.29× 10−8) (Fig.2). MR Egger, simple mode, and weighted mode are shown in supplementary table: Table S3. Similar results were obtained by repeating the analysis with another GWAS dataset. The MR-PRESSO package did not monitor any outliers in this study.

PCSK9 and HMGCR Gene - Simulated Inhibition: Effects on Atrial Fibrillation

Genetic prediction of PCSK9 inhibitors showed obvious AF protective effect in the IVW (OR [95%]= 0.48 [0.22 to 0.75], p=7.12× 10−5) and weighted median (OR [95%]= 0.59 [0.34 to 0.85], P = 0.001) methods. p=7.29× 10−8), while HMGCR did not reach statistical significance (IVW: p=0.04, OR=1.44; Weighted median :p=0.02,OR=1.67)(Fig.2). Results for other MR Analysis methods are presented in supplementary table: Table S3. In addition, we repeated the analysis using another GWAS dataset and reached similar conclusions (supplementary table: Table S4).

DISCUSSION

MR analysis is widely utilized in medical research. The utilization of SNPs selected from GWAS data as instrumental variables in MR Studies offers the advantages of being unaffected by external interference factors and having a large sample size, effectively avoiding the influence of potential confounding factors and reverse causality, thereby enhancing the accuracy and reliability of research results. This study aimed to comprehensively analyze drug targets to investigate the possible causal effects of PCSK9 inhibitors on atrial fibrillation. As depicted in Fig.2, there was a significant association between PCSK9 inhibitor-mediated low-density lipoprotein cholesterol and atrial fibrillation, which was corroborated by multiple MR analysis methods and sensitivity analysis, further bolstering the credibility and stability of the findings.

Clinical trials have demonstrated that PCSK9 inhibitors can effectively reduce low-density lipoprotein cholesterol levels and decrease cardiovascular event occurrences [23, 31]. Apart from their impact on LDL-C levels, PCSK9 inhibitors possess potential pleiotropic effects such as augmenting tumor response to immune checkpoint therapy, inhibiting platelet activation and thrombosis, as well as reducing apoptosis[23, 32, 33]. In recent years, PCSK9 inhibitors have played an increasingly pivotal role in cardiovascular disease treatment[20, 34-39]. Nevertheless，no study has thoroughly investigated the causal relationship between PCSK9 inhibitors and AF. Through drug-target MR analysis，we discovered that PCSK9i could significantly mitigate AF risk. Our findings contribute to a deeper comprehension of the multifaceted effects of PCSK9i，provide insights into potential side effects associated with its use，and offer theoretical guidance for lipid-lowering strategy selection.

Excitingly, our results demonstrated a distinct protective effect of PCSK9 inhibitors (PCSK9i) against atrial fibrillation (AF). Atrial fibrillation is a cardiovascular disease that represents the continuum of most cardiovascular diseases[40]. Lipopolysaccharides (LPS) is emerging as a novel risk factor for cardiovascular events, patients with elevated levels of both PCSK9 and LPS face an increased risk of cardiovascular events[41]. Our study aligns with the conclusions drawn from a recently published drug-targeted Mendelian randomization study[42], further substantiating the protective effect of PCSK9i on atrial fibrillation. Moreover, previous research has reported a positive correlation between PCSK9 levels and the severity of coronary artery disease as measured by Gensini score, suggesting potential influence through lipid and inflammatory pathways[43]. However, the precise mechanism by which PCSK9 participates in AF initiation and progression remains unclear.

While our study found that PCSK9i had a protective effect on AF risk, HMGCR inhibitors (HMGCRi) did not yield similar results. This suggests that PCSK9i may not directly impact LDL cholesterol to reduce AF risk. It has been reported that PSCK9 directly interacts with platelets and activates Nox2 through CD36 receptor in patients with atrial fibrillation; however, this interaction is more pronounced in the presence of low-density lipoprotein cholesterol[22]. Although this conclusion does not directly support our findings, it prompts further contemplation. Furthermore, an increasing number of studies have indicated that PCSK9 affects cardiovascular diseases by influencing platelet function [21, 44-46], which can also inspire future investigations.

In contrast to previous observational studies, our research employed a drug-targeting MR approach to establish an association between PCSK9 inhibitors and AF. The findings suggest that PCSK9 inhibitors may exert a beneficial effect on AF possibly due to their lipid-lowering properties, suggesting that the application of PCSK9 inhibitors may be considered in the future clinical treatment of AF.

CONCLUSION

After performing drug-target MR Analysis, we found that gene-predicted inhibition of PCSK9 significantly reduced the risk of AF. In contrast, genetic prediction that inhibition of HMGCR may be a risk factor for AF.

Abbreviations

PCSK9: Proprotein convertase subtilis kexin 9
MR: Mendelian randomization
SNP: Single nucleotide polymorphism
HMGCR: 3-Hydroxy-3-methylglutaryl-assisted enzyme A reductase
CHD: coronary heart disease
AF: atrial fibrillation

Authors' contributions Study design: Xinlong Zhang, Zihong Yang, Rongjie Huang; data collection and analysis: Xinlong Zhang, Zihong Yang; writing:Xinlong Zhang, Rongjie Huang.

Funding This work was supported by Guangxi Key Laboratory of Precision Medicine in Cardio-cerebrovascular Diseases Control and Prevention.

Availability of data and materials The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Declarations Ethics approval and consent to participate The GWAS summary data used in this study were all from the online public platform (https://gwas.mrcieu.ac.uk/). The study protocols were approved by respective local ethics committees, and participants have provided written informed consent

Competing interests The authors declare no competing interests.

Reference

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk

Factors, 1990-2019: Update From the GBD 2019 Study. (1558-3597 (Electronic)).

2. Lloyd-Jones Dm Fau - Allen NB, Allen Nb Fau - Anderson CAM, Anderson Cam Fau - Black T, Black T Fau - Brewer LC, Brewer Lc Fau - Foraker RE, Foraker Re Fau - Grandner MA, et al. Life's Essential 8: Updating and Enhancing the American Heart Association's Construct of Cardiovascular Health: A Presidential Advisory From the American Heart Association. (1524-4539 (Electronic)).
3. Elliott AA-O, Middeldorp MA-O, Van Gelder IC, Albert CA-O, Sanders PA-O. Epidemiology and modifiable risk factors for atrial fibrillation. (1759-5010 (Electronic)).
4. Rodriguez CJ, Soliman EZ, Alonso A, Swett K, Okin PM, Goff DC, Jr., et al. Atrial fibrillation incidence and risk factors in relation to race-ethnicity and the population attributable fraction of atrial fibrillation risk factors: the Multi-Ethnic Study of Atherosclerosis. (1873-2585 (Electronic)).
5. Alonso A, Agarwal Sk Fau - Soliman EZ, Soliman Ez Fau - Ambrose M, Ambrose M Fau - Chamberlain AM, Chamberlain Am Fau - Prineas RJ, Prineas Rj Fau - Folsom AR, et al. Incidence of atrial fibrillation in whites and African-Americans: the Atherosclerosis Risk in Communities (ARIC) study. (1097-6744 (Electronic)).
6. Ugowe FE, Jackson LR, 2nd, Thomas KL. Racial and ethnic differences in the prevalence, management, and outcomes in patients with atrial fibrillation: A systematic review. (1556-3871 (Electronic)).
7. Martinez C, Katholing A, Wallenhorst C, Granziera S, Cohen AT, Freedman SB. Increasing incidence of non-valvular atrial fibrillation in the UK from 2001 to 2013. (1468-201X (Electronic)).
8. Guo Y, Tian Y, Wang H, Si Q, Wang Y, Lip GYH. Prevalence, incidence, and lifetime risk of atrial fibrillation in China: new insights into the global burden of atrial fibrillation. (1931-3543 (Electronic)).
9. Baman JR, Passman RS. Atrial Fibrillation. (1538-3598 (Electronic)).
10. Hucker WJ, Hanley A, Ellinor PT. Improving Atrial Fibrillation Therapy: Is There a Gene for That? (1558-3597 (Electronic)).
11. Ahmed AS, Miller J, Foreman J, Golden K, Shah A, Field J, et al. Prophylactic Colchicine After Radiofrequency Ablation of Atrial Fibrillation: The PAPERS Study. (2405-5018 (Electronic)).
12. Harrison SL, Lane DA, Banach M, Mastej M, Kasperczyk S, Jóźwiak JJ, et al. Lipid levels, atrial fibrillation and the impact of age: Results from the LIPIDOGRAM2015 study. (1879-1484 (Electronic)).
13. Xue Y, Zhou Q, Shen J, Liu G, Zhou W, Wen Y, et al. Lipid Profile and New-Onset Atrial Fibrillation in Patients With Acute ST-Segment Elevation Myocardial Infarction (An Observational Study in Southwest of China). (1879-1913 (Electronic)).
14. Li X, Gao L, Wang Z, Guan B, Guan X, Wang BA-O, et al. Lipid profile and incidence of atrial fibrillation: A prospective cohort study in China. (1932-8737 (Electronic)).
15. Mourtzinis G, Kahan T, Bengtsson Boström K, Schiöler L, Cedstrand Wallin L, Hjerpe P, et al. Relation Between Lipid Profile and New-Onset Atrial Fibrillation in Patients With Systemic Hypertension (From the Swedish Primary Care Cardiovascular Database [SPCCD]). (1879-1913 (Electronic)).
16. Liu C, Geng J, Ye X, Yuan X, Li A, Zhang Z, et al. Change in lipid profile and risk of new-onset atrial fibrillation in patients with chronic heart failure: A 3-year follow-up observational study in a large Chinese hospital. (1536-5964 (Electronic)).
17. Badheka AO, Rathod A Fau - Kizilbash MA, Kizilbash Ma Fau - Garg N, Garg N Fau - Mohamad T, Mohamad T Fau - Afonso L, Afonso L Fau - Jacob S, et al. Impact of lipid-lowering therapy on outcomes in atrial fibrillation. (1879-1913 (Electronic)).
18. Rosoff DB, Bell AS, Jung J, Wagner J, Mavromatis LA, Lohoff FW. Mendelian Randomization Study of PCSK9 and HMG-CoA Reductase Inhibition and Cognitive Function. (1558-3597 (Electronic)).
19. Glerup S, Schulz R, Laufs U, Schlüter KA-O. Physiological and therapeutic regulation of PCSK9 activity in cardiovascular disease. (1435-1803 (Electronic)).
20. Hummelgaard S, Vilstrup JP, Gustafsen C, Glerup S, Weyer K. Targeting PCSK9 to tackle cardiovascular disease. (1879-016X (Electronic)).
21. Pastori D, Nocella C, Farcomeni A, Bartimoccia S, Santulli M, Vasaturo F, et al. Relationship of PCSK9 and Urinary Thromboxane Excretion to Cardiovascular Events in Patients With Atrial Fibrillation. (1558-3597 (Electronic)).
22. Cammisotto V, Pastori D, Nocella C, Bartimoccia S, Castellani V, Marchese C, et al. PCSK9 Regulates Nox2-Mediated Platelet Activation via CD36 Receptor in Patients with Atrial Fibrillation. LID - 10.3390/antiox9040296 [doi] LID - 296. (2076-3921 (Print)).
23. Ding Z, Pothineni NVK, Goel A, Lüscher TF, Mehta JL. PCSK9 and inflammation: role of shear stress, pro-inflammatory cytokines, and LOX-1. (1755-3245 (Electronic)).
24. Kim K, Ginsberg HN, Choi SH. New, Novel Lipid-Lowering Agents for Reducing Cardiovascular Risk: Beyond Statins. (2233-6087 (Electronic)).
25. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. (1477-0334 (Electronic)).
26. Richardson TA-O, Sanderson EA-O, Palmer TA-O, Ala-Korpela MA-O, Ference BA, Davey Smith GA-O, et al. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomisation analysis. (1549-1676 (Electronic)).
27. Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. (1546-1718 (Electronic)).
28. Wang XF, Xu WJ, Wang FF, Leng R, Yang XK, Ling HZ, et al. Telomere Length and Development of Systemic Lupus Erythematosus: A Mendelian Randomization Study. (2326-5205 (Electronic)).
29. Hemani GA-O, Zheng JA-O, Elsworth B, Wade KA-O, Haberland V, Baird DA-O, et al. The MR-Base platform supports systematic causal inference across the human phenome. LID - 10.7554/eLife.34408 [doi] LID - e34408. (2050-084X (Electronic)).
30. Verbanck M, Chen CA-O, Neale BA-O, Do RA-O. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. (1546-1718 (Electronic)).
31. Bergeron N, Phan BA, Ding Y, Fong A, Krauss RM. Proprotein convertase subtilisin/kexin type 9 inhibition: a new therapeutic mechanism for reducing cardiovascular disease risk. (1524-4539 (Electronic)).
32. Qi Z, Hu L, Zhang J, Yang W, Liu X, Jia D, et al. PCSK9 (Proprotein Convertase Subtilisin/Kexin 9) Enhances Platelet Activation, Thrombosis, and Myocardial Infarct Expansion by Binding to Platelet CD36. (1524-4539 (Electronic)).
33. Liu X, Bao X, Hu M, Chang H, Jiao M, Cheng J, et al. Inhibition of PCSK9 potentiates immune checkpoint therapy for cancer. (1476-4687 (Electronic)).
34. Khan SA-O, Yedlapati SH, Lone AN, Hao Q, Guyatt G, Delvaux N, et al. PCSK9 inhibitors and ezetimibe with or without statin therapy for cardiovascular risk reduction: a systematic review and network meta-analysis. (1756-1833 (Electronic)).
35. Chen B, Shi X, Cui Y, Hou A, Zhao P. A Review of PCSK9 Inhibitors and their Effects on Cardiovascular Diseases. (1873-4294 (Electronic)).
36. Sabatine MS. PCSK9 inhibitors: clinical evidence and implementation. (1759-5010 (Electronic)).
37. Nicholls SJ. PCSK9 inhibitors and reduction in cardiovascular events: Current evidence and future perspectives. (1897-4279 (Electronic)).
38. Wang X, Wen D, Chen Y, Ma L, You C. PCSK9 inhibitors for secondary prevention in patients with cardiovascular diseases: a bayesian network meta-analysis. (1475-2840 (Electronic)).
39. Rosenson RS, Hegele RA, Fazio S, Cannon CP. The Evolving Future of PCSK9 Inhibitors. (1558-3597 (Electronic)).
40. Joglar Ja Fau - Chung MK, Chung Mk Fau - Armbruster AL, Armbruster AL, Benjamin Ej Fau - Chyou JY, Chyou Jy Fau - Cronin EM, Cronin Em Fau - Deswal A, et al. 2023 ACC/AHA/ACCP/HRS Guideline for the Diagnosis and Management of Atrial Fibrillation: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. (1524-4539 (Electronic)).
41. Pastori D, Ettorre E, Carnevale R, Nocella C, Bartimoccia S, Del Sordo E, et al. Interaction between serum endotoxemia and proprotein convertase subtilisin/kexin 9 (PCSK9) in patients with atrial fibrillation: A post-hoc analysis from the ATHERO-AF cohort. (1879-1484 (Electronic)).
42. Rosoff DA-O, Bell AA-O, Mavromatis LA-O, Hamandi A, Park LA-O, Jung JA-O, et al. Evaluating the Cardiovascular Impact of Genetically Proxied PCSK9 and HMGCR Inhibition in East Asian and European Populations: A Drug-Target Mendelian Randomization Study. (2574-8300 (Electronic)).
43. Li S, Zhang Y Fau - Xu R-X, Xu Rx Fau - Guo Y-L, Guo Yl Fau - Zhu C-G, Zhu Cg Fau - Wu N-Q, Wu Nq Fau - Qing P, et al. Proprotein

convertase subtilisin-kexin type 9 as a biomarker for the severity of coronary artery disease. (1365-2060 (Electronic)).

44. Navarese Ep Fau - Kolodziejczak M, Kolodziejczak M Fau - Kereiakes DJ, Kereiakes Dj Fau - Tantry US, Tantry Us Fau - O'Connor C, O'-Connor C Fau - Gurbel PA, Gurbel PA. Proprotein Convertase Subtilisin/Kexin Type 9 Monoclonal Antibodies for Acute Coronary Syndrome: A Narrative Review. (1539-3704 (Electronic)).
45. Navarese EP, Kolodziejczak M, Winter MP, Alimohammadi A, Lang IM, Buffon A, et al. Association of PCSK9 with platelet reactivity in patients with acute coronary syndrome treated with prasugrel or ticagrelor: The PCSK9-REACT study. (1874-1754 (Electronic)).
46. Hadjiphilippou S, Ray KK. Evolocumab and clinical outcomes in patients with cardiovascular disease. (2042-8189 (Electronic)).

Supplementary Table1 | The detail of instrumental variable corresponding to PCSK9 and HMGCR.

	SNP	chr	pos	Beta	SE	p-val	Effect-allele	Other-allele	eaf	Fval
HMGCR	rs75240579	5	74624484	-0.0372115	0.00487202	2.20E-14	T	C	0.048363	58.33594383
	rs2006760	5	74562029	0.03556	0.00261075	3.00E-42	G	C	0.205486	185.5209379
	rs62366588	5	74664987	-0.0271295	0.00433093	3.70E-10	A	C	0.065948	39.23929995
	rs141642272	5	74615209	0.0532822	0.00653971	3.70E-16	C	G	0.026705	66.38153891
	rs55727654	5	74651864	0.042154	0.0029315	6.90E-47	A	G	0.14871	206.7748838
	rs111353455	5	74623949	0.0243909	0.00372718	6.00E-11	A	G	0.085844	42.82475798
	rs2303152	5	74641707	0.0333589	0.00345272	4.40E-22	A	G	0.101442	93.34707672
	rs116153450	5	74729433	-0.0303618	0.00499242	1.20E-09	A	C	0.04623	36.98561134
	rs12916	5	74656539	0.0621175	0.00212705	1.70E-187	C	T	0.400537	852.8497757
	rs17562727	5	74682474	0.0394972	0.00635898	5.30E-10	C	T	0.027617	38.57959819
	rs80324692	5	74717761	-0.0260509	0.00385694	1.40E-11	T	C	0.081157	45.62046397
	rs115845757	5	74563700	0.048608	0.00785612	6.10E-10	A	G	0.019	38.2824115
	rs17648121	5	74650106	0.0619849	0.00619367	1.40E-23	T	C	0.029877	100.1557033
	rs140092661	5	74682600	0.0329927	0.00582168	1.50E-08	T	A	0.034128	32.11730206
	rs12659331	5	74757657	0.0251785	0.00459392	4.20E-08	C	A	0.054326	30.03951048
	rs72633963	5	74630829	0.0564278	0.00316653	4.90E-71	A	G	0.1238	317.5550495
	rs10051965	5	74560487	0.0410063	0.00216518	5.40E-80	T	C	0.369925	358.6849509
	rs35122945	5	74610293	-0.0281057	0.00423755	3.30E-11	C	A	0.067368	43.99052463
	rs4703665	5	74602898	0.0244938	0.00297742	1.90E-16	C	T	0.848709	67.67560269
PCSK9	rs6691964	1	55433978	-0.0234719	0.00358388	5.80E-11	A	G	0.092472	42.89331118
	rs556369867	1	55491135	0.0175746	0.00243048	4.80E-13	T	C	0.330585	52.28616572
	rs72909541	1	55494301	-0.0334061	0.00501479	2.70E-11	T	C	0.046097	44.37578527
	rs150119739	1	55520938	0.0452728	0.00520209	3.20E-18	A	G	0.045318	75.73889898
	rs7525503	1	55522558	0.0454642	0.0075822	2.00E-09	T	G	0.02036	35.95411766
	rs11587071	1	55522674	-0.0282322	0.00279415	5.30E-24	T	C	0.168881	102.0916
	rs10493176	1	55538552	-0.0531381	0.00394676	2.60E-41	G	T	0.07579	181.2719504
	rs3976734	1	55489960	-0.0297494	0.00231882	1.10E-37	G	A	0.374504	164.5971636
	rs200730299	1	55491853	-0.0543492	0.00278155	5.10E-85	C	A	0.195034	381.779469
	rs17192725	1	55496131	0.0305717	0.00365832	6.40E-17	A	G	0.095408	69.83542745
	rs17111503	1	55503448	0.0406795	0.00235743	1.00E-66	G	A	0.268141	297.7649673
	rs7546522	1	55516713	-0.0168117	0.00295297	1.20E-08	T	C	0.155442	32.41195247
	rs2483205	1	55518316	-0.0295845	0.00214514	2.90E-43	T	C	0.438633	190.2029223
	rs11583974	1	55551718	0.0314531	0.00517068	1.20E-09	A	G	0.042146	37.00254444
	rs56349475	1	55576102	-0.0475957	0.00671909	1.40E-12	C	T	0.024601	50.17813553
	rs79396670	1	55588142	-0.0336489	0.00562029	2.10E-09	A	G	0.035496	35.84464574
	rs146273942	1	55453841	-0.0538858	0.00722418	8.70E-14	A	G	0.023188	55.63800546
	rs2479420	1	55492190	-0.0283879	0.0023826	9.90E-33	T	C	0.73803	141.9594338
	rs11810371	1	55496861	-0.0294547	0.00507333	6.40E-09	A	G	0.043743	33.70722366
	rs11591147	1	55505647	-0.348456	0.00793088	1.00E-200	T	G	0.017468	1930.425903
	rs11206513	1	55507649	0.0316517	0.0021463	3.20E-49	T	C	0.600617	217.4769484
	rs11206517	1	55526428	0.0680285	0.00580615	1.00E-31	G	T	0.033149	137.2793731
	rs2495517	1	55448842	0.0177548	0.0025792	5.80E-12	G	A	0.794271	47.38725246
	rs12732125	1	55470153	-0.10344	0.0073736	1.00E-44	T	C	0.020368	196.7967286
	rs2479395	1	55484582	0.0125674	0.00221762	1.50E-08	C	T	0.668453	32.11564266
	rs77875082	1	55485042	0.0481535	0.00605559	1.80E-15	A	G	0.032388	63.23285511
	rs41294821	1	55513183	-0.0386615	0.00705365	4.20E-08	T	C	0.022807	30.04205156
	rs472495	1	55521313	0.0425743	0.00218093	7.30E-85	T	G	0.648959	381.0759835
	rs530804537	1	55583210	-0.192336	0.00997554	7.80E-83	A	G	0.011303	371.7477347
	rs55637835	1	55466303	-0.0187129	0.00324835	8.40E-09	T	C	0.120881	33.18612613
	rs12739979	1	55496648	-0.0202563	0.00254032	1.50E-15	T	C	0.246521	63.58334709
	rs72660548	1	55500978	0.0509816	0.00777535	5.50E-11	G	C	0.018458	42.99193846
	rs45613943	1	55518622	-0.0340702	0.0048672	2.60E-12	C	T	0.048725	48.99942472

** PCSK9, proprotein convertase subtilisin/kexin 9; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase*

Supplementary Table2 | The detail of instrumental variable corresponding to PCSK9 and HMGCR for repeated analysis.

	SNP	chr	pos	Beta	SE	p-val	Effect-allele	Other-allele	eaf	Fval
PCSK9	rs505151	1	55529187	-0.073956	0.0088079	5.30E-17	A	G	0.968021	70.50210387
	rs10493176	1	55538552	-0.0577103	0.00589916	7.30E-23	G	T	0.075768	95.70316772
	rs3976734	1	55489960	-0.02817	0.00345948	3.50E-16	G	A	0.375003	66.30588035
	rs2483205	1	55518316	-0.0334091	0.00320423	8.00E-26	T	C	0.438806	108.7131772
	rs6691964	1	55433978	-0.031203	0.00534137	7.00E-09	A	G	0.092678	34.12615637
	rs17111503	1	55503448	0.0421054	0.00352171	2.00E-33	G	A	0.268334	142.9448173
	rs12732125	1	55470153	-0.11109	0.0110178	2.70E-24	T	C	0.020317	101.6623546
	rs11591147	1	55505647	-0.343211	0.0118485	1.00E-187	T	G	0.017409	839.0650886
	rs11587071	1	55522674	-0.0278058	0.00416174	9.70E-12	T	C	0.169479	44.63966225
	rs77875082	1	55485042	0.053705	0.0090405	2.70E-09	A	G	0.032496	35.28942164
	rs2495500	1	55487668	-0.023115	0.00348575	5.20E-11	T	A	0.726608	43.97393433
	rs4927191	1	55491702	-0.0429521	0.00352763	1.60E-34	C	T	0.272704	148.2527471
	rs72660539	1	55494906	0.0366186	0.00458604	2.80E-15	A	G	0.134551	63.75698858
	rs7543163	1	55515481	0.034815	0.00320188	8.10E-28	T	C	0.613764	118.2286406
	rs472495	1	55521313	0.0447232	0.00325971	1.20E-43	T	G	0.648806	188.2382215
	rs530804537	1	55583210	-0.201771	0.0149329	3.10E-43	A	G	0.01126	182.5699007
HMGCR	rs2006760	5	74562029	0.0318844	0.00389125	1.20E-16	G	C	0.205365	67.13951974
	rs55727654	5	74651864	0.0373204	0.00437044	2.30E-17	A	G	0.148905	72.91926039
	rs6453131	5	74644706	0.0565272	0.00320968	2.30E-69	G	T	0.374716	310.1640618
	rs4704213	5	74682900	0.05243	0.00471174	1.50E-28	A	G	0.124156	123.8217907
	rs10079346	5	74536655	0.0392565	0.00322442	1.90E-34	A	G	0.364046	148.2244817
	rs2303152	5	74641707	0.0311566	0.00514506	7.40E-10	A	G	0.101678	36.67070251
	rs17648121	5	74650106	0.059432	0.00925546	1.00E-10	T	C	0.029659	41.23297533

** PCSK9, proprotein convertase subtilisin/kexin 9; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase*

Supplementary Table3 | The effect of PCSK9 and HMGCR inhibitor on atrial fibrillation

	Outcome	Target	method	NSNP	pval	OR	OR(95%CI)
CHD	PCSK9	MR Egger	28	4.46E-05	0.48	0.19	0.77
		Weighted median	28	3.46E-10	0.47	0.23	0.71
		Inverse variance weighted	28	7.20E-24	0.44	0.27	0.60
		Simple mode	28	0.000184713	0.46	0.11	0.81
		Weighted mode	28	4.42E-07	0.47	0.25	0.69
	HMGCR	MR Egger	19	0.043879267	0.52	-0.07	1.11
		Weighted median	19	0.000110101	0.62	0.38	0.86
		Inverse variance weighted	19	8.29E-08	0.62	0.44	0.79
		Simple mode	19	0.12555868	0.74	0.37	1.11
		Weighted mode	19	0.000899755	0.62	0.39	0.86
AF	PCSK9	MR Egger	29	0.001735523	0.53	0.17	0.89
		Weighted median	29	7.12E-05	0.59	0.34	0.85
		Inverse variance weighted	29	7.29E-08	0.48	0.22	0.75
		Simple mode	29	0.112623682	0.53	-0.24	1.29
		Weighted mode	29	3.33E-05	0.55	0.31	0.79
	HMGCR	MR Egger	17	0.604844305	1.38	0.19	2.56
		Weighted median	17	0.026836854	1.67	1.21	2.12
		Inverse variance weighted	17	0.04036241	1.44	1.09	1.79
		Simple mode	17	0.277606952	1.45	0.80	2.09
		Weighted mode	17	0.054988281	1.63	1.17	2.09

** NSNP, number of single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; PCSK9, proprotein convertase subtilisin/kexin 9; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; CHD, coronary heart disease; AF, atrial fibrillation.*

Supplementary Table 4 | The effect of PCSK9 and HMGCR inhibitor on atrial fibrillation for repeated analysis.

	Outcome	Target	method	NSNP	pval	OR	OR(95%CI)
CHD	PCSK9	MR Egger	13	0.001905771	0.51	0.19	0.84
		Weighted median	13	1.43E-10	0.47	0.25	0.70
		Inverse variance weighted	13	4.06E-18	0.48	0.31	0.64
		Simple mode	13	0.001082433	0.48	0.15	0.82
		Weighted mode	13	9.62E-05	0.47	0.22	0.73
	HMGCR	MR Egger	7	0.25058299	0.55	-0.35	1.45
		Weighted median	7	0.000441311	0.62	0.36	0.89
		Inverse variance weighted	7	1.45E-05	0.64	0.43	0.84
		Simple mode	7	0.049340073	0.61	0.22	1.00
		Weighted mode	7	0.010368977	0.58	0.29	0.87
AF	PCSK9	MR Egger	15	0.003527197	0.55	0.23	0.88
		Weighted median	15	5.16E-05	0.58	0.32	0.84
		Inverse variance weighted	15	8.17E-10	0.49	0.25	0.72
		Simple mode	15	0.015167876	0.48	-0.03	1.00
		Weighted mode	15	0.000304615	0.55	0.30	0.80
	HMGCR	MR Egger	7	0.320280099	2.78	0.96	4.60
		Weighted median	7	0.064026792	1.61	1.11	2.12
		Inverse variance weighted	7	0.107837737	1.39	0.99	1.80
		Simple mode	7	0.860283274	0.94	0.23	1.65
		Weighted mode	7	0.144313966	1.69	1.08	2.30

** NSNP, number of single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; PCSK9, proprotein convertase subtilisin/kexin 9; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; CHD, coronary heart disease; AF, atrial fibrillation.*

Supplementary Table 5 | The result of heterogeneity test and horizontal pleiotropic test.

Outcomes	Drug Target	Heterogeneity test				Horizontal pleiotropic test		
CHD	PCSK9	Method	Q	Q_df	Q_pval	egger_intercept	SE	p-value
		MR Egger	25.77686717	26	0.475421295	0.00476513	0.005609578	0.403377938
		Inverse variance weighted	26.49845464	27	0.49108877			
	HMGCR	MR Egger	16.34534298	17	0.499498612	-0.008046832	0.013190547	0.549900293
		Inverse variance weighted	16.71749825	18	0.542601824			
AF	PCSK9	MR Egger	50.53232866	27	0.003947936	0.008163639	0.01073047	0.453375637
		Inverse variance weighted	51.61559603	28	0.004246598			
	HMGCR	MR Egger	17.08945872	15	0.313544751	-0.002072687	0.0260416	0.937614391
		Inverse variance weighted	17.09667593	16	0.379368079			

** PCSK9, proprotein convertase subtilisin/kexin 9; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; CHD, coronary heart disease; AF, atrial fibrillation.*

Supplementary Table 6 | The result of heterogeneity test and horizontal pleiotropic test for repeated analysis.

Outcomes	Drug Target	Heterogeneity test				Horizontal pleiotropic test		
CHD	PCSK9	Method	Q	Q_df	Q_pval	egger_intercept	SE	p-value
		MR Egger	11.15365901	11	0.430481507	0.003420242	0.00730614	0.648828665
		Inverse variance weighted	11.37586833	12	0.497010651			
	HMGCR	MR Egger	3.022862964	5	0.696460868	-0.006561158	0.020314809	0.759784365
		Inverse variance weighted	3.127175248	6	0.792724119			
AF	PCSK9	MR Egger	18.22731959	13	0.149077455	0.012766168	0.011722662	0.295923393
		Inverse variance weighted	19.89015046	14	0.133642945			
	HMGCR	MR Egger	1.26362084	5	0.938630169	0.032010342	0.041901462	0.479379375
		Inverse variance weighted	1.84723021	6	0.933198825			

** PCSK9, proprotein convertase subtilisin/kexin 9; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; CHD, coronary heart disease; AF, atrial fibrillation.*