Research Square

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Evaluation of the genetic architecture of human blood metabolites on sudden cardiac arrest: A Mendelian randomization analysis

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Abstract

Background

Previous studies supported metabolic disturbances in sudden cardiac arrest (SCA) patients. Still, the evidence about the causal role of metabolites in SCA is lacking. We investigated the causality between the two, aiming at providing novel targets for SCA prevention.

Methods

We performed a bidirectional two-sample Mendelian randomization (MR) analysis based on genome-wide association study (GWAS). The summary dataset for 1,091 metabolites and 309 metabolite ratios was obtained from a GWAS including 8,299 participants. The summary datasets for SCA were obtained from the FinnGen consortium (n_{cases} =2,308 and $n_{controls}$ =191,924) and the meta-analysis (n_{cases} =3,939 and $n_{controls}$ =25,989), respectively. Sensitivity analyses were performed for the assessment of horizontal pleiotropy and heterogeneity. In order to fully verify the causality, we also used Steiger test, linkage disequilibrium score regression (LDSC), and multivariable MR (MVMR).

Results

Through inverse variance weighted (IVW) and sensitivity analysis filtration, five metabolites and one metabolite ratio with causal effects on SCA were identified from the FinnGen consortium, except for one of the metabolites categorized as unknown. After excluding biased SNPs, the causality still remained significant for [N2,N5-diacetylornithine (odds ratio (OR) = 1.12, 95% confidence interval (CI) = 1.02–1.22, *P* = 0.019), ascorbic acid 3-sulfate (OR = 1.16, 95% CI = 1.04–1.30, *P* = 0.009), uridine to pseudouridine ratio (OR = 1.21, 95% CI = 0.598–0.930, *P* = 0.009)]. These metabolites still remained significant associations with SCA when combined with the SCA GWAS of meta-analysis.

Conclusions

This MR study presented a unique perspective that might support the causal relationships between human blood metabolites and SCA, offering valuable opportunities to enhance screening, prevention, and therapeutic strategies for SCA. (253)

1. Background

Sudden cardiac arrest (SCA) is a critical emergency event that poses a significant threat to lives and health, remaining a leading cause of global mortality with associated health and economic burdens. The worldwide incidence of SCA is estimated to be approximately 418 cases per 100,000 individuals, reaching

as high as 10% of total mortality in developing countries[1, 2]. The primary electrophysiologic cause of SCA is known as ventricular fibrillation (VF), whereas the predominant pathological substrate is believed to be coronary artery disease (CAD)[3]. Patients may succumb to cardiovascular failure in the initial days following SCA, and even survivors may endure severe brain damage[4]. In addition, the incidence and outcome of cardiac arrest (CA) are not solely dependent on the patient's condition but also reflect the level of national socioeconomic and health advancement. Mortality rates from CA in developing countries can soar as high as 10%, while even in developed nations such as the United States, the pre-discharge average survival rate varies widely, ranging from less than 1% to over 25%[5]. The high prevalence and recurrence of SCA result in a heavy burden on human society; thus, the prevention of disease is extremely critical.

Due to the acute onset and narrow treatment window of SCA, it is nearly impossible to identify risk factors through randomized controlled trials or retrospective observational studies. Currently, blood neuron-specific enolase (NSE), spectrin breakdown products (SBDP), neurofilament heavy chain (Nf-H), red cell distribution width (RDW)[6–8] and other clinical biomarkers are employed for severity prognosis. However, there is still room for enhancing the sensitivity and specificity of these indicators. Organisms undergo a series of metabolic changes to adapt to various forms of acute or chronic diseases. With the development of metabolomics, research on the metabolomics of SCA is steadily increasing. At the animal level, researchers observed that targeting metabolic pathways such as kynurenine pathway[9], ketone pathway[10], mitochondrial energy[11] can attenuate brain injury after SCA, improve prognosis, and prolong survival in mice. Previous studies have also reported several circulating metabolite biomarkers in SCA patients: N-acetylaspartate/creatinine ratios, lactate levels, glucose level served as predictive indicators for unfavorable outcomes[12, 13]. Although previous research has clearly demonstrated metabolic disorders in SCA, comprehensive and systematic investigations are still needed to determine the exact causal influences between SCA and human blood metabolites.

Mendelian randomization (MR) is an emerging statistical technique in which researchers can use genetic variants that serve as instruments for the exposures of interest to assess the causality of an exposure on an outcome and minimize biases arising from reverse causality or confounding factors[14]. According to ongoing genetic advancements, genome-wide association studies (GWASs) today provide a wealth of potential tools for MR analysis. Previous MR studies have explored the causal relationships between cardiac electrophysiologic factors, anthropometric traits, heart rate variability, sleeping phenotypes, and SCA[3, 15, 16]. The heritability of numerous metabolite levels is high, presenting an opportunity for the implementation of MR. Nevertheless, the genetic association between human blood metabolites and SCA has received limited exploration and warrants further investigation. Consequently, our research aims to provide a novel perspective for exploring the fundamental mechanisms and causal pathways influencing the risk of SCA, inspired by the work of Cai et al.[17].

2. Materials and methods

2. 1 Study design

This MR study was conducted to investigate the causal relationships between blood metabolites and SCA. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) reporting guideline[18]. Specifically, the MR design should rely on three fundamental assumptions, as shown in Fig. 1: (A) Instrumental variables (IVs) should be strongly correlated with human blood metabolites; (B) IVs should be unrelated to potential confounding factors; (C) IVs should be specifically associated with SCA only via human blood metabolites. Among these assumptions, the second and third are commonly referred to as the independence of horizontal pleiotropy, a condition that can be assessed through a variety of statistical methods.

All analyses were performed using the TwoSampleMR (version 0.5.7), MendelianRandomization (version 0.8.0), MRPRESSO package (version 1.0) in R Software version 4.3.1 (https://www. R-project. org) and and LDSC software.

Since the data were analyzed using publicly released summary statistics and individuals provided informed permission in these first trials, no extra ethical approval was needed.

2. 2 Data sources for human blood metabolites

Genetic information for each blood metabolite was obtained from the newest and largest GWAS with high-throughput metabolic profiling by Chen et al., which provides extensive data for GWAS on the human metabolomes[19](**Additional File 2, Table S1**). The GWAS summary statistics were publicly available at GWAS catalog (https://www.ebi.ac.uk/gwas/). Specifically, the extensive GWAS series included the analysis of 1,091 metabolites and 309 metabolite ratios in a cohort of 8,299 European participants from the Canadian Longitudinal Study on Aging. Among these 1,091 metabolites, 850 across eight super pathways are well known, including lipid, amino acid, xenobiotics, nucleotide, cofactor and vitamins, carbohydrate, peptide and energy[20]. Meanwhile, another 241 metabolites remain categorized as unknown, as their chemical identities have yet to be conclusively established.

2. 3 Data sources for sudden cardiac arrest

The most recent and largest GWAS summary statistics for SCA in primary analysis were based on the European population of the Finngen consortium (n = 194,232, EUR), including 2,308 SCA cases and 191,924 controls. FinnGen, a major public-private collaboration, aims to collect and analyze genome and health data from 500,000 participants in the Finnish biobank. We utilized the GWAS data on SCA from a meta-analysis that included 9 studies with a European-descendent population, recruiting 25,989 controls and 3,939 SCA cases, in order to validate our results through replication analysis and meta-analysis[3, 16]. The GWAS statistics are accessible to the public via the website

(https://www.finngen.fi/en/access_results;
http://arkinglab.org/upload/SCA/METAANALYSIS_woHSDS.SUDS_Jun20151.tbl.gz) (Table 1).

Table 1
Details for data source of sudden cardiac arrest (SCA) .

Data source	Sample size	Cases	Non-cases	Publication Years
Finngen_R9_I9_CARDARR	194,232	2,308	191,924	2023
A meta-analysis of GWAS	29,928	3,939	25,989	2018

2. 4 Instrumental variables selection

A series of quality control criteria were applied to select eligible genetic IVs. Specifically, at first, recognizing the constrained number of single-nucleotide polymorphisms (SNPs) attaining genome-wide significance, we selected SNPs with a *p*-value below the significance level ($P<1\times10^{-5}$) in the initial analysis as IVs which was in accordance with the study of Cai et al.[17]. Subsequently, all IVs underwent linkage disequilibrium (LD) clumping ($r^2<0.001$; clumping window size = 10,000 kb) to mitigate the influence of correlated SNPs. Meanwhile, we calculated the *F*-statistic [$R^2(N-2)/(1-R^2)$], which assesses the strength of each IVs, where R^2 represents the proportion of variance explained by IVs and N is the effective sample size of GWAS[21]. The SNPs with an *F*-statistic threshold greater than 10 were chosen for the subsequent MR analysis as they provided a reliable estimate of genetic variation[22]. Then, we identified the SNPs mentioned above in the outcome and checked whether these SNPs were associated with the outcome, and SNPs with a *p*-value of less than 5×10^{-5} in the outcome that needed to be excluded. Subsequently, harmonization procedures were implemented to align the alleles of exposure-SNPs and outcome-SNPs and we excluded palindromic SNPs (where the effective allele is unclear) from our study. Metabolites with more than three SNPs were retained for MR analysis. In addition, Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/) was screened to identify the potential pleiotropic effects[23].

2. 5 Primary MR analysis

We chose FinnGen consortium for the primary analyses. Two-sample MR analysis was carried out to evaluate the causal effects between blood metabolites and SCA. The inverse variance weighted (IVW) method was selected as the main MR analysis to scan preliminary relationships of human blood metabolites with SCA[22], which generates a pooled estimate by combining all of the Wald ratios for each SNP but also prone to pleiotropic bias. The choice of the random-effects and fixed-effects IVW approaches is based on heterogeneity test. In the presence of heterogeneity, we favor the random-effects IVW model.

To enhance the robustness of results, we employed auxiliary causal analysis methods: MR-Egger method, weighted median method, and weighted mode analysis[24–26]. All these auxiliary MR methods have different assumptions regarding the validity of the genetic instruments. The MR-Egger method implies the presence of horizontal multiplicity if its intercept term is significant. Weighted median, which is deemed second only to the IVW in statistical efficacy, allows for the use of invalid instruments under the assumption that at least half of the instruments used in the MR analysis are valid. Weighted mode assumes that all genetic variants exert an influence on a single factor with uniformity of effect.

2. 6 Sensitivity analysis

Sensitivity analyses were performed for the identified significant estimates (IVW, $P < 0.05$) to assess potential biases associated with MR assumptions. To check for heterogeneity in our investigation, we computed the Cochran Q statistic for IVW and MR-Egger. Existing heterogeneity was identified as $P < 0.05$ and $I^2 > 25\%$ using the Cochran-Q method[27]. Horizontal pleiotropy was assessed by estimating the deviation of the MR-Egger intercept as previously described, $P<0.05$ was recognized as existing horizontal pleiotropy[26]. Moreover, we used the leave-one-out (LOO) analysis to determine whether the results were reliable. To look for outliers, the MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) analysis was employed.

As a result, the following criteria were satisfied in order to identify the possible viable candidate metabolites implicated in SCA development: 1) consistent directions and magnitude among the four MR methods; 2) no heterogeneity or pleiotropy was found; 3) SNPs with significant effects found by LOO were removed.

2. 7 Replication and meta-analysis

We repeated IVW analysis using additional independent meta-analysis of GWAS data previously indicated to confirm the robustness of candidate blood metabolites. Subsequently, findings from the 'primary' and 'replication' stages were then meta-analyzed.

2. 8 Confounding analysis and multivariable MR (MVMR)

In order to determine whether the SNPs linked to metabolites were also associated with other common risk factors that might skew the results, such as height, body mass index (BMI), coronary heart disease (CAD)[3], and others, we also scanned the Phenoscanner V2 website (http://www.phenoscanner.medschl.cam.ac.uk/). After removing these confounding SNPs, we repeated IVW and FDR correction to attempt to confirm the robustness of our findings. In order to ensure that no other factor mediates the direct effect of each exposure factor on the outcome, MVMR was chosen simultaneously to assess the combined effects of two or more risk variables that have overlapping

SNPs[28]. To account for their interactions, we used MVMR on the identified metabolites in our investigation. The main MVMR analytical technique was MVMR-IVW, while MVMR-Egger and MVMR-median were also applied. Additionally, sensitivity analyses were carried out as part of the MVMR analyses to assess robustness.

2. 9 Genetic correlation and directionality assessment

We used the Steiger test[17], to verify whether the observed causalities were biased by reverse causation. MR estimates might not precisely reflect causal a relationship in the presence of a genetic correlation between an exposure and the outcome of interest. Therefore, to investigate the impact of shared genetic factors on the identified causal relationships, we assessed genetic associations between the identified metabolites and SCA using linkage disequilibrium scorecausation (LDSC)[29] .

3. Results

3. 1 Genetic instrument for human blood metabolites

After following the steps for instrument selection, a total of 1,400 metabolites were retained for MR analysis. The number of SNPs associated with each metabolite ranges from 11 to 82. The *F*-statistics of IVs are all greater than 10, demonstrating a small possibility of weak IV bias. Additional details regarding the harmonized data are available in **Additional File 2, Table S2**.

3. 2 Causal effects of metabolites on sudden cardiac arrest

The IVW method initially identified 64 metabolites significantly ($P<0.05$) associated with SCA. Among them, 15 metabolites remained chemically unknown, while the remaining 49 metabolites were assigned to amino acid, carbohydrate, energy, lipid, and peptide metabolism. However, only five metabolites and one metabolite ratio exhibited statistically significant positive results in four auxiliary analyses. Hexadecanedioate (C16-DC) played a protective role in SCA (OR = 0.85, 95% CI = 0.76–0.95, $P = 0.006$), while N2, N5-diacetylornithine (OR = 1.11, 95% CI = 1.01–1.22, $P = 0.024$), 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) (OR = 1.11, 95% CI = 1.01–1.21, $P = 0.027$), ascorbic acid 3-sulfate (OR = 1.16, 95% CI = 1.04–1.30, $P = 0.009$), uridine to pseudouridine ratio (OR = 1.24, 95% CI = 1.06–1.46, $P = 0.009$) and X-12798 (OR = 1.13, 95% CI = 1.03–1.24, $P = 0.008$) were associated with an increased risk of SCA (Fig. 2, Fig. 3)

Cochran *Q*-derived *p*-values and I^2 indicated no heterogeneity among those metabolites. Moreover, a pleiotropy test employing the MR-Egger intercept revealed that intercept *p*-values exceeded 0.05, signifying an absence of evidence for pleiotropy in these findings (**Additional File 2, Table S3**). Additionally, LOO analysis did not reveal any high-influence SNPs that could potentially skew the pooled impact estimates (**Additional File 1, Figure S1, Additional File 2, Table S4**). Consequently, these metabolites were identified for further investigation as potential candidate metabolites involved in the

pathogenesis of SCA. Moreover, no outliers were detected by the MRPRESSO approach. Simultaneously, a reverse MR analysis was conducted, revealing no evidence of causal relationships between SCA and the aforementioned five metabolites and one metabolite ratio (**Additional File 2, Table S5**).

3. 3 Replication and meta-analysis

To further verify our findings, we performed a replication analysis using SCA GWAS data from a meta-analysis involving nine studies. As expected, similar trends were observed in certain metabolites (Fig. 4). The combined analysis of the FinnGen datasets and the SCA GWAS data reaffirmed that a genetic liability to higher levels of metabolites predicted a higher risk of SCA [N2, N5-diacetylornithine (OR = 1.12, 95% CI = 1.03–1.23, P<0.05), 1-stearoy1-2-arachidonoyl-GPE (OR = 1.10, 95% CI = 1.02–1.19, P<0.05), ascorbic acid 3-sulfate (OR = 1.18, 95% CI = 1.06–1.31, P<0.05), uridine to pseudouridine ratio (OR = 1.21, 95% CI = 1.05–1.39, P<0.05), X-12798 (OR = 1.11, 95% CI = 1.01–1.22, P<0.05)]. Notably, hexadecanedioate (C16-DC) showed null estimates in the meta-analysis, revealing divergent directions when compared to the SCA meta-analysis GWAS database.

3. 4 Confounding analysis and MVMR

While sensitivity analysis did not reveal any evidence of bias undermining the MR estimates, we conducted a manual investigation into confounding factors (BMI, height, triglyceride, and CAD) associated with the metabolite-associated SNPs (**Additional File 2, Table S6**). Upon examination using Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/), we observed SNPs that were associated with ascorbic acid 3-sulfate did not associate with any of the confounders. For N2, N5-diacetylornithine, two SNPs (*rs3799344* and *rs76672197*); for uridine to pseudouridine ratio, two SNPs (*rs7666824* and *rs4939830*); and for X-12798, three SNPs (*rs1171615,rs1177442* and *rs157572*) were found to be associated with the confounders. After excluding these biased SNPs, the causality still remained significant for N2, N5-diacetylornithine (OR = 1.12, 95% CI = 1.02–1.22, P = 0.019), uridine to pseudouridine ratio (OR = 1.23, 95% CI = 1.03–1.46, P = 0.009), and X-12798 (OR = 1.13, 95% CI = 1.02–1.25, P = 0.019). However, the p-values for two metabolites, hexadecanedioate (C16-DC) (OR = 0.88, 95% CI = 0.74–1.05, P = 0.157) and 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) (OR = 1.08, 95% CI = 0.92–1.27, P = 0.338), were non-significant after eliminating these confounding SNPs. Moreover, the results of MVMR-IVW showed that genetically predicted N2,N5-diacetylornithine, ascorbic acid 3-sulfate, uridine to pseudouridine ratio, and X-12798 exerted independent causal influences on SCA (Table 2)

Table 2
Multivariable MR (MVMR) analysis of the subsequently identified blood metabolites. IVW, inverse-variance weighted; MR, Mendelian randomization.

Exposure	MR Method	SNPs	OR(95% CI)	*P*
N2,N5-diacetylornithine	MVMR-IVW	93	1.115(1.009,1.232)	0.032
	MVMR-Egger	93	1.115(1.002,1.241)	0.045
	MVMR-median	93	1.135(0.974,1.322)	0.105
Ascorbic acid 3-sulfate	MVMR-IVW	93	1.139(1.021,1.271)	0.020
	MVMR-Egger	93	1.139(1.020,1.272)	0.021
	MVMR-median	93	1.135(0.939,1.373)	0.191
Uridine to pseudouridine ratio	MVMR-IVW	93	1.266(1.075,1.492)	0.005
	MVMR-Egger	93	1.266(1.071,1.496)	0.006
	MVMR-median	93	1.429(1.108,1.843)	0.006
X-12798	MVMR-IVW	93	1.149(1.051,1.256)	0.002
	MVMR-Egger	93	1.149(1.051,1.256)	0.002
	MVMR-median	93	1.143(1.012,1.291)	0.031

3. 5 Genetic correlation and directionality assessment

The *p*-values of the Steiger test further supported the aforementioned results (Table 3). In addition, we conducted genetic correlation analysis by using LDSC[29] between SCA and three metabolites and one metabolite ratio: N2,N5-diacetylornithine (rg = 0.0151, se = 0.2534, *P* = 0.9526), ascorbic acid 3-sulfate (rg = 0.2926, se = 0.4071, *P* = 0.4724), X-12798 (rg = 0.5070, se = 0.2933, *P* = 0.0839), and uridine to pseudouridine ratio (rg = 0.0169, se = 0.3467, *P* = 0.9611), indicating that the MR estimates were not confused by common genetic components (**Additional File 2 Table S7**).

Table 3
Steiger direction test from blood metabolites to sudden cardiac arrest (SCA) .

Exposure	Hexadecanedioate	N2, N5-diacetylornithine	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	Ascorbic acid 3-sulfate	Uridine to pseudouridine ratio
p-value	< 0. 001	< 0. 001	< 0. 001	< 0. 001	< 0. 001
Direction	TRUE	TRUE	TRUE	TRUE	TRUE

4. Discussion

Mitochondrial dysfunction, certain signaling pathways, and inflammatory mediators have been found to be putative risk factors for a worse outcome in SCA through animal experiments[30–32]. These discoveries haven't, however, been effectively applied in clinical settings. Several treatment options post-cardiopulmonary resuscitation, specifically targeted temperature management[33] and hyperbaric oxygenation[34] have been documented. Nonetheless, these interventions' efficacy is insufficient. Some observational studies have identified various clinical and sub-clinical risk factors associated with SCA[3], however it is unclear how these associations are causally related. All of this emphasizes the significance and urgency of determining the etiology of SCA. Previous studies have demonstrated that metabolites serve as functional intermediates, offering insights into potential biological mechanisms underlying disease genetics[20, 35]. Some researchers have concentrated on revealing the relationship between systemic metabolic alterations and cardiovascular diseases[10, 36]. Interestingly, several animal and clinical studies have indicated that dynamic changes in specific bioactive blood metabolites, including lactate, glutamate, glycogen, lipids, and others, may influence the outcome of SCA[37]. However, there is still a lack of thorough and systematic investigation into the causal relationships between blood metabolites and SCA. Due to the inherent limitations of conventional observational studies and randomized controlled trials, a definitive characterization of the metabolite spectrum contributing to the initiation and development of SCA remains elusive based on the current evidence.

To the best of our knowledge, our study represents the first attempt to utilize MR analysis in investigating the causal influences between human blood metabolites and SCA. Our results indicate that genetically determined elevations in N2, N5-diacetylornithine, ascorbic acid 3-sulfate, and the uridine to pseudouridine ratio are associated with high SCA risk. The robustness of our findings has been confirmed through multiple-corrected methods.

Our MR study indicates that elevated levels of N2, N5-diacetylornithine, ascorbic acid 3-sulfate, and uridine to pseudouridine ratio played a detrimental effect on SCA, yet there is a dearth of information on these substances. According to untargeted metabolomic research by Razavi et al., a high level of N2, N5-diacetylornithine, a metabolite byproduct of the urea cycle associated with diastolic dysfunction, portends worsening cardiovascular endothelial dysfunction[38]. Luo et al. study also show that elevated levels of N2, N5-diacetylornithine indicate worse chronic renal failure (CKD) outcomes[39]. A new study recently found that CKD is a strong predictor of SCA in Hispanic and Latino populations[40]. It is probably that sub-clinical or clinical-stage kidney disease may lead to the accumulation of N2, N5-diacetylornithine, which initiates in response to metabolic perturbations, potentially coinciding with systemic microvascular and endothelial dysfunction, ultimately contributing to the development of SCA.

Ascorbic acid 3-sulfate represents a notable metabolite of ascorbic acid excreted in human urine and serves as an intermediate in the metabolic sulfation pathway involving L-ascorbic acid 3-sulfate[41]. A substantial amount of research supports ascorbic acid’s critical function in the prevention of SCA. Blood ascorbic acid levels were lower in post-arrest patients compared to healthy controls[42]. The recent study showed that ascorbic acid supplementation was not associated with a good neurologic outcome, while

ascorbic acid administration was significantly associated with a good neurologic outcome in older (≥ 65 years) SCA patients[43]. However, our study found that a high level of ascorbic acid 3-sulfate leads to an increase in SCA risk, indicating the complexity of essential ascorbate metabolism. As mentioned above, ascorbic acid 3-sulfate is a fairly ubiquitous metabolite in human urine[44], and its accumulation likely signifies an abnormality in renal function, ultimately leading to SCA.

Compared to metabolites, the exploration of genetic determinants influencing the ratio of substrates has the potential to provide valuable insights into basic biological processes. In addition to the aforementioned metabolites, notably, our study identified a metabolite ratio, the uridine to pseudouridine ratio, which was found to be associated with an elevated risk of SCA. Pseudouridine, an isomer of uridine, stands out as the most prevalent post-transcriptionally modified nucleotide found in stable RNAs across diverse organisms[45], which has been linked to various diseases. Notably, previous studies have observed significant associations of pseudouridine with cardiovascular disease[46].The uridine to pseudouridine ratio signifies the relative abundance of these nucleotides in RNA and may serve as an indicator of cellular processes or RNA modification dynamics. Pseudouridine synthase providing information on uridine to pseudouridine ratio, could be found in nucleus and mitochondria[45], and mitochondrial function appears to be of particular significance in SCA. Consequently, we hypothesize that pseudouridine synthase may be associated with the development of SCA by regulating the uridine to pseudouridine ratio, thereby influencing mitochondrial energy metabolism.

In the present study, hexadecanedioate (C16-DC) was identified as one of the risk factors for SCA using GWAS data from the FinnGen consortium. However, replication analysis using SCA GWAS data from the meta-analysis yielded discrepant estimates, which might be attributed to the small sample size in the meta-analysis (194232 vs. 29928). Due to conflicting results from two independent data sets, a definitive interpretation of the involvement of hexadecanedioate (C16-DC) in the pathogenesis of SCA could not be made. Additionally, the *p*-values for both hexadecanedioate (C16-DC) and 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) were non-significance after eliminating confounding SNPs; therefore, they are not further discussed. Taken together, the findings of our study are generally consistent with those of previous studies.

Our research possesses several strengths. The first of the strengths of our study lies in the utilization of the latest and most comprehensive GWAS summary data for SCA, incorporating over 190,000 individuals in the SCA sample, thereby ensuring robust statistical power. Secondly, our MR analysis included 1,400 metabolites, marking the first and most thorough investigation to date into the metabolic effects contributing to SCA and offering novel insights into its risk factors. Thirdly, our study minimized the risk of reverse causality by employing the Steiger test and bidirectional MR analysis. Lastly, we employed LDSC to evaluate the heritability of IVs and the genetic association between blood metabolites and SCA, thereby bolstering the credibility of the MR estimates.

Several limitations warrant consideration in our study. Firstly, due to the restricted number of SNPs reaching GWAS significance, we opted to relax the *P* threshold. Secondly, despite investigating 1,400

blood metabolites, only two metabolites and one metabolite ratio demonstrated statistically significant and robust associations. This may be attributed to the relatively limited proportion of SCA cases in the GWAS database. Thirdly, the predominant composition of participants in our study is of European descent. While this choice aids in mitigating population heterogeneity, it is imperative to validate the MR results in diverse populations to confirm their generalizability in future studies. The last limitations include the lack of a non-linear MR analysis and the use of clinical samples.

In conclusion, our MR study suggested potential causal relationships between blood metabolites and the risk of SCA, providing insights into the impact of circulating metabolic disturbances on SCA risk. Notably, levels of N2, N5-diacetylornithine, ascorbic acid 3-sulfate, and uridine to pseudouridine ratio emerge as promising circulating metabolic biomarkers for SCA screening and prevention in clinical practice. Moreover, these metabolites merit further investigation as candidate molecules in future explorations of the underlying mechanisms associated with SCA.

Declarations

Authors' Contributions

H.Y. and X.S.T. conceived and designed the study. H.J.Z. analyzed and collected the data. X.S.T. and H.J.Z. performed visualization. X.S.T. wrote the manuscript. All authors read and approved the final manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

All authors declare that there are no relationships or activities that might bias, or be perceived to bias, this work.

Ethics approval and consent to participate

Our study only utilized publicly available GWAS data, and ethical approval as well as consent to participate can be found in the original GWAS study.

Consent for publication

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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Figures

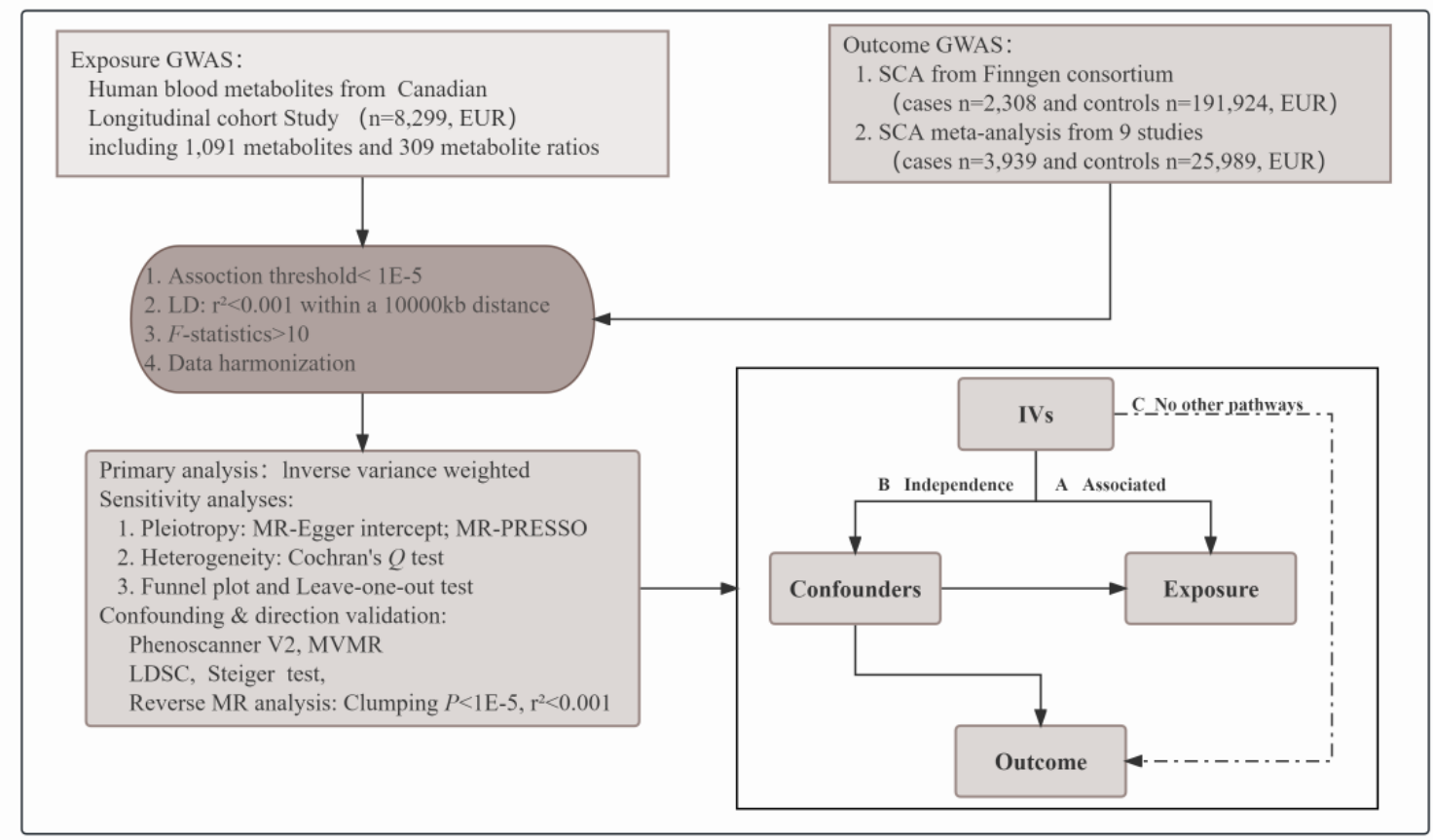

Figure 1

The general framework of this study.

Notes: Assumption A: genetic variants are strongly associated with exposure; Assumption B: genetic variants are not correlated with other confounders; Assumption C: Genetic variants exert their influence on the outcome solely via the specific exposure. MR, Mendelian randomization; MVMR, multivariable MR; LDSC, linkage disequilibrium score regression; SNPs, single nucleotide polymorphisms; GWAS, genome-wide association study.

Exposure	No.of SNP	Method	OR(95% CI)	P
Hexadecanedioate (C16−DC) levels	22	IVW	0.85 (0.76 to 0.95)	0.006
		MR Egger	0.82 (0.69 to 0.98)	0.043
		Weighted median	0.83 (0.72 to 0.95)	0.006
		Weighted mode	0.84 (0.72 to 0.97)	0.025
N2,N5−diacetylornithine levels	27	IVW	1.11 (1.01 to 1.22)	0.024
		MR Egger	1.19 (1.05 to 1.35)	0.013
		Weighted median	1.17 (1.02 to 1.34)	0.021
		Weighted mode	1.17 (1.04 to 1.31)	0.017
1−stearoyl−2−arachidonoyl−GPE (18:0/20:4) levels	32	IVW	1.11 (1.01 to 1.21)	0.027
		MR Egger	1.22 (1.04 to 1.44)	0.024
		Weighted median	1.11 (1.01 to 1.21)	0.027
		Weighted mode	1.22 (1.04 to 1.44)	0.024
Ascorbic acid 3−sulfate levels	20	IVW	1.16 (1.04 to 1.30)	0.009
		MR Egger	1.23 (1.05 to 1.43)	0.019
		Weighted median	1.22 (1.03 to 1.45)	0.020
		Weighted mode	1.21 (1.04 to 1.41)	0.025
Uridine to pseudouridine ratio	26	IVW	1.24 (1.06 to 1.46)	0.009
		MR Egger	1.50 (1.06 to 2.13)	0.032
		Weighted median	1.41 (1.11 to 1.78)	0.004
		Weighted mode	1.45 (1.03 to 2.04)	0.043
X−12798 levels	34	IVW	1.13 (1.03 to 1.24)	0.008
		MR Egger	1.16 (1.02 to 1.32)	0.027
		Weighted median	1.14 (1.02 to 1.27)	0.022
		Weighted mode	1.13 (1.01 to 1.25)	0.038

Figure 2

Forest plot illustrating the causal relationships between metabolites and the risk of sudden cardiac arrest (SCA) derived from IVW. OR, odds ratio; CI, confidence interval; IVW, inverse variance weighted.

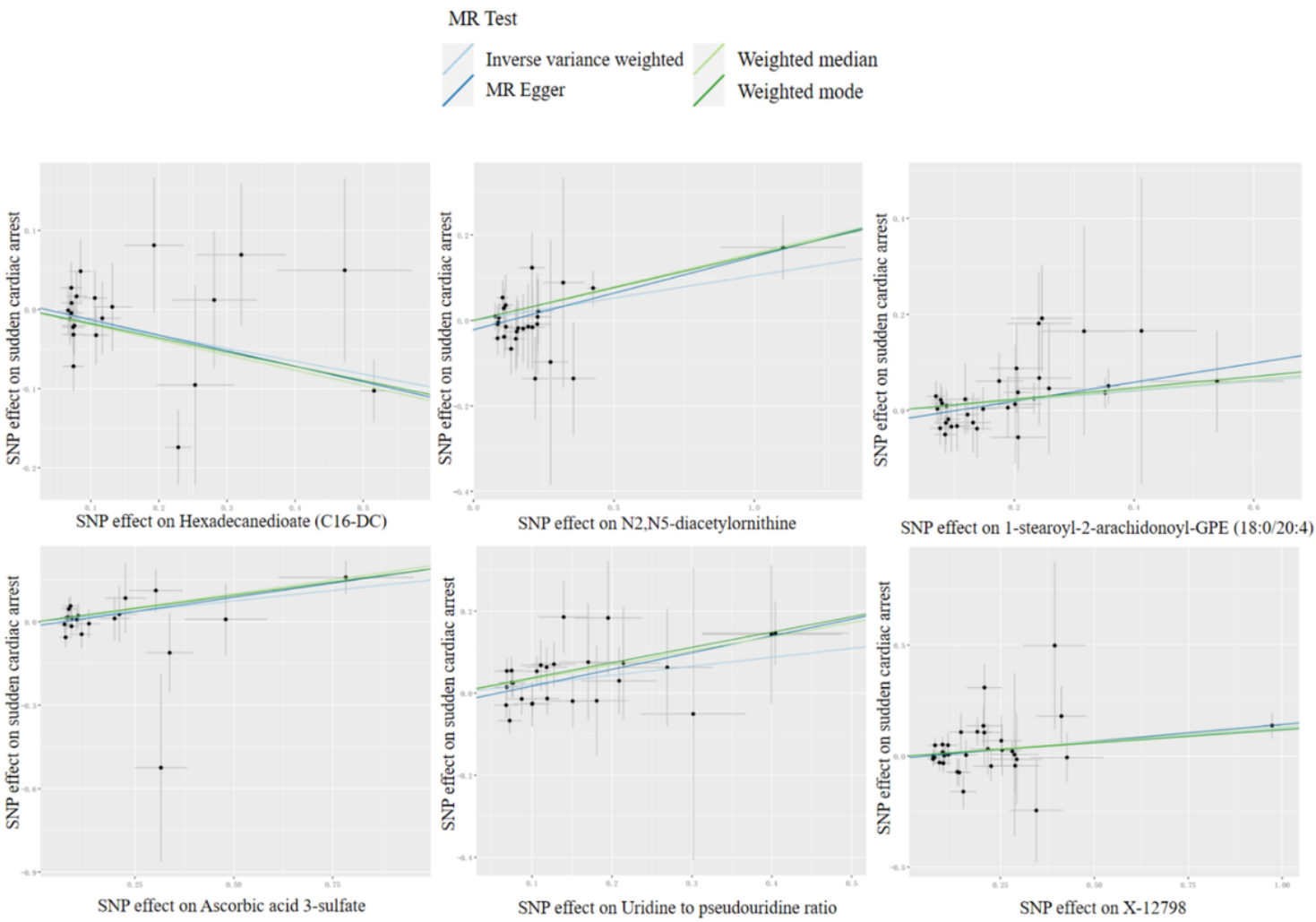

Figure 3

Scatter plot for the significant Mendelian randomization (MR) association (P<0. 05) between metabolites and sudden cardiac arrest (SCA) identified by inverse variance weighted (IVW). SNP, single nucleotide polymorphism.

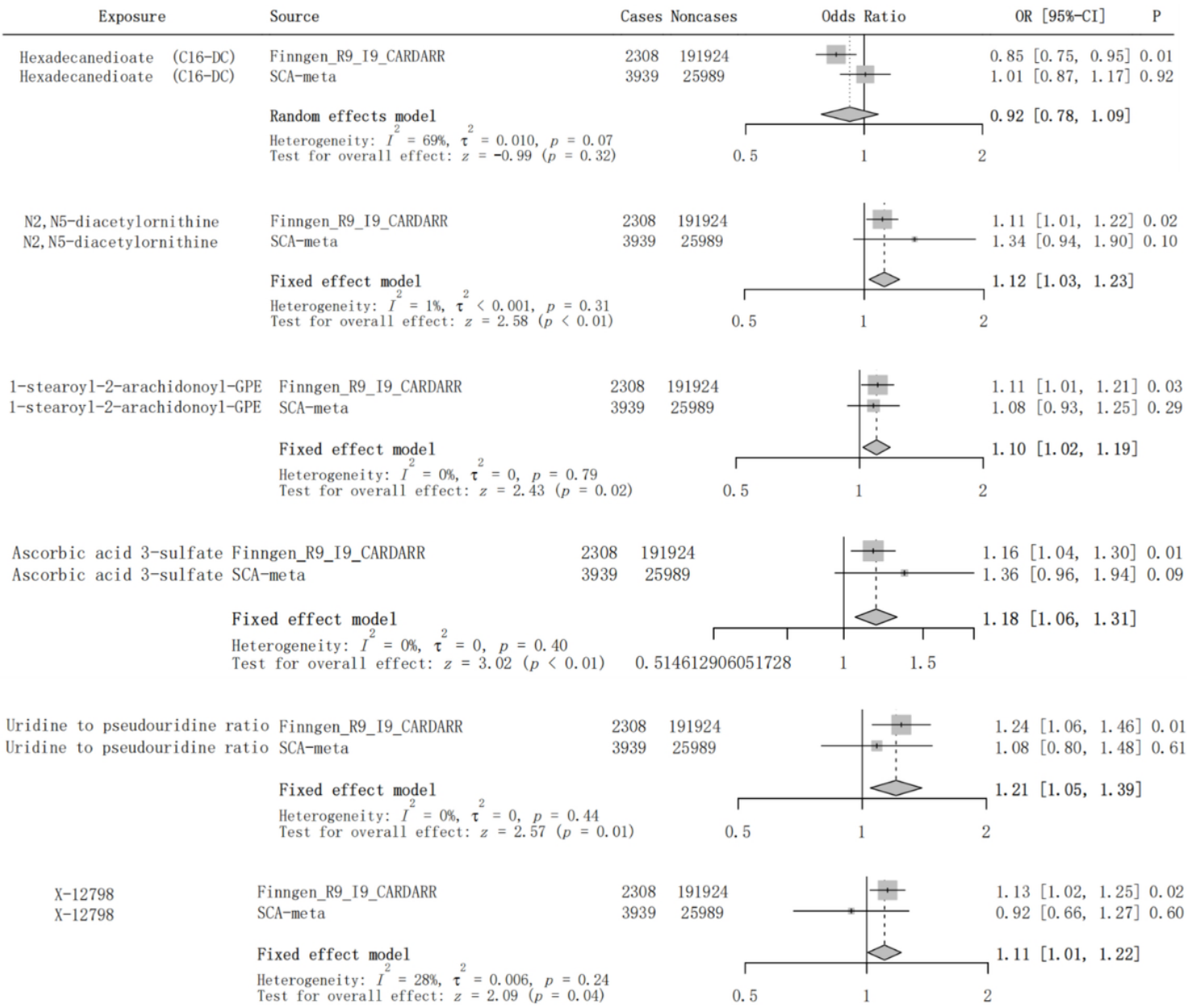

Figure 4

Meta-analysis of the causal associations between metabolites and sudden cardiac arrest (SCA). OR, odds ratio; CI, confidence interval. OR, odds ratio; CI, confidence interval.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx
- Additionalfile2.xlsx