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Original Article

Exploring the impact of metabolites function on heart failure and coronary heart disease: insights from a Mendelian randomization (MR) study

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Abstract: Background: Heart failure (HF) and coronary heart disease (CHD) are major causes of morbidity and mortality worldwide. While traditional risk factors such as hypertension, diabetes, and smoking have been extensively studied, the role of metabolite functions in the development of these cardiovascular conditions has been less explored. This study employed a Mendelian randomization (MR) approach to investigate the impact of metabolite functions on HF and CHD. Methods: To assess the causal impacts of specific metabolite risk factors on HF and CHD, this study utilized genetic variants associated with these factors as instrumental variables. Comprehensive genetic and phenotypic data from diverse cohorts, including genome-wide association studies (GWAS) and cardiovascular disease registries, were incorporated into the research. Results: Our results encompass 61 metabolic cell phenotypes, with ten providing strong evidence of the influence of metabolite functions on the occurrence of HF and CHD. We found that elevated levels of erucate (22:1n9), lower levels of α-tocopherol, an imbalanced citrulline-to-ornithine ratio, elevated γ-glutamyl glycine levels, and elevated 7-methylguanine levels independently increased the risk of these cardiovascular conditions. These findings were consistent across different populations and robust to sensitivity analyses. Conclusion: This MR study provides valuable insights into the influence of metabolite functions on HF and CHD. However, further investigation is needed to fully understand the precise mechanisms by which these metabolite factors contribute to the onset of these conditions. Such research could pave the way for the development of targeted therapeutic strategies.

Keywords: Heart failure, coronary heart disease, Mendelian randomization study

Introduction

Heart failure (HF) and coronary heart disease (CHD) are significant global health concerns, contributing to high morbidity and mortality rates. Understanding the factors involved in their development is crucial for effective prevention and treatment. Recently, altered metabolite profiles have emerged as potential contributing factors to the development of cardiovascular diseases. Adipocytes and skeletal muscle cells play vital roles in regulating energy metabolism and metabolite homeostasis [1, 2]. Therefore, dysfunction in these cell types can disrupt the overall metabolic process, resulting in metabolic disorders and cardiovascular complications [3, 4].

Under pathological conditions, metabolites critically influence the progression of HF and CHD by acting as key regulators of pathophysiological processes and potential biomarkers [5]. For instance, B-type natriuretic peptide (BNP) has been closely linked to HF severity, indicating its

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vital applications as diagnostic and prognostic markers [6]. Similarly, troponin levels, indicative of myocardial injury, are commonly used in diagnosing acute coronary syndromes [7]. Besides, dyslipidemia, characterized by imbalances in low-density lipoprotein (LDL) to high-density lipoprotein (HDL) cholesterol ratio, is a major risk factor for CHD [8]. Furthermore, the expression of inflammatory markers like C-reactive protein (CRP) and interleukin-6 (IL-6) are significantly elevated in HF and CHD patients, suggesting the underlying hyper-activation of inflammatory responses [9].

The mechanisms through which metabolites influence HF and CHD involve their interactions within complex pathways. BNP exerts vasodilatory, renal natriuretic, and diuretic effects, enhancing volume clearance in HF [6]. In contrast, myocardial injury-induced troponin release in the bloodstream indicates heart muscle damage in CHD [7]. Moreover, chronic dyslipidemia increases the risk of atherosclerosis by several orders through the persistent deposition of LDL cholesterol droplets in the arterial walls, leading to plaque formation and arterial blockade [8]. On the other hand, elevated CRP and IL-6 levels in the systemic circulation indicate underlying inflammation-associated endothelial dysfunction and plaque destabilization in HF and CHD [9].

The metabolism of myocardial substrates, particularly fatty acids (FAs), has been extensively studied under healthy as well as diseased conditions [10]. Imbalances in FA metabolism have been strongly associated with the development of a range of cardiovascular complications, including HF and CHD, underscoring the importance of physiological metabolite function. Dysregulated FA metabolism produces toxic lipid intermediates, causing oxidative stress and mitochondrial dysfunction, resulting in cardiomyocyte injuries and impaired cardiac function [11]. Additionally, altered FA utilization perturbs energy production and causes contractile dysfunction and cardiac remodeling, ultimately predisposing individuals to HF and CHD [11]. Exploring the intricate crosstalk among altered FA metabolism, metabolite production, and development of HF and CHD symptoms, researchers and clinicians can identify novel therapeutic targets and personalized treatment strategies to improve patient outcomes and halt the disease progression [12]. The heart's metabolic flexibility in switching between different energy-producing pathways is essential for maintaining cardiac functions [1]. Impaired metabolic flexibility has been widely observed in cardiovascular diseases (CVDs), suggesting a potential connection between metabolite dyshomeostasis and cardiac malfunction [2]. Moreover, conditions such as diabetes can profoundly impact metabolite function and contribute to the onset of diabetic cardiomyopathy, a distinct form of heart disease [13, 14]. Mechanistically, the dysregulation of insulin signaling in diabetic patients is linked to HF-related mortality [15]. This evidence sets the ground for further investigation of metabolite biosynthesis pathways in maintaining cardiovascular health and their disruptions under diseased conditions [16] and the potential application of Mendelian randomization (MR) in deciphering the causal connections among these disease parameters [17].

Materials and methods

Study design

This study aimed to examine the causal relationship between metabolite function and the onset of HF and CHD symptoms using an MR approach [2-4, 16]. MR utilizes genetic variants as instrumental variables (IVs) to represent risk factors, ensuring reliable and reproducible causal inference. The chosen IVs must satisfy the following criteria: (1) the genetic variation must be directly associated with metabolite function, (2) the genetic variation must not be associated with potential confounding factors between metabolite function and diseased conditions, and (3) the genetic variation must not influence the development of CVDs through pathways unrelated to metabolite function.

All included studies in our analysis received the necessary approvals from the Institutional Review Board. Informed consent was collected from each participant, ensuring compliance with ethical guidelines and data confidentiality of the study protocol. Throughout the study, we prioritized ethical considerations and participant consent. Overall, our study utilized an MR approach to examine the causal link between metabolite function and the CVD (such as HF and CHD) onset. Exploiting the genetic variants as IVs, we aimed to meet the prerequisites for reliable causal inference in MR studies. **Figure 1** provides an overview of our study design.

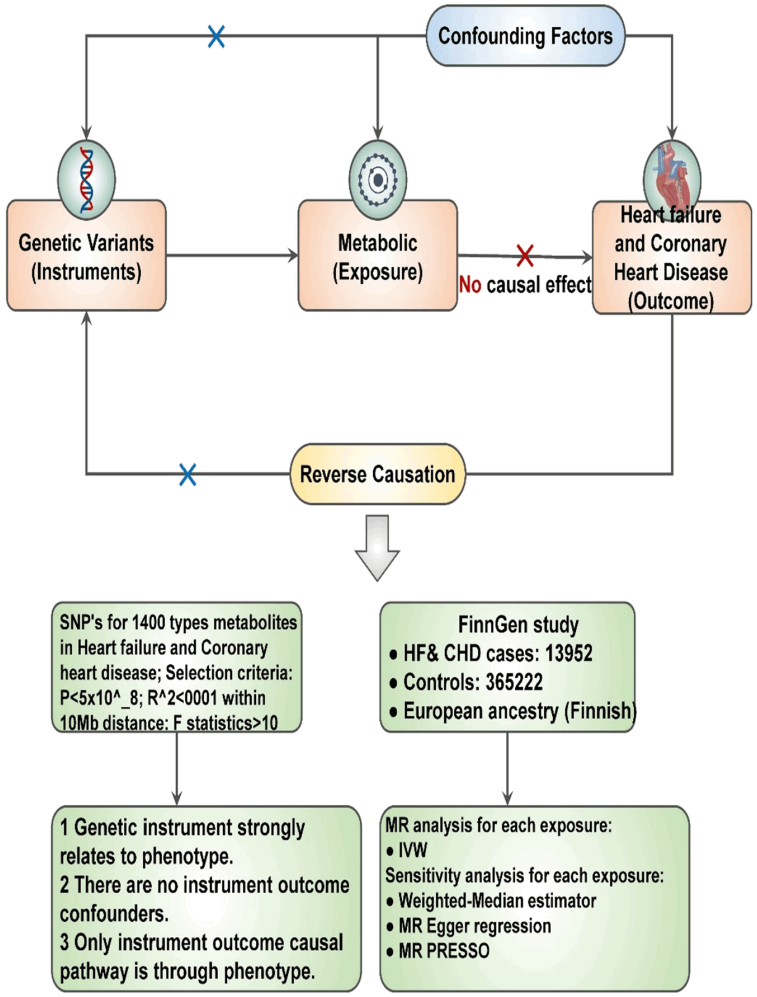

Figure 1. The flow chat of study design. Overview of the overall MR design. Assumption 1, instrument variables are robustly related to exposure; Assumption 2, instrument variables are not related to confounders; Assumption 3, instrument variables are related to outcome only through exposure. SNPs, single-nucleotide polymorphisms; LD, linkage disequilibrium; IVW, inverse variance weighted; LOO, leave-one-out; MR, Mendelian randomization.

In this study, patients were included if they: (1) were diagnosed with both HF and CHD; (2) were carrying HF and CHD-associated specific SNPs (out of 1400 known SNPs) that made significant contributions to the disease progression ($P < 5 \times 10^{-8}$ and $R^2 < 0.001$ within a 10 Mb distance, and $F_{statistics} > 10$), and (3) had relevant data on the crosstalk between genetic variants and the concerned metabolites and its levels. Further, this study recruited patients with documented outcomes for HF or CHD in a statistically significant cohort size to be suitable for MR analysis. Written informed consent was obtained from all eligible study participants. Patients were excluded from the study if they: (1) were missing information on the outcome of their CHD diagnosis and treatment; (2) specified SNPs were not detected; (3) lacked relevant genetic variant and metabolite profiling data, and (4) had known confounding factors that might interfere our results, such as comorbidities or medications affecting the metabolite level or outcome. Additionally, candidates not willing to sign the consent for genetic screening were also eliminated from the study cohorts.

Data sources

We obtained genome-wide association study (GWAS) data for metabolite traits using the code ebi-a-GCST90038595 from publicly available databases, allowing us to generate summary statistics for each metabolic cell type [18]. To identify relevant data for HF and CHD, we used study-specific keywords to search the GWAS catalog (https://gwas.mrcieu.ac.uk/) and selected finngen_R10_I9_HEARTFAIL_AND_CHD. We then downloaded the corresponding data from the European Bioinformatics Institute (https://www.ebi.ac.uk/gwas/), based on the ID of each HF and CHD type. This data was used to analyze the association between 1400 types of metabolites and HF or CHD onset.

To assess approximately 22 million SNPs, genotyped using high-density arrays, we used a reference panel derived from Sardinian sequences [19]. Correlations were assessed after adjusting for covariates. The FinnGen database, which combines genetic data obtained from participants with their detailed health records, served as a rich resource for our research. The comprehensive nature of this data can provide insights into the genetic basis of diseases, potentially leading to new diagnos-

tic methods, treatments, and preventative strategies. The project leverages Finland's unique genetic heritage, extensive biobank infrastructure, and comprehensive national health registries.

Based on the ID of each HF and CHD type, we utilized data from the FinnGen database, which includes 379174 European individuals (13952 case patients and 365222 control participants), to analyze the relationship between metabolite function and CVD development.

Genetic instrument selection

In our study, we implemented strict criteria for selecting genetic IVs concerning SNPs and metabolite traits. Given the large number of SNPs achieving genome-wide significance ($P < 5 \times 10^{-8}$), we adopted even more stringent criteria ($P < 5 \times 10^{-9}$) for IV selection [20]. To identify IVs, we categorized them based on the linkage disequilibrium (LD) reference panel from the 1000 Genomes Project, applying a cutoff of $R^2 < 0.001$ within a distance of up to 1000 kilobases (kb). This allowed us to pinpoint the most relevant IVs for our analysis.

Considering the relatively smaller size of GWAS datasets for metabolites, we used a *p*-value threshold of 5×10^{-8} and a more relaxed clustering threshold ($R^2 < 0.1$ within a distance of 500 kb) [21]. This approach enabled us to capture sufficient numbers of IVs, maintaining statistical rigor. To ensure the strength of our genetic instruments, we only selected IVs with F-statistics greater than 10, identifying potential instruments to generate reliable and reproducible results for our analyses.

We extracted these IVs from the summary data for HF and CHD outcomes. To maintain the integrity of our analysis, we excluded any SNPs with potential pleiotropic effects ($P < 10^{-5}$) on HF and CHD types [22]. To ensure consistency in effect size estimations, we harmonized the SNPs across the datasets for exposures and outcomes, allowing us to perform coherent comparisons and draw accurate conclusions based on the same alleles. Finally, we eliminated SNPs with effect allele frequencies (EAFs) greater than 0.42 or those incompatible with harmonization from our analysis [21]. This careful process of SNP selection and harmonization ensured the integrity and consistency of our MR analysis.

Statistical analysis

The analysis was conducted using R v3.5.3 software (http://www.Rproject.org). Various methodologies, including inverse variance weighting (IVW), weighted median, and mode, were applied to investigate the causal relationship between 1400 types of metabolites and HF and CHD development using the MR v0.4.3 software. Heterogeneity within the chosen IVs was evaluated through Cochran's Q test and the respective *p*-values. If the null hypothesis was rejected, the random-effects IVW method was adopted instead of the fixed-effects IVW method. The MR-Egger method was employed to address horizontal pleiotropy, and the MR-PRESSO method was used to detect and eliminate potential horizontal pleiotropic outliers that could impact the results. To ensure the robustness of our findings, we conducted scatter plots, funnel plots, and leave-one-out analyses. These analyses confirmed that the outcomes were not influenced by outliers and demonstrated a strong and consistent association [23].

In addition to applying stringent participant selection criteria, we validated our genetic instruments through MR analyses. Of note, the IVW method, assuming all genetic variants are valid, may introduce bias when numerous SNPs are affected by horizontal pleiotropy [24]. And, the weighted median approach, effective when fewer than 50% of variants display horizontal pleiotropy, assumes that most genetic variants are valid [25]. To evaluate the strength of our genetic instruments in cases where over 50% of variants could be influenced by horizontal pleiotropy, we analyzed the mean $F_{Statistics}$, with a value below 10 indicating weak instrumental variables [26].

Furthermore, we employed the MR-Egger method to examine potential directional pleiotropy, where a significant intercept indicates a violation of IV assumptions and suggests the presence of directional pleiotropy [27]. Besides, we utilized the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method to minimize heterogeneity in causal effect estimates by excluding disproportionately influential SNPs (with NbDistribution = 1500) [28]. We also utilized the Steiger filtering method to identify and exclude genetic variants that showed a stronger

association with the outcome than with the exposure, suggesting a potential reverse causality [29].

All statistical analyses were conducted using R version 4.3.1 (R Foundation) and specific R packages ("TwoSampleMR" and "Mendelian Randomization") tailored for MR analyses [30, 31].

Results

Causal effects of metabolic phenotypes on HF and CHD onset

Here, we identified 61 metabolic phenotypes that might be associated with HF and CHD onset, as shown in **Table 1**. However, we focused on ten key metabolites, with the results summarized in **Figure 2**. Our MR study unveiled a significant causal effect of elevated Erucate (22:1n9) levels on the risk of HF and CHD (OR: 1.1375, 95% CI: 1.0407-1.2434, P < 0.0045). Higher levels of Erucate (22:1n9) were independently linked to an increased likelihood of developing these CVDs. Furthermore, our MR analysis highlighted several other important findings. We observed a causal relationship between decreased alpha-tocopherol levels and an increased susceptibility to HF and CHD (OR: 1.1117, 95% CI: 1.0124-1.2208, P < 0.0265), underscoring the importance of maintaining adequate alpha-tocopherol level in minimizing CVD risks.

Furthermore, an imbalanced citrulline-to-ornithine ratio was found to be a causal risk factor for HF and CHD (OR: 1.1012, 95% CI: 1.0118-1.1986, P < 0.0256). Deviations from the optimal ratio were linked to an increased risk of CVDs. Also, elevated levels of gamma-glutamyl glycine showed a significant protective effect against HF and CHD (OR: 0.9171, 95% CI: 0.8695-0.9673, P < 0.0014), suggesting that gamma-glutamyl glycine could be a potential biomarker for identifying individuals with reduced risks of CVDs. This MR study revealed a significant correlation between elevated 7-methylguanine levels and higher risks of HF and CHD (OR: 1.0964, 95% CI: 1.0161-1.1830, P < 0.0175), indicating that a higher level of 7-methylguanine can independently be associated with elevated risks of CVDs.

Further investigation revealed significant causal effects of four additional metabolites and identified one as a protective factor for HF and CHD, as shown below:

1) Hippurate: A metabolite produced during the metabolism of aromatic compounds, such as benzoic acid, in the liver. Elevated levels of hippurate were found to be a causal contributor to HF and CHD (OR: 1.0981, 95% CI: 1.0043-1.2006, P < 0.0397). Increased hippurate levels may indicate impaired detoxification processes or oxidative stress, which can contribute to the development of CVDs. 2) N-acetyl aspartate (NAA): It is primarily found in neurons and is a marker of neuronal health and integrity. Low levels of NAA were identified as a causal risk factor of HF and CHD (OR: 0.9286, 95% CI: 0.8764-0.9839, P = 0.0120), further indicating that impaired neuronal function or loss of neuronal integrity may be linked to the development of CVDs. 3) Galactonate: It is derived from galactose metabolism. An elevated level of galactonate was identified as a causal contributing factor for HF and CHD (OR: 1.0853, 95% CI: 1.0016-1.1759, P = 0.0455). The exact mechanism linking galactonate synthesis and cardiovascular health is not clearly understood. However, it is hypothesized that alterations in galactose metabolism or gluconate accumulation in body fluid may indicate metabolic dysregulation and increased risks of CVDs. 4) S-1-pyrroline-5-carboxylate (P5C): It is an intermediate byproduct in proline metabolism. Elevated levels of P5C were identified as causal risk factors for HF and CHD (OR: 1.1097, 95% CI: 1.0092-1.2202, P < 0.0314). Elevated levels of P5C could be involved in oxidative stress and mitochondrial dysfunction, which are established contributors to CVDs.

These findings offer valuable perspectives on the role of these biochemical compounds in the development of HF and CHD. Additionally, an elevated level of X-18779, a compound with less well-understood implications in cardiovascular health, was identified as the contributing factor to cardiac failure and ischemic heart failure (OR: 1.1071, 95% CI: 1.0230-1.1980, P = 0.0115). Further investigation is required to fully understand the role of X-18779 in cardiovascular health.

To ensure the validity of our results, we performed a sensitivity analysis, which confirmed the statistical robustness of the identified causal relationships. The validation was sup-

Table 1. The causal role of metabolites and heart failure and coronary heart disease

Exposure	Method	nsnp	pval	or	or_lci95	or_uci95
Hippurate levels	IVW	18	0.039754231	1.098140568	1.004393649	1.200637526
N-acetylaspartate (naa) levels	IVW	19	0.012080298	0.928626721	0.876449717	0.983909938
Galactonate levels	IVW	16	0.045520435	1.085317635	1.00163129	1.175995979
Campesterol levels	IVW	19	0.038618184	1.08602876	1.004328082	1.174375673
Gamma-glutamylglycine levels	IVW	24	0.001456479	0.917155027	0.869596251	0.967314822
7-methylguanine levels	IVW	22	0.017582285	1.096441523	1.01619534	1.183024529
Glutamine degradant levels	IVW	26	0.032453702	1.088222478	1.007092079	1.175888668
Cysteine-glutathione disulfide levels	IVW	27	0.008594287	0.924560823	0.872022328	0.980264712
Glutarylcarnitine (c5-dc) levels	IVW	31	0.004069546	0.932061449	0.888376708	0.977894329
1-ribosyl-imidazoleacetate levels	IVW	37	0.00943157	1.081343506	1.019344191	1.147113789
Tryptophan betaine levels	IVW	34	0.048683537	1.062336215	1.000348303	1.12816529
Glycocholenate sulfate levels	IVW	35	0.048224261	0.96380153	0.929180202	0.999712851
Androstenediol (3alpha, 17alpha) monosulfate (3) levels	IVW	30	0.014672471	1.039907661	1.007732514	1.073110105
Androstenediol (3beta,17beta) monosulfate (2) levels	IVW	27	0.007550012	1.097430372	1.025071665	1.174896803
2,3-dihydroxyisovalerate levels	IVW	24	0.029898605	0.929379042	0.869926963	0.992894163
Oleoyl-linoleoyl-glycerol (18:1/18:2) [2] levels	IVW	27	0.018020295	1.08054188	1.013359174	1.1521786
Imidazole propionate levels	IVW	21	0.045776616	1.083389176	1.001502701	1.171970986
Mannonate levels	IVW	22	0.046330143	0.965879215	0.933449059	0.999436069
Etiocholanolone glucuronide levels	IVW	20	0.01250081	1.078125249	1.016325415	1.143682955
Tricosanoyl sphingomyelin (d18:1/23:0) levels	IVW	26	0.046658528	1.093552363	1.001321934	1.194278013
Behenoyl dihydrosphingomyelin (d18:0/22:0) levels	IVW	36	0.029003061	1.07047544	1.006993717	1.137959104
Arachidonoylcholine levels	IVW	19	0.005505831	0.900187891	0.835772779	0.969567637
Methylsuccinoylcarnitine levels	IVW	29	0.049677979	1.062662795	1.000084428	1.129156882
Docosahexaenoylcholine levels	IVW	20	0.03457323	0.916122939	0.844632476	0.993664419
Linolenoylcarnitine (C18:3) levels	IVW	24	0.033553978	0.906502453	0.828047745	0.992390477
2-butenoylglycine levels	IVW	27	0.005507112	0.921696113	0.87012732	0.976321172
Sphingomyelin (d17:1/14:0, d16:1/15:0) levels	IVW	24	0.028809343	0.913015728	0.841480136	0.990632678
Tetradecadienedioate (C14:2-DC) levels	IVW	24	0.028178071	0.940574155	0.890497038	0.993467359
Pentose acid levels	IVW	21	0.023034959	1.093179652	1.012338658	1.180476259
Erucate (22:1n9) levels	IVW	19	0.004524608	1.137556406	1.040713031	1.243411524
Alpha-tocopherol levels	IVW	27	0.026529601	1.111751028	1.012414667	1.220834099
4-acetamidobutanoate levels	IVW	19	0.022361271	1.082936087	1.011371944	1.159564072
N1-methyladenosine levels	IVW	32	0.032267464	1.094854137	1.00769371	1.189553501
N-formylmethionine levels	IVW	21	0.047594003	1.085051015	1.000864685	1.17631856
N-acetylputrescine levels	IVW	23	0.031365272	1.051169984	1.004470955	1.100040106
3-(4-hydroxyphenyl)lactate levels	IVW	27	0.022384291	0.90454474	0.829921904	0.985877325

S-1-pyrroline-5-carboxylate levels	IVW	18	0.031491137	1.109763212	1.009282265	1.220247724
Ornithine levels	IVW	24	0.045251399	1.082420002	1.00167493	1.169673939
Serine levels	IVW	36	0.023170281	0.934958368	0.882226501	0.99084209
Salicylate levels	IVW	23	0.044764672	1.091526554	1.002046973	1.188996376
X-15503 levels	IVW	18	0.029048303	1.087222519	1.008573385	1.172004757
X-18779 levels	IVW	19	0.011560797	1.107113035	1.023039086	1.19809623
X-23641 levels	IVW	28	0.004983472	1.069362365	1.020460686	1.120607471
X-24546 levels	IVW	25	0.016715329	0.936002403	0.88664612	0.988106166
X-25422 levels	IVW	23	0.037337364	0.929887431	0.868383283	0.995747675
N2-acetyl,N6-methyllysine levels	IVW	29	0.029325082	0.975558248	0.954084649	0.997515154
Glycine levels	IVW	22	0.020719021	0.947674142	0.905484085	0.991829999
Glycine to alanine ratio	IVW	18	0.034352853	0.935325182	0.879153691	0.995085621
Phosphate to N-acetylneuraminate ratio	IVW	31	0.008337363	1.09509146	1.023623614	1.171549081
Serine to alpha-tocopherol ratio	IVW	28	0.014370366	0.915197456	0.852510727	0.982493659
Spermidine to (N(1) + N(8))-acetylspermidine ratio	IVW	21	0.022564911	0.918180136	0.853235616	0.988067946
Phosphate to N-palmitoyl-sphingosine (d18:1 to 16:0) ratio	IVW	22	0.043799104	0.922441007	0.85280801	0.997759637
Cholate to bilirubin (Z,Z) ratio	IVW	24	0.046393806	0.936168105	0.877333934	0.998947706
Adenosine 5'-monophosphate (AMP) to citrate ratio	IVW	22	0.049835839	1.085254409	1.000057162	1.177709812
Phosphate to glutamine ratio	IVW	27	0.033353181	1.083030153	1.006310188	1.165599163
Phosphate to cysteine ratio	IVW	22	0.04625959	0.919538268	0.846726456	0.998611322
Citrulline to ornithine ratio	IVW	25	0.02561024	1.101291921	1.011830043	1.198663653
N-palmitoyl-sphingosine (d18:1 to 16:0) to N-palmitoyl-sphinganine (d18:0 to 16:0) ratio	IVW	19	0.038487442	0.903173736	0.820134982	0.994620173
Cholesterol to oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] ratio	IVW	32	0.032719034	0.9181736	0.848978611	0.993008245
Cholesterol to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	IVW	30	0.04011378	1.075257425	1.00327797	1.152400994
Glucuronate to etiocholanolone glucuronide ratio	IVW	24	0.048748417	0.933131945	0.871065087	0.999621315

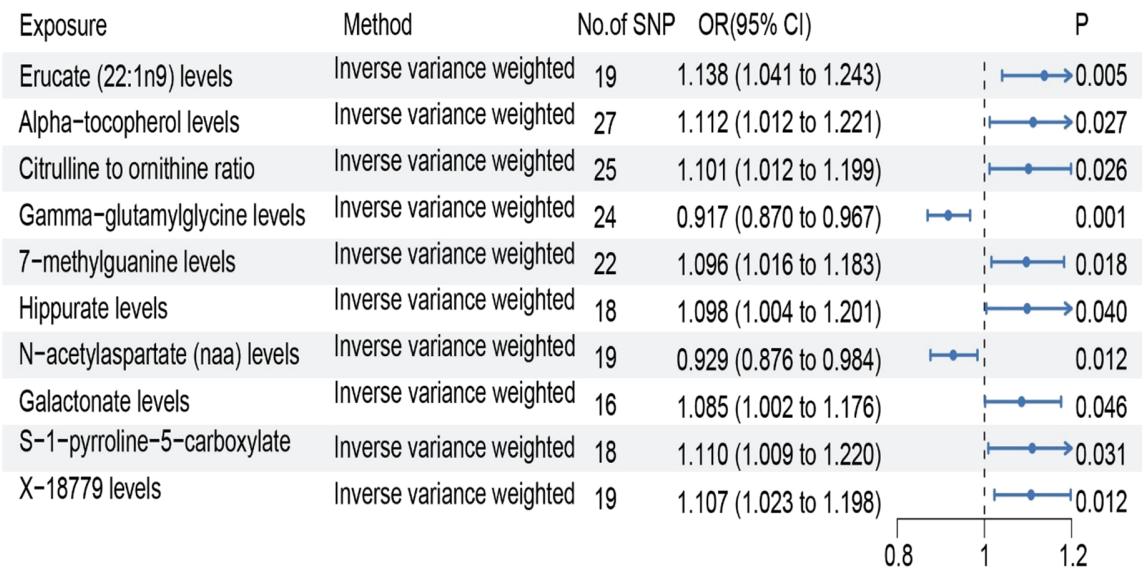

Figure 2. The causal role of metabolites and heart failure and coronary heart disease.

ported by funnel plots and scatter plots. Cochran's Q test showed a substantial heterogeneity with statistical significance ($P < 0.05$). However, the causality estimates remained satisfactory when a random-effects IVW method was applied. Furthermore, the MR-Egger intercept analysis suggested the absence of substantial genetic overlap effects ($P > 0.05$) (Supplementary Table 1A and 1B).

In summary, our study highlights the possible contribution of X-18779 as a causal risk factor for HF and CHD, while further investigation is required to comprehensively understand its implications in cardiovascular health. This article presents the results of the sensitivity analysis, which affirm the robustness of our findings despite some observed heterogeneity.

Discussion

This study aims to explore the impact of metabolite functions on the development of HF and CHD using an MR study design. Our primary goal was to decipher the causal relationships between specific metabolite functions and the occurrence of CVDs. The MR analysis, leveraging genetic variants as instrumental factors, aims to provide robust evidence of potential causal effects of metabolite functions on HF and CHD. The findings of this research have the potential to enhance our understanding of the underlying mechanisms and identify possible targets for intervention in the prevention and management of these CVDs.

Erucate (22:1n9) is a long-chain monounsaturated omega-9 fatty acid predominantly present in the Brassica plant family. Animal feeding trials have shown that erucate (22:1n9) can trigger cardiac steatosis [32, 33]. The Cardiovascular Health and Atherosclerosis Risk in Communities (CH-ARIC) sub-cohort study showed that a higher consumption of monounsaturated omega-9 fatty acids was linked to an increased susceptibility to congestive heart failure [34]. Interestingly, increased levels of erucate are found in the heart tissues of spontaneously hypertensive rats compared to sham animals [35]. Additionally, patients with chronic thromboembolic pulmonary hypertensive disorders exhibit significantly higher plasma erucate levels than healthy controls [36]. A separate study provides initial longitudinal evidence suggesting that changes in erucate levels are positively associated with systolic blood pressure alterations. Despite efforts to reduce erucate (22:1n9) content in edible rapeseed oils, minimal attention has been paid to controlling its levels in other commonly consumed foods such as mustard and salmon [37]. Therefore, hypertensive patients should avoid consuming these foods to prevent abnormal blood pressure changes and potential cardiac complications associated with erucic acid. The Bogalusa Heart Study also confirms that higher erucate

levels could be linked to systolic blood pressure changes and an increased risk of developing hypertensive disorders [38]. Our study also showed that erucate (22:1n9) could be associated with increased risks of HF and CHD, which was in agreement with previous findings.

On the contrary, α-tocopherol, a prevalent biologically active form of vitamin E, is well-characterized for its potent antioxidant properties. Vitamin E is easily absorbed and stored in the body [39]. Multiple studies have shown a reciprocal correlation between α-tocopherol concentrations and the occurrence of coronary artery disease (CAD) [40, 41]. Vitamin E regulates proteins involved in the absorption, transportation, and metabolism of atherogenic lipids, and also exhibits anti-inflammatory properties [42, 43]. Recent studies indicate that elevated levels of α-tocopherol in individuals with severe coronary artery lesions may reflect changes in vitamin E metabolism during periods of increased oxidative stress [38]. Based on the available evidence [44], it is not clear whether α-tocopherol or vitamin E has a causal linkage with CVDs. Although some studies have shown vitamin E's potential benefits in preventing CVD, clinical trials have not consistently shown such positive effects. Moreover, uncontrolled vitamin E supplementation may lead to adverse outcomes. Despite our study revealing a connection between α-tocopherol levels and the risk of HF and CHD, further investigations are warranted to establish the causal relationship.

Citrulline, a proteinogenic amino acid, was first identified by Wada in 1930 and named after *Citrullus*, the scientific term for watermelon [45]. Citrulline serves as the final byproduct of glutamine breakdown and an intermediate compound of the arginine synthesis pathway. It is important to note that this proteinogenic acid is not integrated into any polypeptides [46]. Glutamine is a precursor for ornithine, which is transformed into citrulline in the intestine. Enterocytes can also convert arginine into citrulline [46], but since they lack arginosuccinate synthetase, citrulline is discharged into the portal venous system [34]. Although physiologically, citrulline absorption is very limited in the liver [47], it gains access to the bloodstream and is subsequently converted into arginine in the kidneys. Therefore, circulating citrulline is primarily released from small intestinal enterocytes, predominantly in the proximal sections of the jejunum and duodenum [46].

Ornithine, a non-essential amino acid, is vital in the urea cycle [48]. It is synthesized by enzymatic modification of arginine in the cytoplasm, and urea is produced as a byproduct [49]. Ornithine is then translocated to the mitochondria via ornithine translocase 1 [50], where it participates in mitochondrial metabolism [51]. A survey by Krebs and Bezinger shows that ornithine acts in the cell as a urea cycle-associated enzyme [48, 52]. This proposition challenged a previous understanding of ornithine's role as a catalyst and found that ornithine could synthesize arginine in the presence of ammonia and carbon dioxide [53]. Subsequently, arginine reacts with water to generate urea and ornithine. Later, ornithine was characterized as an intermediary product in the urea cycle as well as a substrate for certain rate-limiting enzymes, including ornithine transcarboxylase (OTC), ornithine aminotransferase (OAT), and ornithine decarboxylase (ODC), which produce citrulline, proline, and polyamines, respectively [48]. Notably, individuals diagnosed with type 2 diabetes retinopathy are found to possess higher plasma citrulline levels than in diabetic controls. Also, proliferative diabetic retinopathy (PDR) patients present increased serum levels of asymmetric dimethylarginine (ADMA) levels in contrast to those with non-proliferative diabetic retinopathy (NPDR) [54]. In line with our findings, another study demonstrated a positive correlation between citrulline and ornithine levels and cardiovascular disease risk [55]. Furthermore, alterations in arginine metabolism, such as the citrulline to ornithine ratio, have been documented in ST-segment elevation myocardial infarction (STEMI) patients [56]. Our findings also indicate that the citrulline-to-ornithine ratio is a critical risk factor for HF and CHD. However, more functional research is needed to determine whether the citrulline-to-ornithine ratio has a causal relationship with CVD risks.

γ-Glutamyl glycine is a dipeptide synthesized by dipeptidyl peptidase from polypeptides. During digestion, dietary proteins are initially hydrolyzed into dipeptides, which then undergo further hydrolysis steps to produce individual amino acid residues. Dipeptides stimulate G-cells in the stomach to release gastrin.

γ-Glutamyl glycine shares a structural similarity with γ-aminobutyric acid (GABA) and functions as a blocker to excitatory amino acid receptors [57]. A study shows that a deficiency in reduced glutathione levels in individuals with CVD impacts their heart functionality and structural integrity [58]. These findings also suggest the potential application of serum glutathione level as a novel diagnostic predictor for identifying symptomatic individuals with anomalies in the heart structure [58]. Our study uncovered a connection between the levels of γ-glutamyl glycine and a factor that enhances the protective factor against HF and CHD, thereby confirming the consistency with previous findings.

7-Methylguanine (7-MG) level is a byproduct of nucleic acid degradation [59] and is found in trace amounts in human urine [60, 61]. 7-MG serves as an indicator of the overall RNA turnover rate [62, 63]. The methyltransferase-mediated methylation of the guanosine cap is crucial for the maturation and translation of mRNA [64, 65]. The rate of 7-MG formation and its levels have been associated with the degree of DNA exposure to methylating agents and biological aging [66, 67]. However, there is no evidence to confirm whether nucleotides are synthesized from free 7-MG bases or 7-MGs are directly incorporated into nucleic acids [68, 69]. Xanthine oxidase drives the conversion of a portion of 7-MG to 8-hydroxy-7-methylguanine [69, 70]. Importantly, levels of 7-MGs can be exploited to gauge the extent of DNA damage caused by methylating agents, including nitrosamines in tobacco [71] and the environmental pollutant methyl bromide [72]. Several studies have exploited increased urinary 7-MG levels as a potential diagnostic predictor of DNA damage in humans [73], especially in active smokers [74]. Elevated levels of 7-MG excretion are also found in individuals diagnosed with colon carcinoma [75]. Notably, we demonstrate that urinary 7-MG level provides valuable insights into the status of oxidative DNA damage, particularly DNA methylation. The excretion level of urinary 7-MG can also serve as a significant indicator for assessing the risk of pulmonary tumors [67]. Importantly, our study reveals an association between 7-MG level and risks of HF and CHD onset. However, further functional investigations are needed to determine whether the &-MG level in serum or urine has a causal relationship with CVD risks.

Limitations

Our research has certain limitations that should be acknowledged. First, despite conducting multiple sensitivity analyses, we were unable to fully assess horizontal pleiotropy. Second, the insufficiency of individual-level information prevented us from conducting stratified observational analysis in this cohort, which could have provided further insights. Third, our study fully relied on a European database, which may impede the broad applicability of the findings to other ethnic groups. Finally, we employed a less stringent gauge to evaluate the outcomes, which might have led to false-positive outcomes included in this analysis. Apart from these shortcomings, this MR-based approach allowed us to comprehensively explore the potential association between CVD risks and altered metabolite profiles.

Conclusions

Our MR study presents compelling evidence of the influence of metabolite functions in the development of HF and CHD. By utilizing genetic markers as instrumental variables, we have identified specific metabolic risk factors with a causal impact on these cardiovascular conditions. These findings underscore the importance of addressing metabolic dysfunction in the prevention and management of HF and CHD.

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Disclosure of conflict of interest

None.

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Supplementary Table 1A. The pleiotropy of casual relation between metabolites and HF & CHD

Exposure	egger_intercept	se	pval
Hippurate levels	-0.005700114	0.01095852	0.610078956
N-acetylaspartate (naa) levels	0.000434982	0.007155707	0.952236872
Galactonate levels	0.012500484	0.015388194	0.430192372
Campesterol levels	-0.004792131	0.014605177	0.746833749
Gamma-glutamylglycine levels	-0.006302273	0.006216028	0.321664031
7-methylguanine levels	0.004762516	0.00969691	0.62867532
Glutamine degradant levels	-0.002527659	0.008774605	0.775769809
Cysteine-glutathione disulfide levels	-0.007787872	0.008841641	0.386806283
Glutarylcarnitine (c5-dc) levels	-0.008566661	0.008338535	0.312745216
1-ribosyl-imidazoleacetate levels	0.006427391	0.009954445	0.522697235
Glycocholenate sulfate levels	-0.001741163	0.006573645	0.792756902
Androstenediol (3alpha, 17alpha) monosulfate (3) levels	-0.001739707	0.005293609	0.744871007
2,3-dihydroxyisovalerate levels	0.009604482	0.007741527	0.227805346
Oleoyl-linoleoyl-glycerol (18:1/18:2) [2] levels	-0.001112184	0.009549172	0.908211317
Imidazole propionate levels	0.013437274	0.010352087	0.20981544
Mannonate levels	-0.002214356	0.005754535	0.704445639
Etiocholanolone glucuronide levels	0.003819452	0.007918587	0.635377665
Tricosanoyl sphingomyelin (d18:1/23:0) levels	0.004605514	0.010922972	0.677043645
Behenoyl dihydrosphingomyelin (d18:0/22:0) levels	0.000290972	0.008758333	0.973691548
Arachidonoylcholine levels	-0.011154591	0.010428518	0.299741458
Methylsuccinoylcarnitine levels	0.013947113	0.008869434	0.12748297
Docosahexaenoylcholine levels	0.010915997	0.010986713	0.333605059
Linolenoylcarnitine (C18:3) levels	-0.002405378	0.013309331	0.858235073
2-butenoylglycine levels	0.001663412	0.011889346	0.889853951
Sphingomyelin (d17:1/14:0, d16:1/15:0) levels	0.002635602	0.010934794	0.81176482
Tetradecadienedioate (C14:2-DC) levels	-0.002627137	0.007686862	0.735767275
Pentose acid levels	-0.011669304	0.010182395	0.266013194
Erucate (22:1n9) levels	0.011434122	0.012999203	0.391339669
Alpha-tocopherol levels	-0.002028143	0.011760933	0.864474235
4-acetamidobutanoate levels	0.003444807	0.009309161	0.715918901
N1-methyladenosine levels	-0.00641372	0.012234202	0.603960331
N-formylmethionine levels	-0.002815058	0.011609872	0.811013309
N-acetylputrescine levels	0.00545761	0.00650642	0.411026265

3-(4-hydroxyphenyl)lactate levels	-0.003758431	0.01257031	0.767417016
S-1-pyrroline-5-carboxylate levels	0.011438607	0.012366682	0.368731042
Ornithine levels	-0.005760178	0.008622928	0.511076945
Serine levels	0.012130953	0.007974928	0.137472279
Salicylate levels	0.007799578	0.011059371	0.488406622
Cytidine levels	-0.01230232	0.007291415	0.103079899
X-15503 levels	0.006287607	0.010882316	0.571453771
X-18779 levels	0.013056066	0.011634791	0.277395609
X-23641 levels	-0.004538138	0.007476756	0.549137625
X-24546 levels	-0.005347462	0.007197019	0.464997363
X-25422 levels	0.004100583	0.011128895	0.71621661
N2-acetyl,N6-methyllysine levels	0.005892266	0.006048359	0.338608789
Glycine levels	-0.001348955	0.005460062	0.807381252
Glycine to alanine ratio	0.000595385	0.010095123	0.953700381
Phosphate to N-acetylneuraminate ratio	0.007828457	0.008369984	0.357356607
Serine to alpha-tocopherol ratio	0.003655104	0.009467099	0.702575119
Spermidine to (N(1) + N(8))-acetylspermidine ratio	-0.00616181	0.009214777	0.511737855
Phosphate to N-palmitoyl-sphingosine (d18:1 to 16:0) ratio	-0.009814502	0.009056328	0.291382323
Cholate to bilirubin (Z,Z) ratio	0.001276305	0.011114016	0.909615659
Adenosine 5’-monophosphate (AMP) to citrate ratio	0.002208278	0.011300368	0.847038572
Phosphate to glutamine ratio	0.013153499	0.010616598	0.226868362
Phosphate to cysteine ratio	-0.004332823	0.010981162	0.697337219
Citrulline to ornithine ratio	-0.001301382	0.011773228	0.912942111
N-palmitoyl-sphingosine (d18:1 to 16:0) to N-palmitoyl-sphinganine (d18:0 to 16:0) ratio	0.011931667	0.013976689	0.405149331
Cholesterol to oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] ratio	-0.001195282	0.010805495	0.912656023
Cholesterol to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	0.00059159	0.009788013	0.952234349
Glucuronate to etiocholanolone glucuronide ratio	0.003384109	0.009064036	0.712457254
Androstenediol (3beta,17beta) monosulfate (2) levels	-0.003321211	0.008398318	0.695856777

Supplementary Table 1B. The heterogeneity of casual relation between metabolites and HF & CHD

Exposure	Method	Q	Q_df	Q_pval
Hippurate levels	Inverse variance weighted	14.74436276	17	0.613889025
N-acetylaspartate (naa) levels	Inverse variance weighted	13.26120465	18	0.775827731
Galactonate levels	Inverse variance weighted	9.057717402	15	0.874480283
Campesterol levels	Inverse variance weighted	35.92987072	18	0.007203397
Gamma-glutamylglycine levels	Inverse variance weighted	28.59007899	23	0.194286998
7-methylguanine levels	Inverse variance weighted	16.72338673	21	0.727731583
Glutamine degradant levels	Inverse variance weighted	31.45976395	25	0.174243565
Cysteine-glutathione disulfide levels	Inverse variance weighted	24.98594702	26	0.519770831
Glutarylcarnitine (c5-dc) levels	Inverse variance weighted	31.84023619	30	0.374994474
1-ribosyl-imidazoleacetate levels	Inverse variance weighted	33.3692745	36	0.594357639
Glycocholenate sulfate levels	Inverse variance weighted	46.52168102	34	0.074536797
Androstenediol (3alpha, 17alpha) monosulfate (3) levels	Inverse variance weighted	44.14065909	29	0.035589781
Androstenediol (3beta,17beta) monosulfate (2) levels	Inverse variance weighted	14.8377578	26	0.96022945
2,3-dihydroxyisovalerate levels	Inverse variance weighted	23.41311821	23	0.43687502
Oleoyl-linoleoyl-glycerol (18:1/18:2) [2] levels	Inverse variance weighted	31.13421887	26	0.223308524
Imidazole propionate levels	Inverse variance weighted	13.42799298	20	0.858275121
Mannonate levels	Inverse variance weighted	20.00032795	21	0.521240478
Etiocholanolone glucuronide levels	Inverse variance weighted	15.94254763	19	0.661107799
Tricosanoyl sphingomyelin (d18:1/23:0) levels	Inverse variance weighted	32.86652687	25	0.134438864
Behenoyl dihydrosphingomyelin (d18:0/22:0) levels	Inverse variance weighted	43.40836506	35	0.15564827
Arachidonoylcholine levels	Inverse variance weighted	10.15783858	18	0.926632509
Methylsuccinoylcarnitine levels	Inverse variance weighted	42.06770266	28	0.042726236
Docosahexaenoylcholine levels	Inverse variance weighted	22.49671101	19	0.260237281
Linolenoylcarnitine (C18:3) levels	Inverse variance weighted	32.29650167	23	0.094132551
2-butenoylglycine levels	Inverse variance weighted	25.44577043	26	0.493869315
Sphingomyelin (d17:1/14:0, d16:1/15:0) levels	Inverse variance weighted	21.21275514	23	0.56809653
Tetradecadienedioate (C14:2-DC) levels	Inverse variance weighted	21.52964981	23	0.548776453
Pentose acid levels	Inverse variance weighted	15.35796131	20	0.755571714
Erucate (22:1n9) levels	Inverse variance weighted	17.65085631	18	0.478866134
Alpha-tocopherol levels	Inverse variance weighted	47.21746	26	0.00664558
4-acetamidobutanoate levels	Inverse variance weighted	20.53678523	18	0.303423204
N1-methyladenosine levels	Inverse variance weighted	44.19750588	31	0.05865241
N-formylmethionine levels	Inverse variance weighted	18.34483491	20	0.564703653

N-acetylputrescine levels	Inverse variance weighted	25.73998928	22	0.263012843
3-(4-hydroxyphenyl)lactate levels	Inverse variance weighted	36.52304255	26	0.082461092
S-1-pyrroline-5-carboxylate levels	Inverse variance weighted	18.86801669	17	0.336160299
Ornithine levels	Inverse variance weighted	27.54976381	23	0.233287481
Serine levels	Inverse variance weighted	40.3997067	35	0.243930561
Salicylate levels	Inverse variance weighted	23.89377665	22	0.352822033
Cytidine levels	Inverse variance weighted	33.88655633	28	0.204633301
X-15503 levels	Inverse variance weighted	7.787679561	17	0.970878248
X-18779 levels	Inverse variance weighted	13.62769447	18	0.753018802
X-23641 levels	Inverse variance weighted	22.46082952	27	0.713609314
X-24546 levels	Inverse variance weighted	21.10461069	24	0.632546457
X-25422 levels	Inverse variance weighted	25.25652613	22	0.284965305
N2-acetyl,N6-methyllysine levels	Inverse variance weighted	31.36299753	28	0.301199726
Glycine levels	Inverse variance weighted	23.53194976	21	0.316284654
Glycine to alanine ratio	Inverse variance weighted	25.58438448	17	0.082372832
Phosphate to N-acetylneuraminate ratio	Inverse variance weighted	27.52221593	30	0.595739336
Serine to alpha-tocopherol ratio	Inverse variance weighted	32.2622208	27	0.222511882
Spermidine to (N(1) + N(8))-acetylspermidine ratio	Inverse variance weighted	19.81222132	20	0.469729958
Phosphate to N-palmitoyl-sphingosine (d18:1 to 16:0) ratio	Inverse variance weighted	17.87080565	21	0.65716471
Cholate to bilirubin (Z,Z) ratio	Inverse variance weighted	25.83739184	23	0.308602522
Adenosine 5’-monophosphate (AMP) to citrate ratio	Inverse variance weighted	18.56434354	21	0.613064794
Phosphate to glutamine ratio	Inverse variance weighted	29.17291027	26	0.303277854
Phosphate to cysteine ratio	Inverse variance weighted	28.55112607	21	0.125201776
Citrulline to ornithine ratio	Inverse variance weighted	28.14560438	24	0.253946381
N-palmitoyl-sphingosine (d18:1 to 16:0) to N-palmitoyl-sphinganine (d18:0 to 16:0) ratio	Inverse variance weighted	32.69796086	18	0.01815081
Cholesterol to oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] ratio	Inverse variance weighted	59.31879146	31	0.001622351
Cholesterol to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	Inverse variance weighted	44.88335993	29	0.030200737
Glucuronate to etiocholanolone glucuronide ratio	Inverse variance weighted	29.3767385	23	0.168141894