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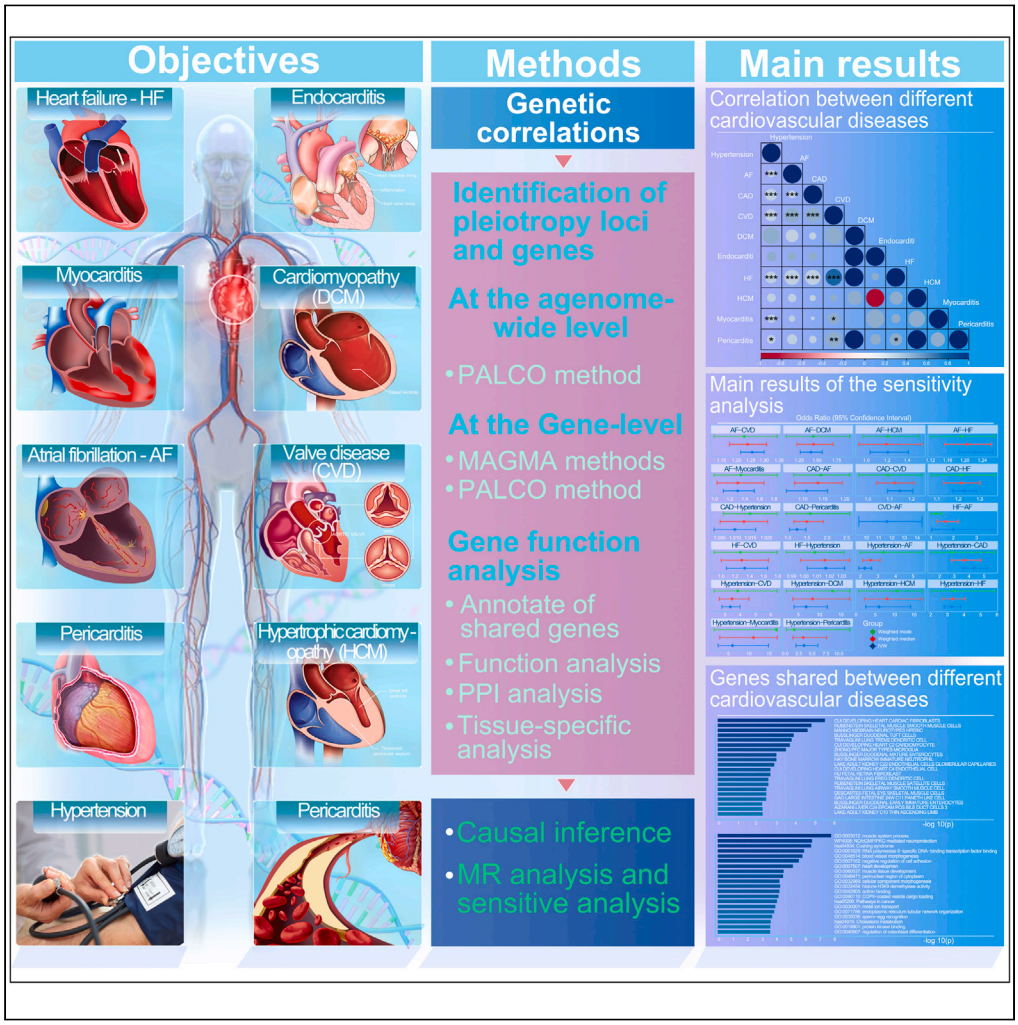

Zeye Liu, Jing Xu, Jiangshan Tan, ..., Yuan Huang, Shoujun Li, Xiangbin Pan

clevelandhuangyuan@163.com (Y.H.)
drlishoujunfw@163.com (S.L.)
panxiangbin@fuwaihospital.org (X.P.)

Highlights

We conducted a comprehensive gene-centric pleiotropic association analysis for 10 CVDs

Over two-thirds of the CVDs exhibit common genes and SNPs

Identified genes and pathways could provide novel targets for CVDs prevention

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Genetic overlap for ten cardiovascular diseases: A comprehensive gene-centric pleiotropic association analysis and Mendelian randomization study

Zeye Liu,[1,2,3,4,9] Jing Xu,[5,9] Jiangshan Tan,[6,9] Xiaofei Li,[7,9] Fengwen Zhang,[1,2,3,4] Wenbin Ouyang,[1,2,3,4] Shouzheng Wang,[1,2,3,4] Yuan Huang,[8,*] Shoujun Li,[8,*] and Xiangbin Pan[1,2,3,4,10,*]

SUMMARY

Recent studies suggest that pleiotropic effects may explain the genetic architecture of cardiovascular diseases (CVDs). We conducted a comprehensive gene-centric pleiotropic association analysis for ten CVDs using genome-wide association study (GWAS) summary statistics to identify pleiotropic genes and pathways that may underlie multiple CVDs. We found shared genetic mechanisms underlying the pathophysiology of CVDs, with over two-thirds of the diseases exhibiting common genes and single-nucleotide polymorphisms (SNPs). Significant positive genetic correlations were observed in more than half of paired CVDs. Additionally, we investigated the pleiotropic genes shared between different CVDs, as well as their functional pathways and distribution in different tissues. Moreover, six hub genes, including *ALDH2*, *XPO1*, *HSPA1L*, *ESR2*, *WDR12*, and *RAB1A*, as well as 26 targeted potential drugs, were identified. Our study provides further evidence for the pleiotropic effects of genetic variants on CVDs and highlights the importance of considering pleiotropy in genetic association studies.

INTRODUCTION

Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality worldwide.[1] According to the World Health Organization (WHO), CVDs accounted for 17.9 million deaths in 2019, representing 32% of all global deaths.[2] The burden of CVDs is expected to increase further in the coming years, primarily due to population aging, lifestyle changes, and the increasing prevalence of risk factors such as obesity, diabetes, and hypertension. The prevalence of CVDs has nearly doubled from 271 million in 1990 to 523 million in 2019, and the number of CVDs deaths has steadily increased from 12.1 million in 1990 to 18.6 million in 2019.[3] Genetic and genomic factors play a vital role in the development and progression of CVDs. Indeed, previous research has identified a multitude of common and rare genetic variants that contribute to the risk of disease and impact outcomes.[4,5]

Common polymorphisms (with a minor allele frequency >1%) are the primary focus of contemporary genetic studies investigating complex diseases. It is estimated that there are over 10 million common single nucleotide polymorphisms (SNPs) in the human genome.[6] Due to the presence of common alleles in polymorphisms, multiple combinations of susceptibility alleles at different loci in an individual are possible, and some of these combinations may impact the risk of CVDs in a manner that cannot be predicted from the isolated effects of each variant.[7] This poses a significant challenge in characterizing the genetics of complex traits and supports the proposal to investigate gene systems rather than individual genes.[8,9] Despite significant advances in our understanding on the genetic basis of CVDs, a considerable proportion of the heritability remain unexplained.[10] Genome-wide association studies (GWASs) have identified numerous loci associated with CVDs, but the majority of the identified variants has modest effect sizes and explain only a small fraction of the total heritability. This suggests that there may be additional genetic factors that contribute to the risk of CVDs, which are yet to be discovered.

[1]Department of Structural Heart Disease, National Center for Cardiovascular Disease, China & Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100037, China
[2]National Health Commission Key Laboratory of Cardiovascular Regeneration Medicine, Beijing 100037, China
[3]Key Laboratory of Innovative Cardiovascular Devices, Chinese Academy of Medical Sciences, Beijing 100037, China
[4]National Clinical Research Center for Cardiovascular Diseases, Fuwai Hospital, Chinese Academy of Medical Sciences, Beijing 100037, China
[5]State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Fuwai Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College, Beijing, China
[6]Key Laboratory of Pulmonary Vascular Medicine, National Clinical Research Center of Cardiovascular Diseases, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, China
[7]Department of Cardiology, Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China
[8]State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Pediatric Cardiac Surgery Center, Fuwai Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College, Beijing, China
[9]These authors contributed equally
[10]Lead contact
*Correspondence: clevelandhuangyuan@163.com (Y.H.), drlishoujunfw@163.com (S.L.), panxiangbin@fuwaihospital.org (X.P.)
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Table 1. Characteristics of the included studies

Phenotypes	Cases	Controls	Sample Size	Reference
Heart failure	47,309	930,014	977,323	Shah et al.[17]
Coronary artery disease	122,733	424,528	547,261	van der Harst et al.[18]
Cardiac valve disease	25,070	440,457	465,527	Sakaue et al.[19]
Hypertension	129,909	354,689	484,598	Dönertaş et al.[20]
Dilated cardiomyopathy	1,444	353,937	355,381	Sakaue et al.[19]
Hypertrophic cardiomyopathy	507	489,220	489,727	Sakaue et al.[19]
Atrial fibrillation	60,620	970,216	1,030,836	Nielsen et al.[21]
Myocarditis	633	427,278	427,911	Sakaue et al.[19]
Endocarditis	267	456,081	456,348	Jiang et al.[22]
Pericarditis	1,795	453,370	455,165	Sakaue et al.[19]

Recent studies suggest that pleiotropic effects, in which a single genetic variant influences multiple traits, may be a key feature underpinning the genetic architecture of complex diseases.[11] Pleiotropy has been observed for numerous genetic variants associated with CVDs, such as the APOE gene, which is associated with both Alzheimer's disease[12] and CVDs,[13] and the PCSK9 gene, which is associated with lipid metabolism[14] and CVDs.[15] A gene-centric approach that accounts for the pleiotropic effects of genetic variants may therefore help identify novel associations with CVDs and provide insight into the underlying biological mechanisms.[11] A gene-centric approach focuses on genes rather than individual genetic variants and aggregates information from multiple variants within a gene to increase statistical power and reduce the multiple testing burden.[16] However, there is still much to be learned about the complex interplay between genetic pleiotropy and CVDs development, highlighting the need for further research in this field.

In this study, we conducted a comprehensive gene-centric pleiotropic association analysis for ten CVDs using GWAS summary statistics. Our aim was to identify genetic loci and pathways that are associated with multiple CVDs and to investigate the extent of pleiotropy among the identified loci. The ten CVDs included in our analysis were heart failure (HF), coronary artery disease (CAD), cardiac valve disease (CVD), hypertension, dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), atrial fibrillation (AF), myocarditis, endocarditis, and pericarditis. By identifying pleiotropic genes and pathways that are associated with multiple CVDs, our study may provide insight into the shared biological mechanisms that underlie different CVDs types. These findings may have important implications for the development of novel therapeutic interventions and personalized treatments for CVDs.

RESULTS

Basic characteristics of the included trials

As shown in Table 1, a total of 10 trials were included in the present analysis. The range in sample size varied from 267 to 129,909 for patients and 353,937 to 970,216 for controls. All the individuals included in the study, both patients and controls, were drawn from the European cohort, which reduces genetic bias due to race.

Estimated cross-trait genetic correlation analysis

In the current study, we utilized PLACO to identify pleiotropic genes between every CVDs pair. Our analysis revealed several genes that demonstrated pleiotropic associations between different CVDs, suggesting potential shared genetic mechanisms underlying the pathophysiology of these diseases. As illustrated in Figure 1, a substantial majority of CVDs (77.78% or 35 out of 45) share common genes (Figure 1A), with a noteworthy 84.4% (38 out of 45) of CVDs exhibiting shared SNPs (Figure 1B). The most pronounced genetic linkages were observed between hypertension and AF, hypertension and CAD, as well as AF and HF.

Furthermore, more than half of the CVDs pairs (82.2% or 37 out of 45) exhibited positive genetic correlations, with an average coefficient of 0.365 and individual correlation coefficients ranging from −0.962 between HCM and endocarditis to the correlation of 1.000 between DCM and HF, DCM and pericarditis, as well as HCM and pericarditis. Notably, approximately 55.6% (25 out of 45) of these genetic correlation estimates had p values <0.05, and 22.2% (10 out of 45) remained statistically significant even after Bonferroni correction (Figure 2). The genetic correlation analysis results between every two CVDs are shown in Table S1. Using PLACO to identify the multi-effect genes between every two diseases and the multi-effect SNPs between every two diseases are shown in the attachment Tables S2 and S3.

Causal estimation between paired CVDs

As shown in Tables S4 and S5, genetically predicted AF was causally associated with higher risks of CVD with an odds ratio (OR) of 1.256 (95% confidence interval (CI) = 1.216–1.298; $p < 0.001$), DCM (OR = 1.409; 95% CI = 1.269–1.565; $p < 0.001$), HCM (OR = 1.210; 95% CI = 1.037–1.412; $p = 0.015$), HF (OR = 1.225; 95% CI = 1.186–1.264; $p < 0.001$), and myocarditis (OR = 1.305; 95% CI = 1.114–1.529; $p < 0.001$). Genetically predicted CAD was causally associated with higher risks of AF (OR = 1.11; 95% CI = 1.062–1.160; $p < 0.001$), CVD (OR = 1.147; 95% CI = 1.091–1.207; $p < 0.001$), HF (OR = 1.229; 95% CI = 1.176–1.284; $p < 0.001$), hypertension (OR = 1.010; 95% CI = 1.003–1.017; $p = 0.003$), and

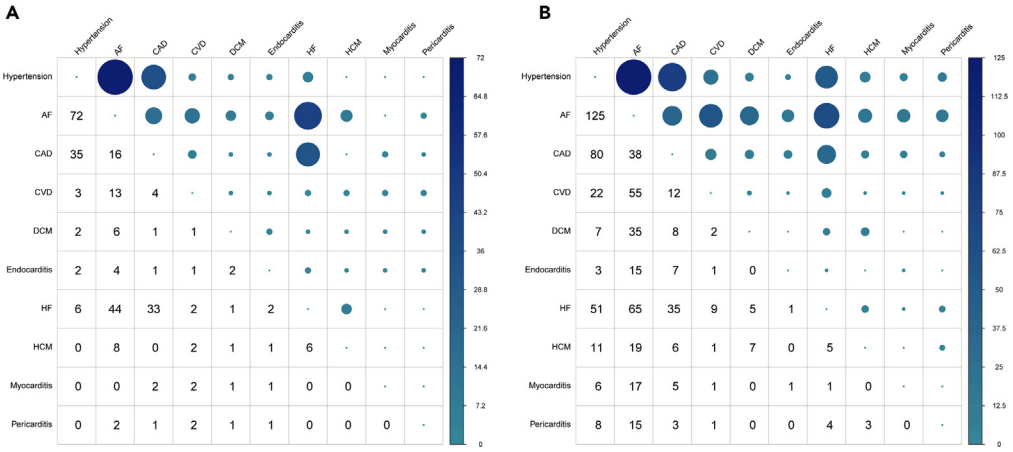

Figure 1. Numbers of genes and SNPs shared among cardiovascular diseases

(A) Numbers of genes shared among cardiovascular diseases.

(B) Numbers of SNPs shared among cardiovascular diseases.

SNP, single-nucleotide polymorphism; HF, heart failure; CAD, coronary artery disease; CVD, cardiac valve disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; AF, atrial fibrillation.

pericarditis (OR = 1.232; 95% CI = 1.041–1.458; p = 0.015). Genetically predicted CVD was causally associated with a higher risk of AF with an OR of 11.633 (95% CI = 9.403–14.392; p < 0.001). Genetically predicted HF was causally associated with higher risks of AF (OR = 1.979; 95% CI = 1.032 = 3.795; p = 0.040), CVD (OR = 1.332; 95% CI = 1.073–1.653; p = 0.009), and hypertension (OR = 1.017; 95% CI = 1.002–1.032; p = 0.024). Genetically predicted hypertension was causally associated with higher risks of AF (OR = 2.231; 95% CI = 1.894–2.627; p < 0.001), CAD (OR = 4.312; 95% CI = 3.58–5.193; p < 0.001), CVD (OR = 3.355; 95% CI = 2.819–3.994; p < 0.001), DCM (OR = 6.778; 95% CI = 3.915–11.733; p < 0.001), HCM (OR = 5.914; 95% CI = 2.492–14.032; p < 0.001), HF (OR = 3.271; 95% CI = 2.810–3.808; p < 0.001), myocarditis (OR = 5.944; 95% CI = 2.382–14.836; p < 0.001), and pericarditis (OR = 3.469; 95% CI = 1.993–6.038; p < 0.001). The Beta values are shown in Figure 3.

In the sensitivity analysis, the weighted median and weighted mode showed similar results to those in the inverse-variance weighted (IVW) Mendelian randomization (MR) analysis (Figure 4), suggesting that the causal estimation in the present MR analysis was robust. Most causal relationships persisted after Bonferroni correction, except for the causal relationship between AF and myocarditis, AF and HCM, CAD and hypertension, CAD and pericarditis, HF and CVD, HF and hypertension, and HF and AF. The summaries of instrumental variables (IVs) and sensitivity analysis results are shown in Tables S6 and S7, respectively. In addition, we performed multiple corrections on the results, and the significant results after *Bonferroni* correction are highlighted in Table S7. The selected threshold for the correction was 0.05/45 = 0.0011.

Enrichment analysis for identified pleiotropic genes

Twenty-four genes were expressed in at least five CVDs (Figure 5A). Most of the shared genes in the heart and vascular tissues had significantly increased expression abundances, especially *SWAP70*, *CAMK2G*, *NDST2*, *CFDP1*, *SEC24C*, *CHCHD1*, and *XPO1* (Figure 5B). Furthermore, cell type signatures corresponding to "Cui developing heart cardiac fibroblasts," "Rubenstein skeletal muscle smooth muscle cells," and "Manno midbrain neurotypes hperic" were the most enriched (Figure 6A). In addition, the results of the Gene Ontology (GO) analysis revealed significant enrichment in three functional categories, including biological processes (BP), cellular components (CC), and molecular functions (MF) (Figure 6B). In the BP category, the common genes exhibited enrichment in muscle system process, blood vessel morphogenesis, negative regulation of cell adhesion, and heart development. In the CC category, significant enrichment was observed in the perinuclear region of the cytoplasm. For the MF term, the common genes were predominantly enriched in RNA polymerase II-specific DNA-binding transcription factor binding, histone H3K9 demethylase activity, actinin binding, and protein kinase binding. Furthermore, the enrichment analysis of signaling pathways using Kyoto Encyclopedia of Genes and Genomes (KEGG) revealed that these common genes were primarily involved in Cushing syndrome, cancer, and cholesterol metabolism. In the WikiPathways, our results showed that 6 genes were enriched in the pathway of NO/cGMP/PKG-mediated neuroprotection.

Protein interaction and module analysis

After generating a list of genes, we utilized the STRING website to construct PPI networks (Figure 7A). Subsequently, we employed the molecular complex detection (MCODE) application to identify a significant gene module, which consisted of 6 genes/nodes and 12 edges, including *ALDH2*, *XPO1*, *HSPA1L*, *ESR2*, *WDR12*, and *RAB1A* (Figure 7B).

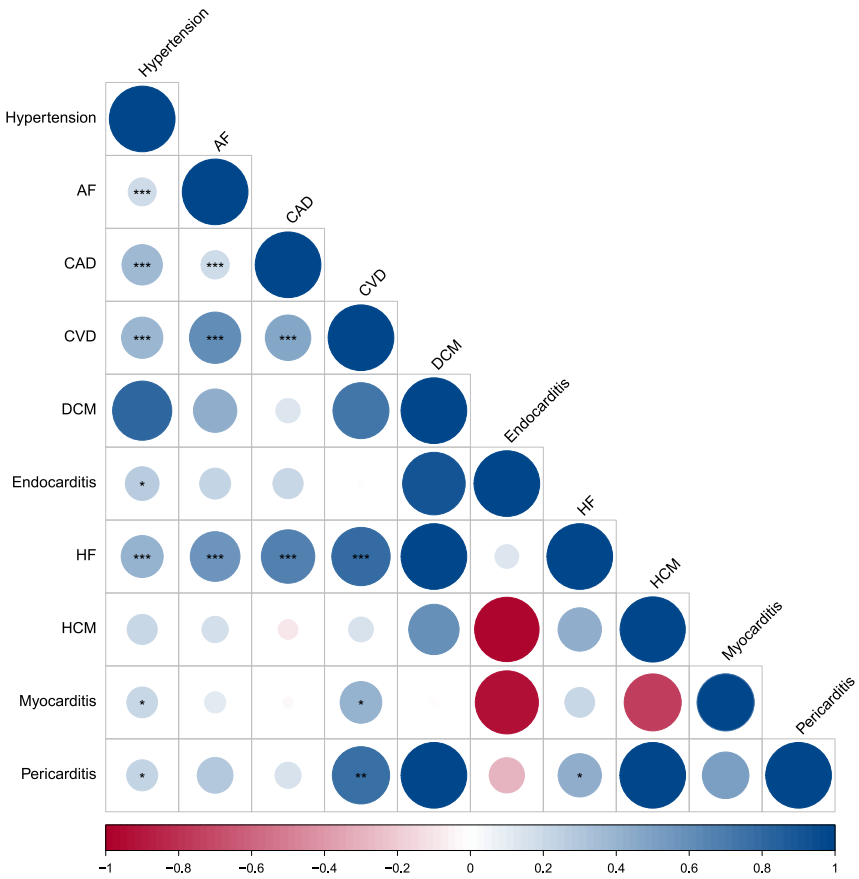

Figure 2. Heatmap showing the correlation between different cardiovascular diseases

The color intensity of bubbles represents the strength of the correlation, with significant values denoted as * = $p < 0.05$; ** = $p < 0.01$; and *** = $p < 0.001$. HF, heart failure; CAD, coronary artery disease; CVD, cardiac valve disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; AF, atrial fibrillation.

Drug-gene interaction analysis of potential genes

To streamline our analysis and identify potential high-efficiency drugs for the treatment of CVDs, the hub genes were selected for further investigation. Finally, 26 potential existing drugs were identified in 4 hub genes, which were categorized into 7 different drug-gene interaction types. The potential gene targets of these drugs included *ALDH2*, *ESR2*, *HSPA1L*, and *XPO1*. Of the 26 drugs, 4 targeted *ALDH2*, 16 targeted *ESR2*, 1 targeted *HSPA1L*, and 5 targeted *XPO1*. All of these drugs have initial drug indications that are approved by the FDA (Table 2).

DISCUSSION

In this study, we conducted a comprehensive gene-centric pleiotropic association analysis of 10 CVDs using GWAS summary statistics. Furthermore, we identified 6 hub genes associated with multiple CVDs and 26 potential drugs. Additionally, we found a wide range of causal associations between different CVDs types, which suggests that the different CVDs may represent manifestations of the same pathophysiological state. Therefore, treatment should involve a holistic approach rather than narrowly focus on one manifestation. In other words, on the basis of focusing on a specific manifestation, more attention should be given to targeting the underlying cause of CVDs.

In this paper, several bioanalytical methods were performed to analyze public summary statistics and GWAS data, which provide important insight into the genetic background underlying different CVDs. Genetics exerts a prominent influence on cardiovascular development and disease, encompassing various aspects ranging from the structural integrity of blood vessels to intercellular communication within the heart.[46] A familial history of premature CAD has been shown to increase the odds of developing CVD by approximately 50%, irrespective of traditional clinical risk factors.[47] Furthermore, twin studies comparing monozygotic twins with dizygotic twins have demonstrated that the variance in susceptibility to CAD,[48] AF,[49] and diabetes[50,51] can be attributed to common genetic variations. These findings collectively suggest that genetics contributes additively to risk prediction for CVDs.[47,49,51,52] Our findings provide further evidence supporting the substantial heritability and shared genetic components of various CVDs at the genome-wide level, and suggest that these disorders may represent extreme manifestations of underlying continuous heritable traits. This observation also offers a plausible explanation for the frequently observed comorbidity among CVDs in epidemiological studies.[53,54] While some genetic correlations were not statistically significant, these null genetic

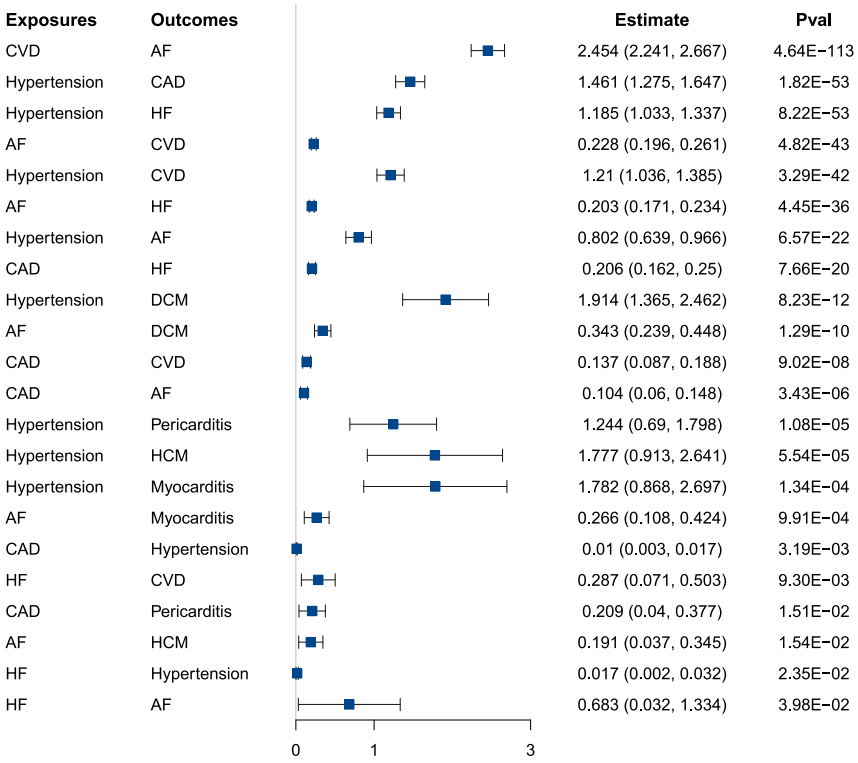

Figure 3. Main results of the MR analysis

MR, Mendelian randomization; HF, heart failure; CAD, coronary artery disease; CVD, cardiac valve disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; AF, atrial fibrillation.

correlations may have been influenced by the considerable estimation uncertainty resulting from the limited sample sizes of certain CVDs included in the analyses.

Furthermore, our findings based on estimated genetic correlations revealed the existence of distinct subgroups among these CVDs, challenging the biological validity of current diagnostic approaches that primarily rely on expert opinions, subjective descriptions, patient experiences, and observational or syndromic systems of diagnosis and classification. This genetic overlap among CVDs also suggests the potential for alternative nosology informed by the similarity of disease genetic architecture, in addition to clinical manifestations. By employing PLACO for pleiotropy association analysis, we identified a substantial number of potential pleiotropic genes associated with these CVDs. Moreover, our MR analysis uncovered a wide range of substantial causal associations among CVDs, suggesting that these disorders may actually cause each other and that genetic overlap and causality may underlie the observed coexistence of CVDs.[53,54]

Our study presents two distinct features that differentiate it from previous pleiotropy studies of CVDs that have primarily focused on individual SNPs.[55,56] First, we employed a gene-centric analysis approach based on a set of local SNPs rather than individual genetic variants. This strategy considers that a gene is a more biologically meaningful functional unit in living organisms and typically contains multiple association signals. As such, SNP-set analysis, as an effective alternative strategy, is generally more powerful than single-marker analysis due to the aggregation of multiple weak association signals and reduced burden of multiple testing.[57–64] Second, we explicitly addressed the issue of pleiotropy identification from a statistical perspective of a composite null hypothesis and employed PLACO, a novel method, to detect genes with pleiotropic effects.[16] In contrast to previous methods where error rate control for familywise error rate (FWER) was not well studied, PLACO demonstrated well-calibrated error control and superior power compared to existing methods. Notably, PLACO remains valid even when overlapping subjects exist between diverse GWASs, which is not uncommon in large-scale *meta*-GWAS for phenotypically correlated traits.[16] It is important to highlight that overlapping subjects can inflate test statistics of association signals,[65–67] and therefore, our pleiotropy analysis implemented with PLACO is less likely to be biased by overlapping subjects.

Most importantly, our results have important implications for understanding the genetic architecture underlying CVDs. By identifying pleiotropic loci associated with multiple CVDs, we provide new insight into the shared genetic mechanisms that underlie these diseases. These findings may help to identify new drug targets and inform the development of personalized therapies for CVDs. Furthermore, our study highlights the importance of gene-centric approaches in identifying pleiotropic loci that are missed by traditional single-SNP analyses.

In this study, the drug-gene interaction analysis revealed six key genes and 26 potential drugs that correlate with CVDs in varied ways. For instance, disulfiram, a medication for alcohol dependency, reportedly has cardioprotective effects, such as improving left ventricular function and diminishing oxidative stress.[68,69] However, its use is highly contraindicated for patients with significant CAD or HF, and it may trigger

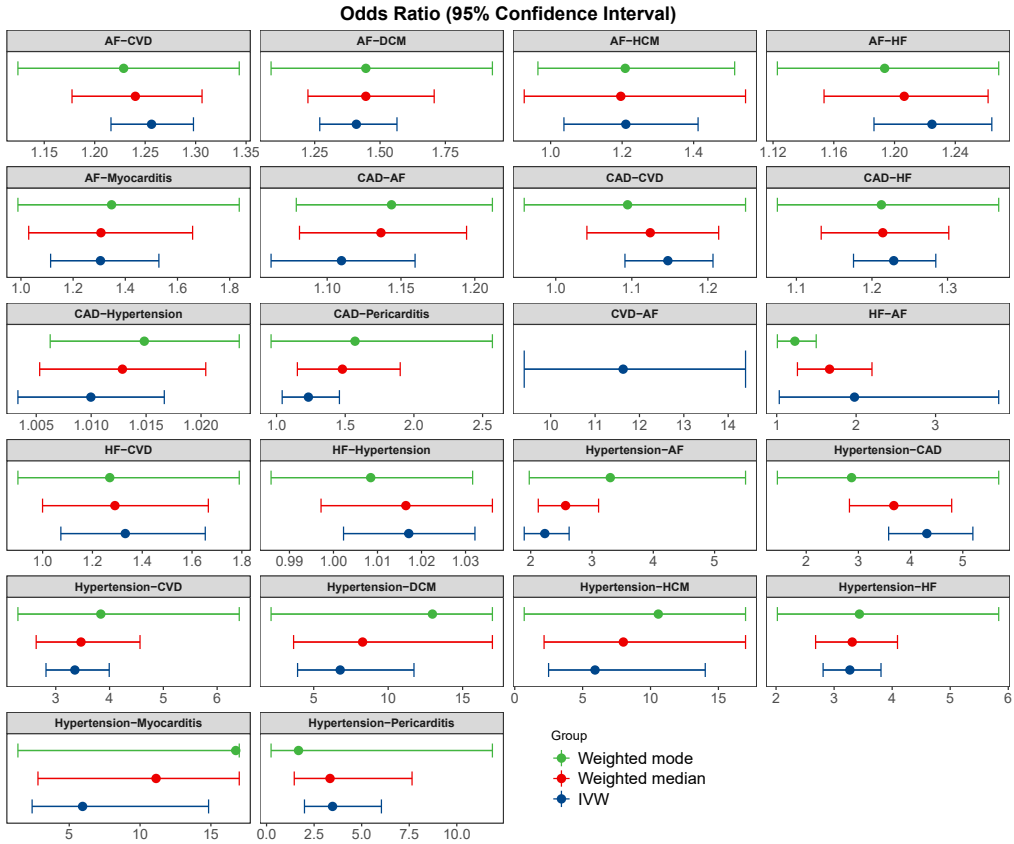

Figure 4. Main results of the sensitivity analysis

HF, heart failure; CAD, coronary artery disease; CVD, cardiac valve disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; AF, atrial fibrillation.

severe reactions in CVDs patients.[70] Diacetylmorphine, or heroin, underwent scrutiny for its cardiovascular effects.[71] Evidence suggests no cardiovascular or respiratory side effects from oral and rectal administration, yet reports argue heroin-related fatalities and the intricacies of heroin metabolism are underreported.[72] The toxic metabolite of alcohol, acetaldehyde, is linked with escalating oxidative stress and inflammation, known contributors to CVDs development.[73] Notably, alcohol consumption, in chronic, heavy forms, fosters the development of hypertension, arrhythmias, and cardiomyopathy.[73] On the other hand, moderate consumption might offer some protection. Medications like tamoxifen, fulvestrant, erteberel, raloxifene, and others used for breast cancer treatment have diverse CVD effects.[74,75] Some enhance cardiovascular event risks, while others lessen them.[76] Tamoxifen reportedly both shields and harms CVDs, subject to dose, treatment duration, and other conditions.[77–79] Additionally, letrozole, gemcitabine, and carbamazepine correspond to higher cardiovascular event risks. Conversely, selinexor, leptomycin B, osthole, and goniothalamin show potential cardioprotective effects in early stage studies.[80–83] Isoniazid, used for tuberculosis, shows minimal cardiovascular system correlation.[84] Notably, the effects of these drugs on CVDs may vary depending on the dose, duration of treatment, patient characteristics, and other factors. Further research is needed to fully understand their potential risks and benefits. Additionally, some of the other drugs listed may have indirect effects on CVDs (e.g., through interactions with other medications or conditions) but do not have direct cardiovascular effects.

Limitations of the study

There are several potential limitations in the present study. First, this study relied on summary statistics from existing GWAS, which is limited by sample size, population ancestry, and coverage of genetic variants. Second, this study focused on common genetic variants and may miss rare or low-frequency variants that could have important associations with CVDs. Additionally, the quality control processes and covariate inclusion differ among the different datasets. However, due to the lack of individual-level information, we are unable to correct for these differences. In order to ensure an adequate sample size, there is inevitably some sample overlap between the CVD groups, which may result in some false positives. Further studies with larger sample sizes in different populations are required to address this issue.

This study conducted a gene-centric pleiotropic association analysis and revealed six hub genes that are associated with multiple CVDs, along with 26 potential drugs. Furthermore, this study found causal associations between different CVDs, suggesting that various CVDs

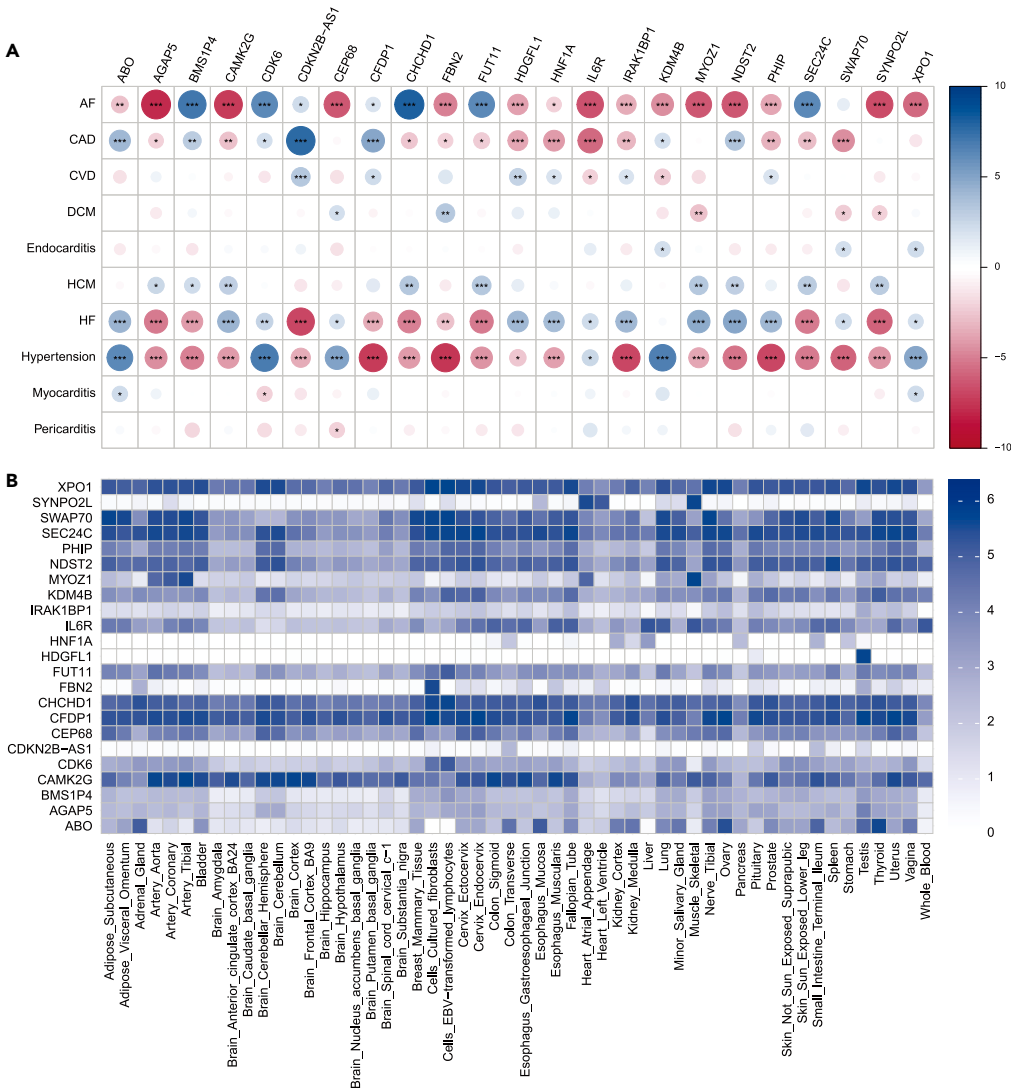

Figure 5. Genes shared between different cardiovascular diseases and the distribution of these pleiotropic genes in different tissues

(A) Genes shared between different cardiovascular diseases.

(B) The distribution of these pleiotropic genes in different tissues.

Significant values denoted as * = $p < 0.05$; ** = $p < 0.01$; and *** = $p < 0.001$. Circle size represents significance level, where larger circles correspond to smaller p values. Colours indicated in the legend on the right represent the sign of the Z value. HF, heart failure; CAD, coronary artery disease; CVD, cardiac valve disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; AF, atrial fibrillation.

subtypes may represent different manifestations of the same underlying pathophysiological state. These findings highlight the need for a holistic approach to treating CVDs, rather than narrowly focusing on one manifestation. Healthcare professionals should pay attention to the underlying cause of CVDs, rather than simply addressing individual symptoms. By understanding the complex interplay between different CVDs, healthcare providers can tailor treatment strategies to the specific needs of each patient, ultimately improving outcomes and reducing the burden of CVDs. Further research is needed to explore the identified hub genes and drugs as potential therapeutic targets for CVDs.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability

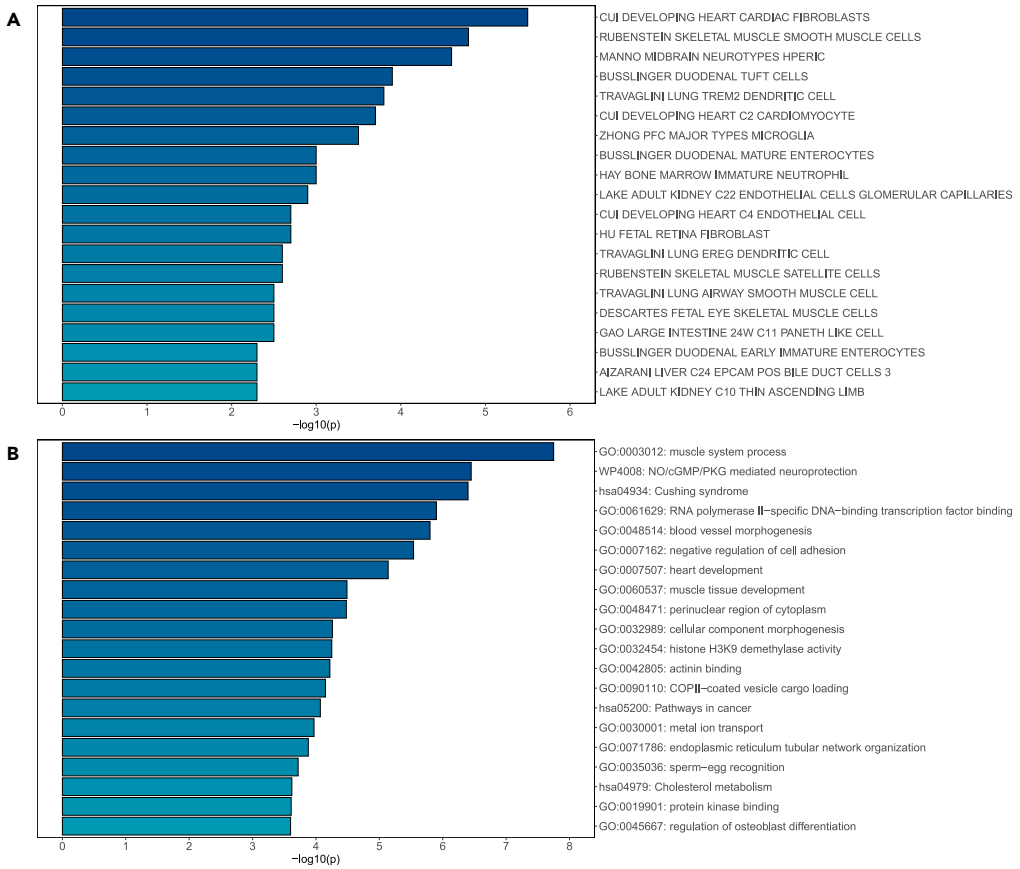

Figure 6. Enrichment analysis of cell type signatures and KEGG and GO analysis of the shared genes

(A) Enrichment analysis of cell type signatures.

(B) KEGG and GO analysis of the shared genes.

Functional annotation of biological processes (BP), molecular functions (MP), and cellular components (CC).

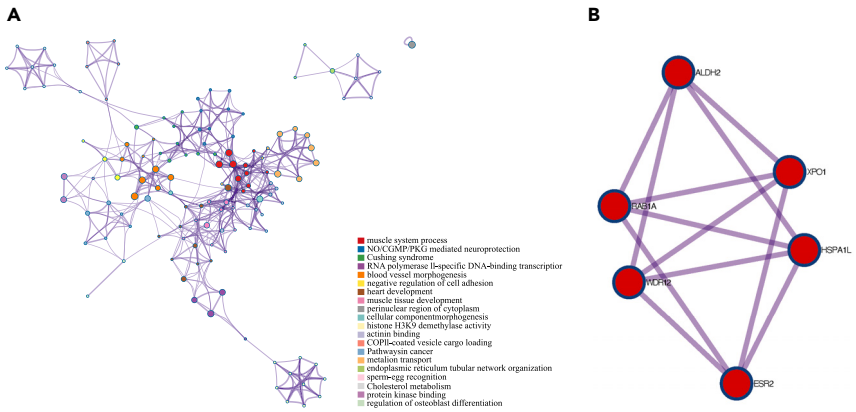

Figure 7. PPI network of the shared genes and the identified hub genes

(A) PPI network of the shared genes.

(B) The identified hub genes.

PPI, protein−protein interaction.

Table 2. Drug candidates associated with hub genes

Gene	Drug	Interaction types	Sources	Reference
ALDH2	DISULFIRAM	inhibitor	TdgClinicalTrial|ChemblInteractions|TEND	Mackenzie et al.[23] and Ho et al.[24]
ALDH2	DIACETYLMORPHINE		PharmGKB	Wanget al.[25]
ALDH2	ACETALDEHYDE		PharmGKB	Lee et al.[26]
ALDH2	ALCOHOL		NCI|PharmGKB	Matsumoto et al.[27]
ESR2	TAMOXIFEN	antagonist|agonist	DTC|TdgClinicalTrial|TEND|PharmGKB	Mc Ilroy et al.[28]
ESR2	FULVESTRANT	antagonist	TALC|DTC|ChemblInteractions|PharmGKB	Calogeropoulou et al.[29]
ESR2	ERTEBEREL	agonist	ChemblInteractions|TTD	Berman et al.[30]
ESR2	RALOXIFENE	agonist|antagonist	DTC|TdgClinicalTrial|TEND|PharmGKB	Jain et al.[31]
ESR2	BAZEDOXIFENE	antagonist	TdgClinicalTrial	Komm et al.[32]
ESR2	ESTRADIOL	agonist	DTC|TdgClinicalTrial|TEND	Jain et al.[31]
ESR2	GENISTEIN	agonist	TTD	Berman et al.[30]
ESR2	PRINABEREL	agonist	ChemblInteractions|TTD	Berman et al.[30]
ESR2	AUS-131	agonist	TdgClinicalTrial|ChemblInteractions|TTD	Setchell et al.[33]
ESR2	TRILOSTANE	allosteric modulator	TTD	Barker et al.[34]
ESR2	AFIMOXIFENE	modulator	ChemblInteractions	Reed et al.[35]
ESR2	NARINGENIN	partial agonist	TTD	Kuiper et al.[36]
ESR2	LETROZOLE		PharmGKB	Oesterreich et al.[37]
ESR2	CHEMBL2332580		DTC	Min et al.[38]
ESR2	GEMCITABINE		PharmGKB	Woo et al.[39]
ESR2	CHEMBL1222035		DTC	Jain et al.[31]
HSPA1L	CARBAMAZEPINE		PharmGKB	Alfirevic et al.[40]
XPO1	SELINEXOR	inhibitor	ChemblInteractions|TTD	Turner et al.[41]
XPO1	LEPTOMYCIN B		DTC	Van Neck et al.[42]
XPO1	OSTHOLE		DTC	Tamura et al.[43]
XPO1	GONIOTHALAMIN		DTC	Wach et al.[44]
XPO1	ISONIAZID		PharmGKB	Nanashima et al.[45]

TEND, Trends in the Exploration of Novel Drug Targets; PharmGKB, Pharmacogenetics and Pharmacogenomics Knowledge Base; NCI, National Cancer Institute; DTC, Drug Target Commons; TALC, Targeted Agents in Lung Cancer; TTD, Therapeutic Target Database.

- Genetic correlation analysis
- The pleiotropic analysis under composite null hypothesis (PLACO)
- Gene-level analysis
- Mendelian randomization analysis
- Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses
- Protein interaction analysis
- Drug–gene interactions

- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.108150.

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AUTHOR CONTRIBUTIONS

X.B.P., S.J.L., and Y.H. conceived and planned the study and supervised the analyses. Z.Y.L., J.X., J.S.T., and X.F.L. conducted the analyses and wrote the first draft. F.W.Z., W.B.O.Y. and S.Z.W. critically reviewed the integrity and plausibility of the data analysis. X.B.P., S.J.L. and Y.H. revised the manuscript and were responsible for the integrity of data acquisition and statistical analyses. All authors agreed to submit the manuscript, read and approved the final draft and take full responsibility of its content, including the accuracy of the data and the fidelity of the trial to the registered protocol and its statistical analysis. Every author has unrestricted access to all data.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Analysis of the genetic correlation between two types of cardiovascular diseases	This study	Supplemental information
Identification of pleiotropic genes between every two diseases using PLACO	This study	Supplemental information
Identification of pleiotropic SNPs between every two diseases using PLACO	This study	Supplemental information
Mendelian randomization (MR) analysis between two types of cardiovascular diseases via the inverse-variance weighted (IVW) method	This study	Supplemental information
Mendelian randomization (MR) analysis between every two types of cardiovascular diseases (utilizing seven different MR methods for each analysis)	This study	Supplemental information
Instrumental variables summary	This study	Supplemental information
Summary of sensitivity analysis results	This study	Supplemental information

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Xiangbin Pan (panxiangbin@fuwaihospital.org).

Materials availability

This study did not generate new unique reagents.

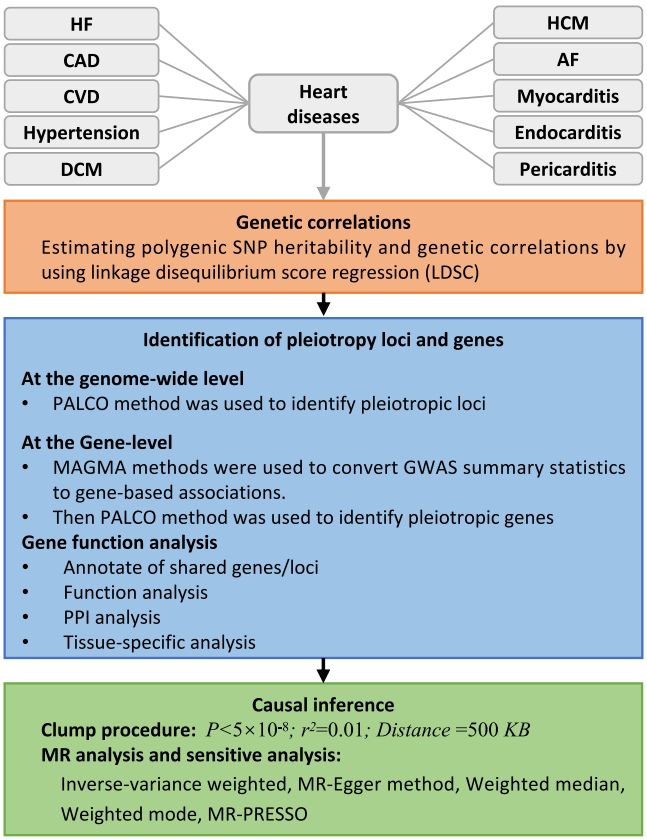
Data and code availability

- This paper does not report the original code.
- The sources of the datasets supporting the current study are presented in the "key resources table" and "STAR Methods" sections.
- Any additional information required to reanalyze the data reported in this paper or reproduce the results is available from the lead contact upon request.

METHOD DETAILS

Protocol design

This study assessed the complex genetic relationships among 10 CVDs using the linkage disequilibrium score regression (LDSC) approach to determine genetic correlations among disease subtypes. Furthermore, the pleiotropic approach to identify corresponding pleiotropic loci and genes, and the two-sample bidirectional MR approach to assess causal associations between them (see figure below).



The study flow chart

HF, heart failure; CAD, coronary artery disease; CVD, cardiac valve disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; AF, atrial fibrillation.

Source of summary statistics

GWAS summary datasets for the 10 cardiovascular diseases (HF, CAD, CVD, hypertension, DCM, HCM, AF, myocarditis, endocarditis, and pericarditis) were obtained from the GWAS catalogue (https://www.ebi.ac.uk/gwas/). Summary statistics were calculated using only European samples, and strict quality control measures were implemented, including the exclusion of nondual allele SNPs, those with strand-ambiguous alleles, and SNPs without rs tags. Duplicate SNPs or those not included in the 1000 Genomes Project or whose alleles did not match with their SNPs were also removed, and SNPs with minor allele frequencies (MAF) less than 0.01 were excluded. The meta-analysis of HF data was conducted as part of a study by the HERMES consortium, which included participants of European ancestry from 26 cohorts (in total, 29 different datasets). These participants were involved in either case-control or population-based study designs. The case group comprised participants clinically diagnosed with HF of any etiology, without inclusion criteria based on left ventricular ejection fraction; whereas the control group consisted of individuals without HF. The entire meta-analysis covered 47,309 cases of HF and 930,014 data points from the control group. All included studies obtained ethical approval from local institutional review boards, and all participants provided written informed consent. The GWAS meta-analysis of aggregated-level estimates for the participating studies was conducted following the research protocol guidelines provided by the Research Ethics Committee of University College London. The CAD data originated from a GWAS study conducted on 34,541 cases of CAD (coronary artery disease) and 261,984 controls from the UK Biobank resource. Subsequently, replication was performed on 88,192 cases and 162,544 controls from the CARDIoGRAMplusC4D dataset. In the meta-analysis, 75 replicable and genome-wide significant loci (p < 5 × 10^-8) were identified, including 13 loci that had not been reported before. GWAS results for hypertension were obtained from the UK Biobank (UKBB) genetic and health-related data comprising almost half a million participants. A total of 129,909 cases and 354,689 controls were included in the study, using gender as a covariate in the GWAS model. The data on AF were tested for the association between 34,740,186 genetic variants and atrial fibrillation. These data were obtained from six studies of European ancestry (The Nord-Trøndelag Health Study (HUNT), deCODE, the Michigan Genomics Initiative (MGI), DiscovEHR, UK Biobank, and the AFGen Consortium), comprising a total of 60,620 cases and 970,216 controls. Endocarditis data are from GWAS summary statistics of 2,989 binary traits from the fastGWA-GLMM analysis of UKB imputed data. Contains 267 cases of European ancestry, 456,081 controls of European ancestry. GWAS analysis was performed after adjusting for age, age^2, gender, age×sex, age^2×sex, and the first 20 principle coponents provided by UKB. Finally,

data for DCM, HCM, myocarditis, CVD, and pericarditis came from a meta-analysis of UK Biobank and FinnGen (ntotal = 628,000), which identified approximately 5,000 new loci, improving the resolution of the genome map of human traits. Meta-analysis was performed using the inverse variance method in METAL software, and heterogeneity was estimated using Cochran's Q test.)

Genetic correlation analysis

The LDSC approach was used to assess shared polygenic structure between traits by calculating LD scores from a sample of individuals of European descent from the Thousand Genomes Project, which was used as a reference group.[85] Strict quality control measures were implemented for SNPs, and SNPs with nondual alleles, stranded ambiguous alleles, MAF less than 1%, missing rs numbers, duplicate rs IDs, or not present or whose alleles did not match stage 3 of the Thousand Genomes Project were removed.

The pleiotropic analysis under composite null hypothesis (PLACO)

PLACO is a powerful statistical method that enables the identification of genes exhibiting pleiotropic effects, thereby influencing multiple diseases or traits.[11] SNP-Level PLACO was used to investigate pleiotropic motifs among complex traits using only aggregated level genotype-phenotype association statistics.[16] Z scores for each variant were calculated, and SNPs with extremely high Z^2 values (>80) were removed. The Z correlation matrix was estimated, which considered potential correlations between different cardiovascular diseases, and the horizontal alpha crossover union test (IUT) method was used to test the hypothesis of no pleiotropy. The final p value of the IUT test was then the maximum of the p values of the test H_0 versus H_1. Further pleiotropic biomarker identification was performed at the gene level using the PLACO method. The genetic correlation and pleiotropy methods we currently use follow a published study.[86]

Gene-level analysis

Genomic annotation multiple marker analysis (MAGMA v.1.07b) was used to converge a set of SNP-level associations into a single gene-level association signal.[87] The analysis was limited to 18,563 protein-coding genes on autosomes, and an annotation window of $\pm$500 kb was set to assign adjacent SNPs to the same gene. Gene location information was obtained from the matched Ensembl build (GRCh37) and 1000 G EUR data. Further pleiotropic biomarker identification was performed at the gene level using the PLACO method.[86] Functional mapping and annotation of genome-wide association studies (FUMA) were used to determine the biological functions of pleiotropic motifs.[88] MAGMA motif set analysis was performed to investigate the biological functions of the lead SNPs.[87] The identified motifs were then mapped to nearby genes, and pathway enrichment analyses were used to determine the function of the mapped genes based on the Molecular Signature Database (MSigDB).[89]

Mendelian randomization analysis

We utilized Mendelian randomization studies to investigate the causal relationship between cardiovascular disease and genetic variants.[90] To identify instrumental variables (IVs) that were significantly associated with CVDs, we employed the clump program in PLINK software,[91] utilizing a stringent threshold of $p < 5 \times 10\text{-}8$, a r^2 threshold of 0.001, and a 500 kb window. To ensure the robustness of the IVs, we assessed the r^2 and F statistics for each IV. The F statistic was calculated as following:

$$F = \left(\frac{n - 1 - k}{k}\right)\left(\frac{r^2}{1 - r^2}\right)$$

Where n denotes the sample size and k denotes the number of SNPs. r^2 was calculated as following:

$$r^2 \approx \frac{\left(\widehat{\beta}_j^x\right)^2}{\left(\widehat{\beta}_j^x\right)^2 + \mathrm{var}\left(\widehat{\beta}_j^x\right) \times N_j}$$

Where $\widehat{\beta}_j^x$ and $\mathrm{var}(\widehat{\beta}_j^x)$ represent the estimated effect size and variance of the instrumental variable j.[92,93] We primarily utilized the inverse variance weighting (IVW) method for our Mendelian randomization analysis, which requires the IVs to satisfy the following three assumptions: (1) correlation between IVs and exposure; (2) no association between IVs and confounding factors of the exposure and outcome associations; and (3) the effect of the IVs on the outcome is solely through the exposure. Although IVW is a powerful method for MR when these assumptions are met, if some instruments violate the assumptions, MR analysis may produce erroneous results, even if the result is null, indicating no causal association. Therefore, we conducted several sensitivity analyses, including Q-tests using IVW and the MR–Egger test to detect violations of assumption via heterogeneity of the association between individual IVs and MR–Egger to assess horizontal pleiotropy based on its intercept to ensure genetic variance was independently associated with exposure and outcome.[94] Furthermore, we employed additional analyses, such as weighted median and weighted mode, with different modeling assumptions and strengths of the MR approach to increase the stability and robustness of the results. Finally, the results were presented as odds ratios (ORs) with 95% confidence intervals (CIs). The Bonferroni correction was applied in the current MR analysis to account for multiple comparisons. Findings for which the p value was less than 0.0006 (0.05 divided by 90), signified a significant causal association. Additionally, p value that fell between 0.0006 and 0.05 were considered

suggestive of a causal association. Statistical analyses were conducted using R (version 3.5.3) software and the MendelianRandomization package for MR analysis.[95]

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses are popular computational methods in bioinformatics and functional genomics that are used to gain insights into the biological functions and pathways associated with a set of genes or proteins of interest.[96] The GO database provides a standardized and structured ontology that categorizes genes and gene products into functional groups, such as biological processes (BP), molecular functions (MP), and cellular components (CC), allowing for functional annotation and enrichment analysis.[97] On the other hand, KEGG is a comprehensive database that maps genes to biological pathways and networks, providing a systematic and integrated view of cellular functions and interactions.[96] In the present MR analysis, GO and KEGG analyses were used to elucidate the functional relevance and potential roles of shared genes in specific biological processes, molecular functions, or pathways and to identify overrepresented or enriched functional categories or pathways within a set of genes of interest.

Protein interaction analysis

We extracted the protein–protein interaction (PPI) network from the BioGrid database.[98] Compared to manual approaches and precompiled processes, protein networks provide a complementary method for the dynamic identification of potentially function-related protein groups. Specifically, proteins connected within a PPI network may collaborate (e.g., to form signaling pathways or molecular complexes) to carry out various related biological processes. To predict and establish the PPI network of the shared genes, we utilized the Search Tool for the Retrieval of Interacting Genes (version 11.5), available at http://string-db.org/.[99] Then, we employed Cytoscape software and the Metascape database[46,100] to visualize the PPI networks. The Molecular Complex Detection (MCODE) algorithm (version 1.6.1) was utilized to screen the hub genes based on topology and to identify densely connected regions in large PPI networks. This automated kit facilitates the identification of molecular clusters or complexes.

Drug–gene interactions

The Drug Gene Interaction database, available at http://www.dgidb.org, was employed for further investigation of drug-gene interactions based on the final list of genes identified as potential therapeutic targets for CVDs.[96] The candidate drugs that target these genes or pathways associated with CVDs may hold promise as potential treatment options.

QUANTIFICATION AND STATISTICAL ANALYSIS

This study obtained GWAS summary datasets for 10 cardiovascular diseases from the GWAS catalog and applied strict quality control measures. The LDSC approach was used to assess shared polygenic structure between traits,[85] and the PLACO method was used to investigate pleiotropic motifs among complex traits.[11] Gene-level analysis was performed using MAGMA v.1.07b,[87] and pleiotropic biomarker identification was performed using the PLACO method. The study utilized MR analysis to investigate the causal relationship between cardiovascular disease and genetic variants.[90] Finally, GO and KEGG analyses were performed to determine the biological functions and pathways associated with the genes or proteins of interest.