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ARTICLE

Mendelian Randomization Study Reveals Causal Pathways for Hypertrophic Cardiomyopathy, Cardiovascular Proteins, and Atrial Fibrillation

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Abstract

Aims/Background Research evidence has demonstrated a significant association between hypertrophic cardiomyopathy (HCM) and atrial fibrillation (AF), but the causality and pattern of this link remain unexplored. Therefore, this study investigated the causal relationship between HCM and AF using a two-sample and bidirectional Mendelian randomization (MR) approach. Additionally, this assessed the role of cardiovascular proteins (CPs) associated with cardiovascular diseases between HCM and AF by applying a two-step MR analysis.

Methods Data for HCM, AF, and 90 CPs were obtained from the Finn Gen and IEU Open GWAS Project databases. MR-Egger, inverse variance weighting (IVW), weighted median estimator (WME), weighted mode, and simple mode were used to estimate causal inferences. Furthermore, Cochran's Q test, MR-Egger's intercept terms, and Leave-one-out methods determined the heterogeneity, horizontal pleiotropy, and sensitivity. Additionally, mediation effects were used to assess the role of CPs in the relationship between HCM and AF.

Results Two-sample and bidirectional MR analysis revealed HCM as a risk factor for AF (odds ratio (OR) = 1.008, 95% confidence interval (CI): 1.001–1.016, p = 0.029) and AF was found to increase the risk of developing HCM (OR = 1.145, 95% CI: 0.963–1.361, p = 0.126). Moreover, Two-step MR analyses indicated that 5 CPs were causally associated with HCM; 12 CPs with AF and 1 CP (Melusin) with both HCM and AF. Additionally, Melusin was observed as a protective factor for both HCM and AF and may serve as a mediator variable for these two conditions (mediation effect 0.0004, mediation ratio 5.5178%, 95% CI: 5.4624–5.5731).

Conclusion HCM may increase the risk of developing AF, with Melusin serving as a mediator for this risk.

Key words: Mendelian randomization; hypertrophic cardiomyopathy; atrial fibrillation; cardiovascular protein; mediation effect

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Introduction

Hypertrophic cardiomyopathy (HCM) is a frequently inherited cardiac condition characterised by asymmetrical thickening of the ventricular walls, impacting individuals across all age groups (Dungu et al, 2024). HCM is one of the most prevalent genetic heart disorders, with an estimated prevalence of 1:500–1:200 (Maron et

al, 2022). This disease can result in impaired cardiac pumping function and arrhythmias, and has become a significant cause of sudden death in young and middle-aged individuals, as well as a leading cause of heart failure across all age groups (Goff and Calkins, 2019; Thakkar et al, 2023). Research indicates that around 60% of individuals with HCM carry mutations in genes encoding cardiac sarcomere proteins (Maron et al, 2022). For example, variations in the β-myosin heavy chain (*MYH7*) gene are associated with an increased risk of atrial fibrillation (AF), and HCM patients carrying specific gene mutations exhibit a significantly higher incidence of AF (Pioner et al, 2022).

The incidence and prevalence of atrial fibrillation are rising globally, especially with increasing age, and in 2020, approximately 50 million individuals were affected by AF worldwide (Joglar et al, 2024). Among HCM patients, AF is the most common sustained arrhythmia (Yuan et al, 2024). Statistics indicate that about 20%–27% of individuals with HCM develop AF (Mistrulli et al, 2024; Zörner et al, 2024), a prevalence rate four to six times higher than that in the general population of similar age groups (Losi et al, 2024; Rowin et al, 2023; Yuan et al, 2024). Furthermore, in individuals with inherited HCM but no evident myocardial disease phenotype, AF often occurs as the initial manifestation (Wang et al, 2023). Considering the high incidence and risk associated with HCM coupled with AF as well as limited and inconsistent clinical evidence, further identification of their relationship and potential pathogenesis is essential for accurate risk assessment and personalised treatment approaches.

Residual confounding factors and potential reverse causation often limit causal inferences in observational studies, hindering the optimization of diagnostic and therapeutic strategies. Proteins play key roles in regulating molecular pathways, with plasma proteins frequently used as biomarkers for diagnosing and predicting diseases. Studies on the circulating proteome have identified numerous circulating proteins associated with the onset, progression, and remission of HCM and AF (Castiglione et al, 2022; Egerstedt et al, 2019; Ferreira et al, 2019; Shimada et al, 2021; Sonnenschein et al, 2021). Folkersen et al (2020) identified 90 proteins, including Heat shock protein 27 (Hsp27), Kallikrein-6, Hepatitis A virus cellular receptor 1 (HAVCR1), and Matrix metalloproteinase-10 (MMP10), collectively termed as cardiovascular proteins (CPs), which play a crucial role in cardiovascular processes, enhancing understanding of mechanisms linking HCM and AF.

Mendelian randomization (MR) has emerged as an effective method for assessing the causal effects of protein levels on disease outcomes (Rasooly et al, 2023). MR employs genetic variants strongly associated with exposure factors as instrumental variables (IVs) to infer causal effects between exposure factors and study outcomes, thereby eliminating the impact of common confounding factors such as postnatal environment and lifestyle factors (Davey Smith et al, 2020). This strategy addresses confounding, reverse causation, and regression dilution bias in causal inference (Davey Smith and Hemani, 2014; Lawlor et al, 2008) while also overcoming the constraints of representativeness and feasibility occur in randomized controlled trials (RCTs) (Hemani et al, 2018).

Therefore, the two-sample and bidirectional MR analyses were performed using publicly available Genome-Wide Association Study (GWAS) data for HCM, AF, and CPs. Additionally, a two-step MR approach was applied to explore the role of CPs in the relationship between HCM and AF, aiming to investigate the pathogenic associations among HCM, AF, and CPs, thereby ensuring the biological validity of our genetic findings.

Methods

Study Design

Fig. 1A depicts the comprehensive study design. Specific criteria were used to select Single Nucleotide Polymorphisms (SNPs) from GWAS datasets as IVs to evaluate the causal relationship between HCM and AF. Two-sample and bidirectional MR methods were employed to examine the causal relationships between HCM and AF. Conversely, a two-step MR method was used to calculate the mediating effects of CPs on HCM and AF (Fig. 1C). The MR study design adhered to three core assumptions (Gagliano Taliun and Evans, 2021) (Fig. 1B) and followed the requirements of Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) (Skrivankova et al, 2021).

Data Sources

The genotype detection platforms, quality control standards, and statistical analysis methods used in the selected GWAS datasets are detailed in the studies conducted by Kurki et al (2023), Nielsen et al (2018), and Folkersen et al (2020). GWAS data for HCM (finn-b-I9_HYPERTROCARDMYOP), 90 CPs (Folkersen et al, 2020), and AF (ebi-a-GCST006414) were obtained from the IEU OpenGWAS project website (https://gwas.mrcieu.ac.uk/) on 11 March 2024 (Table 1). As we accessed the data from the public databases, and ethics committee approval and informed consent of participants were already obtained in the original studies; therefore, no additional approval was necessary.

Table 1. Brief information of selected GWAS databases.

Data source	Phenotype	Sample size	Cases	Population
IEU Open GWAS project (finn-b-I9 HYPERTROCARDMYOP)	HCM	218,792	556	European
IEU Open GWAS project	CPs	-	-	European
IEU Open GWAS project (ebi-a-GCST006414)	AF	1,030,836	60,620	European

GWAS, Genome-Wide Association Study; HCM, hypertrophic cardiomyopathy; CPs, cardiovascular proteins; AF, atrial fibrillation.

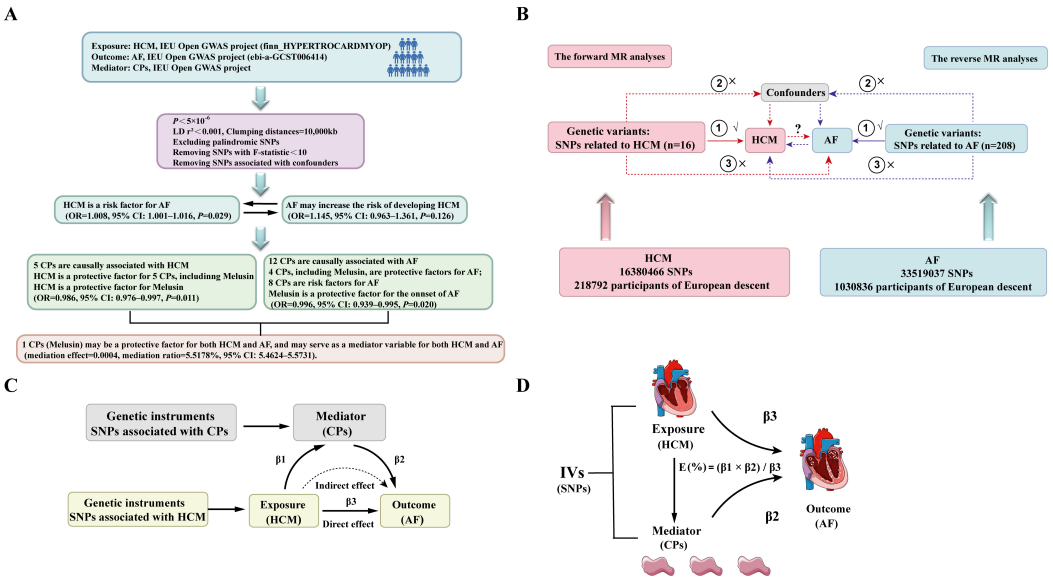

Fig. 1. Study design diagram. (A) Flowchart of the study design (overall framework of the study). (B) Schematic representation of the bidirectional two-sample Mendelian randomization (MR) study design. The red section represents forward MR analysis, with hypertrophic cardiomyopathy (HCM) as the exposure and atrial fibrillation (AF) as the outcome. The blue section represents reverse MR analysis, with AF as the exposure and HCM as the outcome. MR analysis relies on three core assumptions: ① Relevance: the genetic variant is associated with the exposure; ② Independence: the genetic variant is not related to any confounding factors of the exposure-outcome association; ③ Exclusion restriction: the genetic variant does not affect outcome except through its potential effect on the exposure. (C) Schematic diagram of mediation MR. $\beta 1$ represents the MR effect of HCM on cardiovascular proteins (CPs) (mediator), $\beta 2$ represents the MR effect of CPs on AF, and $\beta 3$ is the MR effect of HCM on AF, i.e., the total impact. (D) A flowchart for calculating mediation effects. Fig. 1 is drawn by Adobe Illustrator (Adobe Inc., San Jose, CA, USA) software. SNPs, Single Nucleotide Polymorphisms; IVs, instrumental variables; GWAS, Genome-Wide Association Study; CI, confidence interval; OR, odds ratio; LD, linkage disequilibrium.

Setting of IV Filter Conditions

To increase the number of included SNPs, we used a selection criterion of $p < 5 \times 10^{-6}$ (Bowden and Holmes, 2019; Xiao et al, 2024). Furthermore, a threshold of $F > 10$ was adopted to exclude weak instruments, thereby minimizing potential bias from weak instruments and ensuring a robust association between the IVs and the exposure. This approach aimed to increase the power of the analysis (Bowden and Holmes, 2019; Li et al, 2023a). Moreover, to ensure the independence of IVs and reduce the effects of linkage disequilibrium (LD), we employed standard criteria which incorporated an LD coefficient (r^2) of <0.001 and a physical distance threshold of 10,000 kb for LD. Additionally, we excluded SNPs associated with confounding factors and outcomes by utilizing LDlink (https://ldlink.nih.gov/?tab=ldtrait). Finally, a minimum r^2 threshold of >0.8 was applied to select highly correlated SNPs to replace missing SNPs and exclude SNPs without alternative loci, including palindromic SNPs.

MR Analysis

In bidirectional and two-sample MR analyses, methods such as the inverse-variance weighted (IVW) approach, weighted median estimator (WME), Mendelian Randomization-Egger Regression (MR-Egger) regression, simple mode, and weighted mode are widely used. IVW results are typically the primary determinant of causal associations. When horizontal pleiotropy is observed, MR-Egger regression offers a more precise causal estimation. However, for cases with $\leq$3 SNPs, the Wald ratio method assesses the effect of individual SNPs on the outcome, while the fixed-effects IVW method is used otherwise. For analyses involving three or more SNPs, the random-effects IVW method is applied.

This study primarily examined heterogeneity among SNPs using Cochran's Q test, with a $p < 0.05$ indicating heterogeneity. MR-Egger regression interpreted IVW heterogeneity through the intercept term. In sensitivity analysis, MR-Egger better fits the data, explaining more heterogeneity as the magnitude of its intercept increases and moves away from zero. Moreover, if the MR-Egger regression intercept significantly differs from zero ($p < 0.05$), it indicates horizontal pleiotropy among SNPs (Bowden and Holmes, 2019). During sensitivity analysis, the "leave-one-out" method further validates the robustness by systematically excluding each SNP and observing their individual impacts on the results. All analyses were conducted using the TwoSampleMR package in R 4.1.0 software (R Foundation for Statistical Computing, Vienna, Austria), with an $\alpha = 0.05$. Additionally, the scatter plots, funnel plots, and leave-one-out plots were generated through the TwoSampleMR package (https://github.com/MRCIEU/TwoSampleMR), and the forest plot with the forestplot package (https://cran.r-project.org/web//packages/forestplot/index.html).

Two-Step MR Analysis and Mediation Effect Analysis

Given the critical role of cardiovascular proteins in AF, a two-step MR analysis was conducted to observe whether cardiovascular proteins mediate the impact of HCM on AF. In the first step, SNPs associated with the exposure factor were used to estimate their causal effect on the mediator, examining whether the exposure has a causal association with the mediator. In the second step, SNPs associated with the mediator were used to assess their potential causal effect on the outcome measure. Causal effect estimates in the two-step MR analysis, along with methods for determining heterogeneity, horizontal pleiotropy, and sensitivity, aligned with those used in bidirectional and two-sample MR analyses (see **MR analysis**). Finally, the direct effect of the exposure factor on AF was estimated, followed by evaluation of the indirect effect through the mediator and the proportion mediated.

We estimated three parameters in the mediation analysis: the total effect (the impact of exposure on the outcome through all potential pathways), the direct effect (the effect of exposure on the outcome independent of the mediator), and the indirect effect (the pathway from exposure to the outcome through the mediator). When total, direct, and indirect effects operate in the same direction, the "mediation proportion (E%)"—the proportion of the total effect explained by the mediator—can be calculated. A mediator is considered significant when E% $>$5%. In this study,

E% is calculated using the formula E (%) = (β1 × β2)/β3 (Carter et al, 2021), where β1 represents the effect of HCM on CPs through MR, β2 indicates the effect of CPs on AF through MR, and β3 represents the total effect of HCM on AF through MR (Fig. 1D).

Results

IVs Selection

After screening, 16 SNPs from the HCM dataset (**Supplementary Table 1**), and 208 SNPs from the AF dataset (**Supplementary Table 2**) were included to explore the causal link between HCM and AF. Additionally, in the MR analysis of HCM and 90 CPs, 1508 SNPs were identified, with 12–17 SNPs used as IVs for each CP (**Supplementary Table 3**). For the MR analysis of 90 CPs and AF, 1894 SNPs were identified, with 7–41 SNPs used as IVs for each CP (**Supplementary Table 4**).

The Increased Risk of HCM is Associated with AF

In the forward MR analysis, HCM was identified as a risk factor for AF (OR = 1.008, 95% CI: 1.001–1.016, p = 0.029). In contrast, the reverse MR analysis revealed that AF might increase the risk of developing HCM (OR = 1.145, 95% CI: 0.963–1.361, p = 0.126) (Table 2, **Supplementary Fig. 1**).

The heterogeneity test results from the forward MR analysis showed no heterogeneity among SNPs for HCM and AF (Cochran's Q = 7.107, p = 0.931). However, in the reverse MR analysis, the heterogeneity test indicated potential heterogeneity among SNPs linked to AF and HCM (Cochran's Q = 254.740, p = 0.012). Therefore, the results from the IVW analysis should be considered as conclusive (Cochran's Q = 254.904, p = 0.013) (Table 2, Fig. 2).

In the horizontal pleiotropy and sensitivity analyses, no horizontal pleiotropy was observed among SNPs for HCM and AF (p = 0.333). Similarly, no horizontal pleiotropy was detected among SNPs associated with AF and HCM (p = 0.716) (Table 3). Following the Leave-one-out test, where SNPs linked to AF and HCM were removed sequentially, the results for the remaining SNPs were similar to those obtained when all SNPs were included (**Supplementary Fig. 2**). Additionally, no SNPs were found to significantly impact the causal association estimates, thereby ensuring the robustness of the MR results in this study.

CPs can Act as a Mediating Variable between HCM and AF

Based on the findings from bidirectional and two-sample MR analyses, this study aimed to explore potential mediating pathways from HCM to AF. Initially, SNPs associated with HCM were used to estimate its causal impact on CPs. The MR analysis revealed significant causal relationships between HCM and five CPs, all of which act as protective factors for HCM. These CPs include kallikrein-11 (OR = 0.976, 95% CI: 0.955–0.998, p = 0.029), Tissue factor (OR = 0.992, 95% CI: 0.986–0.998, p = 0.009), Urokinase plasminogen activator surface receptor (OR = 0.992, 95% CI: 0.985–0.999, p = 0.025), Melusin (OR = 0.986, 95% CI: 0.976–0.997, p = 0.011), and Caspase 8 (OR = 0.992, 95% CI: 0.985–0.998, p = 0.013).

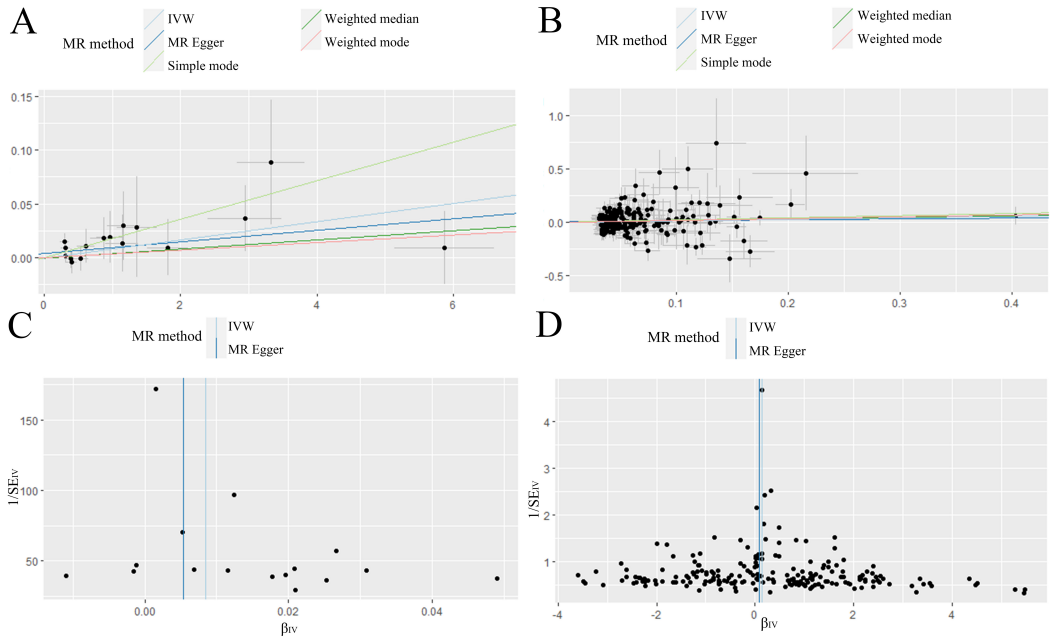

Fig. 2. Scatterplot and funnel plot of HCM and AF. (A) Scatterplot of HCM and AF (forward MR analysis). (B) Scatterplot of AF and HCM (reverse MR analysis). (C) Funnel plot of HCM and AF (forward MR analysis). (D) Funnel plot of AF and HCM (reverse MR analysis). IVW, inverse variance weighting; MR, Mendelian randomization; AF, atrial fibrillation; HCM, hypertrophic cardiomyopathy.

Heterogeneity and horizontal pleiotropy tests indicated no signs of heterogeneity or horizontal pleiotropy among SNPs for HCM and these five CPs, suggesting the reliability of the results (Table 4).

Furthermore, we assessed the causal effect of CPs on AF using SNPs associated with CPs. MR analysis revealed causal relationships between 12 CPs and AF, with Interleukin-6 (OR = 0.921, 95% CI: 0.849–0.999, $p = 0.046$), Renin (OR = 0.891, 95% CI: 0.820–0.968, $p = 0.007$), Melusin (OR = 0.966, 95% CI: 0.939–0.995, $p = 0.020$), and Tumour Necrosis Factor Receptor Superfamily Member 5 (OR = 0.959, 95% CI: 0.928–0.991, $p = 0.012$) identified as protective factors for AF. Conversely, Interleukin-1 Receptor Antagonist (OR = 1.036, 95% CI: 1.001–1.072, $p = 0.044$), Tumour Necrosis Factor Receptor 1 (OR = 1.097, 95% CI: 1.030–1.168, $p = 0.004$), Resistin (OR = 1.053, 95% CI: 1.007–1.100, $p = 0.024$), Myoglobin (OR = 1.095, 95% CI: 1.027–1.167, $p = 0.006$), Dickkopf-related Protein 1 (OR = 1.082, 95% CI: 1.007–1.162, $p = 0.031$), Proheparin-binding epidermal growth factor (EGF)-like Growth Factor (OR = 1.081, 95% CI: 1.007–1.160, $p = 0.032$), Spondin-1 (OR = 1.057, 95% CI: 1.001–1.115, $p = 0.046$), and C-X3-C motif chemokine ligand 1 (CX3CL1) (OR = 1.051, 95% CI: 1.009–1.095, $p = 0.016$) were identified as risk factors for AF. Heterogeneity tests indicated possible heterogeneity among SNPs related to Interleukin-6, Renin, Dickkopf-related Protein 1, and Proheparin-binding EGF-like Growth Factor ($p < 0.05$). Additionally, MR-Egger results revealed no evidence of horizontal pleiotropy among the SNPs associated with CPs and AF ($p > 0.05$) (Table 5).

Table 2. Results of two-sample and bidirectional MR analysis of HCM with AF.

Exposure	SNP	Methods	β	SE	OR (95% CI)	p	Cochran's Q	p
HCM	16	MR-Egger	0.005	0.005	1.005 (0.996–1.015)	0.300	7.107	0.931
		Weighted median	0.004	0.005	1.004 (0.994–1.015)	0.416		
		IVW	0.008	0.004	1.008 (1.001–1.016)	0.029	8.112	0.919
		Simple mode	0.018	0.009	1.018 (0.999–1.037)	0.078		
		Weighted mode	0.004	0.005	1.004 (0.994–1.014)	0.500		
AF	208	MR-Egger	0.082	0.170	1.086 (0.779–1.514)	0.628	254.740	0.012
		Weighted median	0.142	0.158	1.153 (0.846–1.572)	0.367		
		IVW	0.135	0.088	1.145 (0.963–1.361)	0.126	254.904	0.013
		Simple mode	0.199	0.336	1.221 (0.632–2.358)	0.553		
		Weighted mode	0.176	0.154	1.192 (0.882–1.611)	0.254		

β, beta value; SE, standard error of β; OR, odds ratio; IVW, inverse-variance weighted.

Table 3. Results of MR-Egger regression analysis of HCM and AF.

Exposure	Intercept term	SE	p
HCM	0.004	0.004	0.333
AF	0.004	0.011	0.716

SE, standard error of β; OR, odds ratio; MR, Mendelian randomization; AF, atrial fibrillation; HCM, hypertrophic cardiomyopathy.

However, combining the results of the first two MR analyses, one CP (Melusin) showed a significant causal relationship with both HCM and AF, with a mediating effect of 0.0004 (E% = 5.5178%, 95% CI: 5.4624%–5.5731%), thereby serving as a protective factor for both conditions (Fig. 3A,B and Table 6).

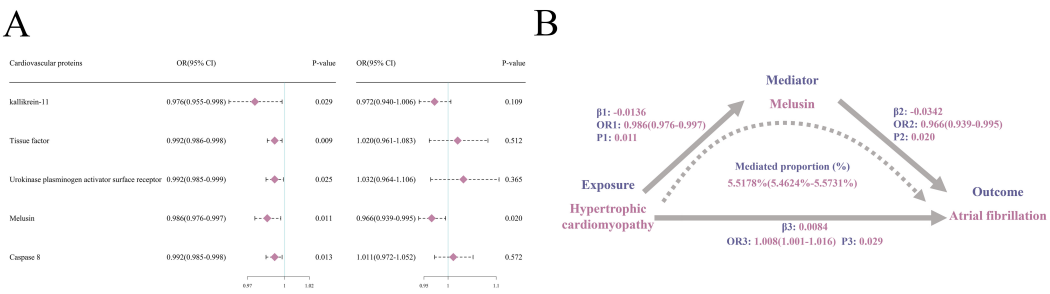

Fig. 3. Plot of two-step MR analysis with mediated effects analysis. (A) Forest plot of the two-step MR analysis results taking the intersection. (B) Melusin's mediation effect analysis plot. OR, odds ratio.

Discussion

This study observed a causal relationship between HCM and AF, demonstrating that HCM may increase the risk of developing AF. However, further research is

needed to elucidate risk direction between AF and HCM. CPs, particularly Melusin, have been identified as mediators in the increased AF risk caused by HCM. These observations enhance our understanding of how HCM contributes to AF and may help guide therapeutic strategies for treating AF resulting from HCM.

Previous studies suggest that AF is a common complication of HCM, with an annual incidence rate of 22.3%, which is closely associated with poor outcomes in HCM patients. Additionally, AF in the context of HCM is linked to increased risks of thromboembolism, heart failure, sudden death, and overall mortality (Alphonse et al, 2021; Falasconi et al, 2020; Ye et al, 2023). This supports the close association between HCM and AF, though causal research on their relationship remains limited. This study reaffirms the association between HCM and AF, validating that HCM may increase the incidence of AF while also addressing gaps in understanding the causal relationship between the two conditions. Notably, previous studies and this research have identified HCM as a risk factor for AF (Philipson et al, 2021). However, whether AF acts as a risk factor for HCM requires further investigation (Chrispin and Marine, 2021). The reverse MR analysis performed in this study suggests that AF may increase the risk of HCM; however, the statistical *p*-value does achieve a level of significance to definitively establish AF as a contributing factor to the increased risk of HCM. Currently, research on assessing the effect of AF on HCM is limited. Future studies evaluating the link between AF and HCM will be beneficial for gaining a deeper understanding of their mechanisms and enhancing treatment approaches for both conditions.

Gaining a deeper understanding of how HCM influences the pathogenesis of AF could improve the diagnosis and treatment of the disease. This study found that HCM may mediate the development of AF through CPs. We observed a close link between HCM and five CPs, while twelve CPs were associated with the incidence of AF. Notably, kallikrein-11 and Urokinase plasminogen activator surface receptors have not been previously explored in HCM research, and Melusin, Tumour necrosis factor receptor superfamily member 5, and Proheparin-binding EGF-like growth factors have not been investigated in relation to AF. This study addresses these gaps and confirms their causal relationships. Previous research has demonstrated that the Tissue factor is altered in HCM, contributing to the pathogenesis of the disease and serving as a potential therapeutic target for HCM (Chaffin et al, 2022; Dimitrow et al, 2007; Laird et al, 2023). However, these studies did not clarify the specific causal direction. Our research establishes the causal role of Tissue factor in HCM, providing insights into targeted therapies for the condition. Caspase-8 is recognized to impact the apoptotic and non-apoptotic pathways in myocardial cells, processes implicated in cardiac fibrosis and hypertrophy in HCM. Additionally, Caspase-8 effects inflammatory signaling and apoptosis of the cells, further exacerbating pathological changes in HCM (Li et al, 2024; Othman et al, 2022; Zhao et al, 2006). There is ongoing debate on the role of Caspase-8 in HCM. Our findings suggest Caspase-8 as a protective factor against HCM development, but further research is needed to clarify its precise mechanisms.

Table 4. Causal association analysis of HCM with CPs in MR regression.

Exposure	Outcome	Nsnp	MR		Heterogeneity		Horizontal pleiotropy		
			OR (95% CI)	*p*	Cochran's Q	*p*	Egger intercept	SE	*p*
HCM	kallikrein-11	16	0.976 (0.955–0.998)	0.029	10.521	0.786	0.006	0.011	0.583
HCM	Tissue factor	17	0.992 (0.986–0.998)	0.009	13.840	0.611	–0.007	0.006	0.243
HCM	Urokinase plasminogen activator surface receptor	17	0.992 (0.985–0.999)	0.025	21.348	0.166	0.009	0.006	0.115
HCM	Melusin	15	0.986 (0.976–0.997)	0.011	15.051	0.375	0.020	0.011	0.089
HCM	Caspase 8	17	0.992 (0.985–0.998)	0.013	15.944	0.457	–0.002	0.007	0.769

SE, standard error; OR, odds ratio; CI, confidence interval; MR, Mendelian randomization; HCM, hypertrophic cardiomyopathy.

Table 5. Causal association results of CPs with AF in MR regression.

Exposure	Outcome	Nsnp	MR		Heterogeneity			Horizontal pleiotropy		
			OR (95% CI)	*p*	I^2 (%)	Cochran's Q	*p*	Egger intercept	SE	*p*
Interleukin–receptor antagonist	AF	24	1.036 (1.001–1.072)	0.044	0	22.945	0.464	0.005	0.004	0.199
Interleukin-6	AF	14	0.921 (0.849–0.999)	0.046	67	39.474	<0.001	0.006	0.009	0.503
Tumor necrosis factor receptor 1	AF	20	1.097 (1.030–1.168)	0.004	13	21.835	0.293	0.007	0.005	0.152
Resistin	AF	41	1.053 (1.007–1.100)	0.024	35	61.981	0.014	0.003	0.003	0.387
Renin	AF	21	0.891 (0.820–0.968)	0.007	61	51.319	<0.001	–0.012	0.007	0.110
Myoglobin	AF	25	1.095 (1.027–1.167)	0.006	28	33.203	0.100	0.005	0.006	0.405
Melusin	AF	18	0.966 (0.939–0.995)	0.020	1	17.167	0.443	–0.005	0.005	0.331
Dickkopf-related protein 1	AF	25	1.082 (1.007–1.162)	0.031	72	87.119	<0.001	–0.007	0.008	0.375
Tumor necrosis factor receptor superfamily member 5	AF	16	0.959 (0.928–0.991)	0.012	23	19.453	0.194	0.002	0.004	0.579
Proheparin-binding EGF-like growth factor	AF	26	1.081 (1.007–1.160)	0.032	61	64.668	<0.001	–0.001	0.009	0.875
Spondin-1	AF	18	1.057 (1.001–1.115)	0.046	60	42.742	0.001	0.001	0.007	0.872
CX3CL1	AF	21	1.051 (1.009–1.095)	0.016	0	14.119	0.824	0.001	0.004	0.709

SE, standard error; OR, odds ratio; CI, confidence interval; MR, Mendelian randomization; AF, atrial fibrillation; CX3CL1, C-X3-C motif chemokine ligand 1; EGF, epidermal growth factor.

Table 6. Mediating effect of HCM on AF through cardiovascular proteins.

Mediator	β1	β2	Mediating effect	Direct effect	Total effect	E (%)
Melusin	–0.0136	–0.0342	0.0004	0.008	0.0084	5.5178 (5.4624–5.5731)

β, beta value; E%, mediation proportion.

Interleukin-1 receptor antagonists have been associated with inflammatory mechanisms and mortality during AF and reported as a risk factor for AF (Amdur et al, 2016; Lappegård et al, 2013). It is believed that tumor necrosis factors can induce adverse changes in arteries and increase AF susceptibility during intense exercise, interfering with cell-autonomous effects and atrial cell crosstalk (Lakin et al, 2023). Resistin levels are higher in AF patients (Samanidis et al, 2020) and may cause postoperative AF (Rachwalik et al, 2019). A previous study has shown that Resistin may induce cardiovascular disease by inducing acute positive inotropic effects but may not cause AF by inducing arrhythmias (Aitken-Buck et al, 2020). Our study is consistent with these findings and reaffirms these three CPs as risk factors for AF, which could help enhance AF treatment.

Additionally, factor receptor 1 is also found to play a crucial role in sodium/calcium homeostasis in AF and may potentially serve as a biomarker for AF (Chang et al, 2021; Pol et al, 2022). Dickkopf-related protein 1 is associated with cardiovascular events (including AF) and mortality among dialysis patients (Stavrinou et al, 2021). Furthermore, Spondin-1 has been reported as the most significantly upregulated biomarker in AF patients (Santema et al, 2022). Elevated levels of CX3CL1 have been linked with the prognosis of AF (Guo et al, 2014). They could serve as a potential biomarker for increased risk of thrombosis and bleeding in AF populations (Tai et al, 2022).

Similarly, increased Myoglobin expression levels have been observed in the hypercoagulable state of AF (De With et al, 2022). This study confirms that these CPs are all associated with an increased risk of AF, but their underlying mechanisms require further studies. Indeed, this study confirms Renin as a factor likely to reduce AF risk (Menichelli et al, 2024; Sun et al, 2024). We observed inconsistent results regarding the role of Interleukin-6 (IL-6) in AF. A previous study has indicated IL-6 as a risk factor for AF and reducing its levels or inhibiting its signalling pathway could serve as a potential strategy for treating this disease (Li et al, 2023b). However, this study suggests that IL-6 is a protective factor against AF. Mechanisms associated with inflammation within AF are complex and yet to be fully explored (Zhang and Dhalla, 2024). As a crucial inflammatory marker and regulatory factor, IL-6 exhibits considerable diversity and complexity. Currently, research has primarily viewed IL-6 as a pro-inflammatory factor (Jelinek and Duris, 2023), with most studies being observational and with limitations for exploring causality. Consequently, our study suggests that previous observational studies have some false-positive results, and further clinical trials are needed to establish definitive results.

This study confirms the protective role of Melusin in regulating the risk of HCM and AF. This means that Melusin might mediate the increased risk of AF

associated with HCM. As a muscle-specific signaling protein, Melusin plays a crucial role in cardiac stress response through the sensing and adaptation of myocardial cells to mechanical stress. Disruption in the regulation of Melusin may affect myocardial fibrosis and functional disturbances (Arina et al, 2022; Sun et al, 2019; Vitadello et al, 2020). This is consistent with the findings of our study. Previous research has suggested that overactivation of Melusin leads to pathological myocardial hypertrophy, which can progress to HCM. Unfortunately, there is limited research on Melusin in the pathogenesis of AF. This study hypothesizes that Melusin could influence the stability of the myocardial structure, including extracellular matrix remodeling and myocardial fibrosis, and electrophysiological properties, such as the regulation of ion channel expression and function, by modulating myocardial cell adaptation to mechanical stress and anti-apoptotic signaling, thus promoting or inhibiting AF development (Arina et al, 2022; Sun et al, 2019). In the pathogenesis of HCM, Melusin may bind to integrins, thus activating downstream signaling pathways, such as Extracellular Signal-Regulated Kinases 1/2 (ERK1/2) and Protein Kinase B (AKT), that promote myocardial cell proliferation and survival. This process can mediate the myocardial hypertrophic response to cardiac pressure overload (Sorge and Brancaccio, 2016; Vitadello et al, 2020). It is important to note that the E% of Melusin between HCM and AF is only 5.5178%. This suggests that Melusin plays a regulatory role in the link between HCM and AF but may not be through the primary pathway. HCM and AF pathogenesis are influenced by multiple factors and pathways, and the presence of Melusin may reveal subtle yet significant signal transduction or cellular processes, particularly within the microenvironment of myocardial functional adaptation and pathological changes. It might have a more pronounced effect in certain subgroups of patients or under particular pathological conditions. This can enhance the understanding of the mechanistic links between HCM and AF, providing a clue for a multidimensional understanding of complex disease mechanisms. Moreover, this study indicates that beyond Melusin, there are more critical mediating factors possibly undiscovered or unexplored, thereby offering direction for future studies of other potential biomarkers or mechanisms. Future research is warranted to explore the specific role of Melusin in HCM and AF and its potential as a therapeutic target.

MR is a powerful tool for addressing problems in human biology and epidemiology. MR analysis methods draw statistical techniques from economics, allowing researchers to assess the impacts of environmental factors, pharmacological interventions, and other aspects of human biology and disease (Birney, 2022). Mediation analysis in MR decomposes the effects of exposure on an outcome into direct effects and effects mediated through intermediary variables. This method estimates causal effects among exposure, intermediary variables, and outcomes, with the possibility of their subsequent disaggregation. This approach retains the advantages of using genetic instruments for causal inference, while reducing confounding related biases. It can estimate the effect sizes required for mediation analysis (Sanderson, 2021). We used MR analysis methods to minimize confounding factors inherent in basic and observational research on cardiovascular diseases, thereby reducing the likelihood of reverse causation.

Additionally, this method diminishes biases introduced by subjective measurement errors such as self-reports, enhancing the reliability of causal inference. Compared to non-instrumental variable mediation methods, instrumental variable mediation approaches provide a better causal inference on mediation analysis (Carter et al, 2021). The study further validates potential therapeutic targets for HCM and AF that can guide drug development (Daghlas and Gill, 2023; Evans, 2022; Levin and Burgess, 2024).

However, besides several promising outcomes, this study has some limitations. We used a threshold p-value of $<5 \times 10^{-6}$ for selecting genes with strong associations, which may make the link between IVs and exposures weaker, leading to inefficient causal inference (Burgess et al, 2017). Such a weak instrument bias is crucial in MR analysis. Furthermore, post-hoc selection of instruments, genetic models, or measurement-based F-statistics may exacerbate bias. Research indicates that decreased expected F-statistic can increase the bias; however, it can be reduced by using simpler genetic association models, such as not over-parameterized, and adjusting for covariates in the measurements. Also, where the observed F-statistic exceeds the expected value, causal estimates are more likely to indicate associations with lesser standard errors. This correlation between causal estimates and standard errors introduces a second source of bias in MR research. Again, the heuristic rule of $F > 10$ as a source to avoid bias in IV analyses may also fail (Burgess and Thompson, 2011).

Moreover, genetic variants in MR studies may be the source of confounding outcomes through multiple pathways, which can confound causal relationships (van Kippersluis and Rietveld, 2018). Genetic effects in MR assumptions are considered independent of environmental factors, but the effects of genes can alter due to environmental conditions (Spiller et al, 2019). Since MR analysis depends on the sample representativeness, there is a risk of selection bias (Gkatzionis et al, 2024; Minică et al, 2020; Swanson, 2019; Wang and Han, 2021). MR analysis is primarily applied to exposures that are linked to genetic variations and is not applicable to all types of exposures (Glynn, 2010; Relton and Davey Smith, 2015; Smith and Ebrahim, 2004). Therefore, to mitigate the possibility of bias, this study needs to incorporate individual-level data and combine genetic effects to cross-study sets for further experimental validation. Future MR research should focus on developing new computational tools that enhance the selection and validation of instrumental variables, developing more precise genetic instruments to improve the credibility of causal inference, and new methods to correcting and detecting the impact of genetic pleiotropy in MR (Guo et al, 2014; Guo et al, 2023; Liu et al, 2023; Sheehan and Didelez, 2020). Expanding the scope of MR to include a wider range of phenotypes and environmental exposures and integrating genomics with transcriptomics and metabolomics will enhance the depth of MR analysis (Auwerx et al, 2023).

Conclusion

HCM may increase the risk of AF. However, the association of AF with the increased HCM risk requires further investigation. Additionally, CPs may be in-

volved in increased AF risk that occurs with HCM. Among CPs, Melusin could be a crucial protein implicated in the development of AF due to HCM. However, further investigation into the role of Melusin in HCM-induced AF would contribute to developing novel therapeutic modalities for managing HCM.

Key Points

- AF is the most common sustained arrhythmia in patients with HCM, but the causal relationship between the two remains unclear.
- Genetic prediction of HCM may increase the risk of developing AF, but further research is needed to understand the increased risk of AF associated with HCM.
- Genetic prediction of CPs might be associated with an increased risk of developing AF in patients with HCM.
- Melusin may be a crucial CPs implicated in the development of AF caused by HCM.

Availability of Data and Materials

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

Author Contributions

YFZ and WXY conceived and designed this project and gave critical revision of the manuscript. CYG, LXW and LW helped with the analysis of data. JL and XH assisted with data collection and assembly. YFZ drafted the manuscript. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://www.magonlinelibrary.com/doi/suppl/10.12968/hmed.2024.0504.

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