scientific reports

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Assessing the causal relationship between metabolic biomarkers and coronary artery disease by Mendelian randomization studies

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The development of coronary artery disease (CAD) is significantly affected by impaired endocrine and metabolic status. Under this circumstance, improved prevention and treatment of CAD may result from knowing the connection between metabolites and CAD. This study aims to delve into the causal relationship between human metabolic biomarkers and CAD by using two-sample Mendelian randomization (MR). Utilizing two-sample bidirectional MR analysis, we assessed the correlation between 1400 blood metabolites and CAD, and the metabolites data from the CLSA, encompassing 8299 participants. Metabolite analysis identified 1091 plasma metabolites and 309 ratios as instrumental variables. To evaluate the causal link between metabolites and CAD, we analyzed three datasets: ebi-a-GCST005195 (547,261 European & East Asian samples), bbj-a-159 (29,319 East Asian CAD cases & 183,134 East Asian controls), and ebi-a-GCST005194 (296,525 European & East Asian samples). To estimate causal links, we utilized the IVW method. To conduct sensitivity analysis, we used MR-Egger, Weighted Median, and MR-PRESSO. Additionally, we employed MR-Egger interception and Cochran's Q statistic to assess potential heterogeneity and pleiotropy. What's more, replication and reverse analyses were performed to verify the reliability of the results and the causal order between metabolites and disease. Furthermore, we conducted a pathway analysis to identify potential metabolic pathways. 59 blood metabolites and 27 metabolite ratios nominally associated with CAD (P < 0.05) were identified by IVW analysis method. A total of four known blood metabolites, namely beta-hydroxyisovaleroylcarnitine (OR 1.06, 95% CI 1.027–1.094, FDR 0.07), 1-palmitoyl-2-arachidonoyl (OR 1.07, 95% CI 1.029–1.110, FDR 0.09), 1-stearoyl-2- docosahexaenoyl (OR 1.07, 95% CI 1.034–1.113, FDR 0.07) and Linoleoyl-arachidonoyl-glycerol, (OR 1.07, 95% CI 1.036–1.105, FDR 0.05), and two metabolite ratios, namely spermidine to N-acetylputrescine ratio (OR 0.94, 95% CI 0.903–0.972, FDR 0.09) and benzoate to linoleoyl-arachidonoyl-glycerol ratio (OR 0.87, 95% CI 0.879–0.962, FDR 0.07), were confirmed as having a significant causal relationship with CAD, after correcting for the FDR method (p < 0. 1). A causal relationship was found to be established between beta -hydroxyisovalerylcarnitine and CAD with the validation in other two datasets. Moreover, multiple metabolic pathways were discovered to be associated with CAD. Our study supports the hypothesis that metabolites have an impact on CAD by demonstrating a causal relationship between human metabolites and CAD. This study is important for new strategies for the prevention and treatment of CAD.

Keywords Cardiovascular disease, Plasma metabolites, Mendelian randomization, Biomarker, Risk assessment

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Coronary artery disease (CAD) is the predominant form of cardiovascular disease, responsible for 9.14 million deaths globally in 2019[1] CAD poses a significant burden on modern societies, with morbidity and mortality rates comparable to those of cancer[2]. Despite advancements in pharmacologic and surgical interventions, mortality rates resulting from CAD remain unacceptably high. Effective prevention and treatment of CAD are crucial in reducing morbidity and disability. The exploration of biological mechanisms is essential for achieving this goal, with lifestyle, environmental, and genetic factors being identified as risk factors for cardiovascular disease development[3]. Over the past decade, significant progress has been made in identifying alleles that increase the risk of CAD[4]. However, the fundamental mechanisms underlying this complex disease remain incompletely understood. Although etiologic studies of cardiovascular disease have greatly benefited from genetic advancements, particularly genome-wide association studies (GWAS)[5–8], there are still substantial obstacles in linking these genetic discoveries to biological processes.

Recent advancements in genomics tools, such as metabolomics, have provided new opportunities to investigate disease pathways. Metabolomics, by identifying intermediate metabolites and altered metabolic pathways, can offer new insights into the molecular mechanisms underlying diseases[9,10]. Global genomic studies of metabolites have recently identified loci associated with diseases, proposing mechanisms for disease onset and disease-related traits[11–13]. Moreover, the importance of metabolites in disease is progressively gaining attention, with studies establishing causal relationships between metabolites and delirium, osteoarthritis, bone fracture, thyroid cancer, and type 2 diabetes[14–18].Several studies have also shown the utility of metabolite intermediates in elucidating potential molecular pathways associated with cardiovascular disease[19–23].

In metabolomics research, a metabolic pathway refers to the ordered series of metabolic reactions involved in an organism's normal functioning. The analysis of interactions and correlations between metabolites can uncover potential metabolic pathways, which may play critical roles in the occurrence and progression of CAD. The most recent and comprehensive GWAS, the Canadian Longitudinal Study of Aging, with a sample size of 8299 participants, identified 1091 plasma metabolites and 309 plasma metabolite ratios as metabolite instrumental variables. Among the 1091 plasma metabolites tested, 850 had known identities within eight super pathways, including lipids, amino acids, exogenous substances, nucleotides, cofactors, vitamins, carbohydrates, peptides, and energy. This study has advanced our understanding of the genetic regulation of human metabolism[24]. However, there is still a lack of analyses utilizing these instrumental variables to explore the biological mechanisms and pathways of CAD, necessitating further in-depth studies to determine the roles played by genetic variants and the effects among 1091 plasma metabolites and 309 plasma metabolite ratios in the biological mechanisms of CAD.

Mendelian Randomization (MR) studies provide a genetics-based approach to epidemiologic research. MR studies offer several advantages over other epidemiologic research methods, including the avoidance of problems such as reverse causality and genetic polymorphisms, resulting in a more accurate assessment of the causal relationship between metabolites and disease. Our study design aims to assess the causal impact between metabolites and CAD, utilizing three datasets. The preliminary analysis utilized the first dataset, identified as ebi-a-GCST005195, which included 547,261 European and East Asian samples, with a total of 7,934,254 single-nucleotide polymorphisms (SNPs) identified. The second CAD cohort, labeled bbj-a-159, included 29,319 cases of East Asian ancestry and 183,134 controls of East Asian ancestry, identifying a total of 8,881,048 SNPs. The third cohort, ebi-a-GCST005194, consisted of 296,525 European and East Asian samples and identified 7,904,237 SNPs. By employing MR research methods, we aim to obtain more accurate estimates of the impact of metabolites on disease risk and identify potential metabolic pathways. Furthermore, utilizing the results of MR studies can aid in understanding disease mechanisms and evaluating potential therapeutic strategies. Through these analyses, we hope to provide novel perspectives on the etiology and mechanisms of CAD and contribute valuable insights for prevention and treatment.

Materials and methods

MR design

The study design followed the STROBE-MR checklist to ensure a logical approach. In MR analyses, instrumental variables (IVs) must satisfy three assumptions: (1) IVs should be associated with the exposure (metabolite); (2) IVs should be associated with the outcome (CAD) solely through the exposure (metabolite); and (3) IVs should be independent of any confounding factors[25]. The research design ideas are illustrated in Fig. 1.

Data source

Three CAD datasets were utilized in this study. The initial dataset, tagged with accession number ebi-a-GCST005195, was used for preliminary analysis. This dataset originated from a meta-analysis conducted by Harst et al[26], which included 547,261 individuals and identified 7,934,254 SNPs. Two additional datasets, labeled bbj-a-159 and ebi-a-GCST005194, were employed for replication analysis and meta-analysis. Instrumental variables for 1091 plasma metabolites and 309 plasma metabolite ratios were obtained from a comprehensive study by Chen et al.[24], which explored metabolic genetic influences in humans. The study included 8299 individuals from the Canadian Longitudinal Study of Aging cohort and reported outcomes such as body mass index (BMI), ischemic stroke, and type 2 diabetes mellitus (T2D) affected by metabolism.

Selection of instrumental variables (IVs)

First, genetic variations were extracted using an association threshold of $P < 1 \times 10^{-5}$[27,28] to ensure the inclusion of more significant variations. Next, independent variants were identified using the clumping program in the R software, with an r^2 threshold of < 0.001 and a kilobase pair (kb) distance of 10,000 to account for linkage disequilibrium (LD) effects. The potency of IVs, representative of metabolite levels, was assessed based on explained variance (R^2) and F-statistical parameters. A threshold of $F > 10$ was utilized for MR analysis.

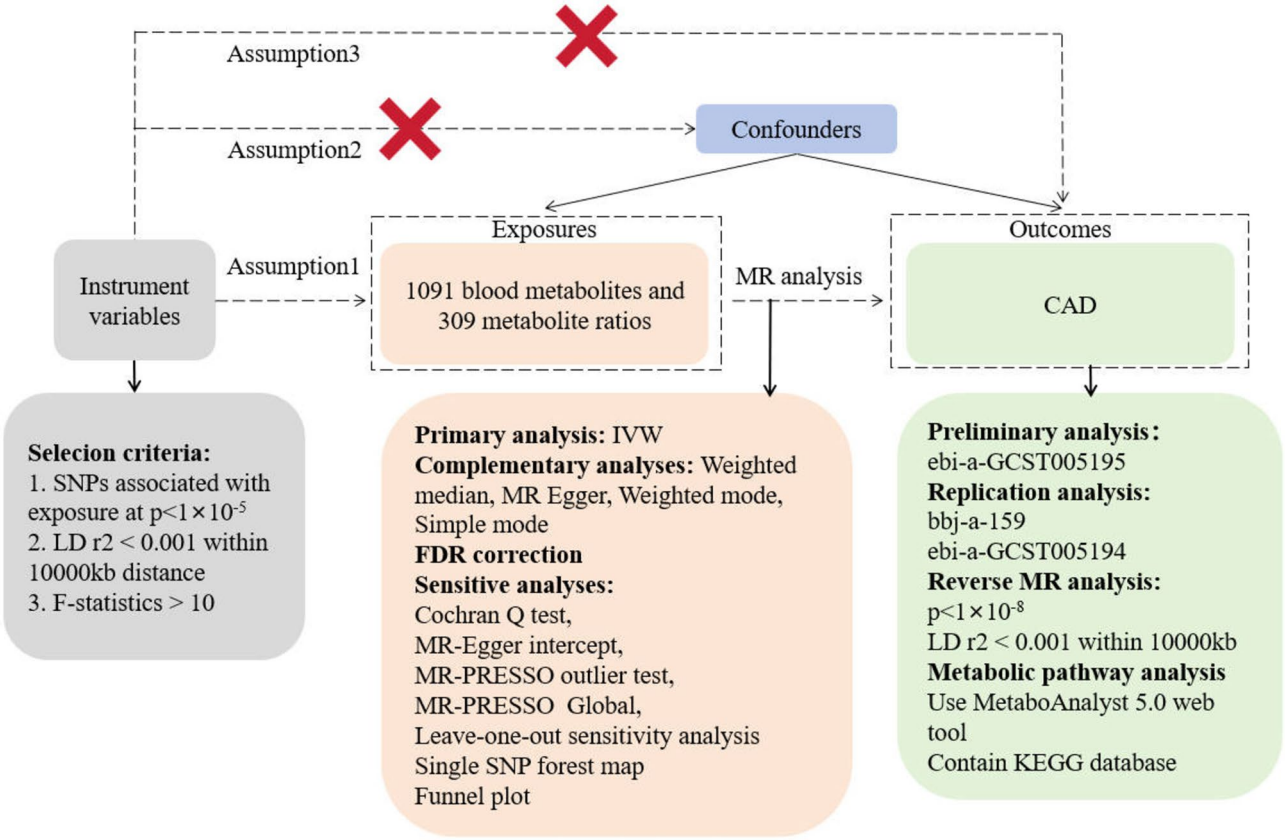

Figure 1. The research design ideas of the study.

MR analyses

The causal effects in two-sample MR analyses were assessed using the instrumental variable weighted (IVW) approach. The IVW fixed-effects model was applied in the absence of heterogeneity, while the IVW random-effects model was used when heterogeneity was present. The IVW method allows for consistent evaluation of exposure causality when all variables fulfill the three assumptions. Slope estimates from weighted linear regression were used for IVW analysis[25]. The weighted median method was employed when at least 50% of instrumental variables were valid[29]. For causal inference in the presence of potential pleiotropy or a large number of invalid instrumental variables, MR Egger regression analysis was conducted[30]. In addition, the weighted mode and simple mode are two methods that relax the assumptions further, but they possess lower testing efficacy compared to the previous three methods[31]. At the same time, in order to verify whether the research results are affected by multiple tests, the study also uses Q-value program to correct the False Discovery Rate (FDR) when the q value of FDR is < 0.1, a significant association is indicated[32,33].

Sensitivity analysis

Sensitivity analyses were performed using the MR-Egger method, which provides consistent estimates even when the instrument is not valid. MR-Egger can detect violations of IV assumptions and estimate effects unaffected by these violations[30,34]. Heterogeneity of SNPs was assessed using the Cochran Q test. MR-PRESSO was utilized to identify significant outliers in the study results and exclude them if necessary. The leave-one-out sensitivity test was conducted to observe any significant changes after removing each SNP.

Metabolic pathway analysis

Metabolic pathways were analyzed using the web-based Metaconflict 5.0 tool (https://www.metaboanalyst.ca/)[35]. Functional enrichment analysis and pathway analysis modules were utilized to identify potential metabolite groups or pathways associated with CAD biological processes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used in this study, with a significance level set at 0.10 for pathway analysis. Additionally, the obtained pathways were further categorized and searched within their higher-level belonging pathways.

Ethical approval

No experiments involving patients and/or animals have been performed.

Consent to participate

Data were obtained from public databases and did not involve clinical participants.

Result

Strength of the instrumental variables

A two-sample MR analysis utilizing GWAS summary statistics evaluated the causal role of 1091 plasma metabolites and 309 ratios in CAD. After rigorous screening, the final number of instrumental variables (IVs) ranged from 12 to 93 for metabolites and 13–39 for ratios. X-15523 and X-12462 respectively had the highest and lowest IVs among metabolites, while the glutamine/alanine and ADP/uridine ratios had the most and fewest IVs. The F-statistic range (19.50-5308.35) suggested low likelihood of weak IVs and validity for MR analyses (Supplementary Table 1).

Causal relationship between metabolites and CAD

Five MR analysis methods were employed to determine the causal association between the blood metabolites and metabolite ratios and CAD. A total of 224 exposures showed significant associations with CAD ($P < 0.05$ for at least one MR analysis method), including 167 blood metabolites (139 unique metabolites) and 57 metabolite ratios. This result was visualized using a circos plot (Fig. 2).

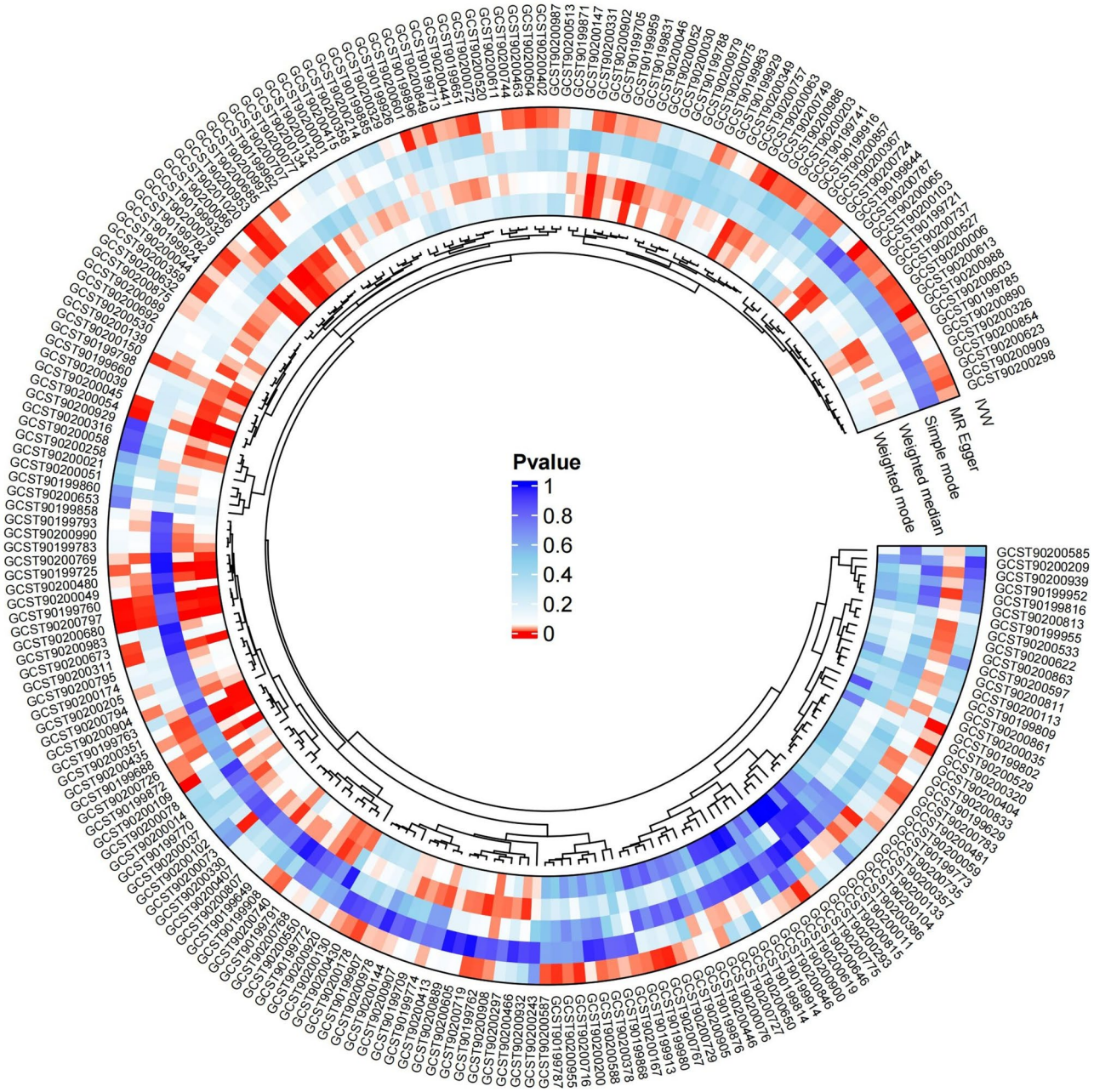

Figure 2. Circos plot of MR analysis results for 167 blood metabolites and 57 metabolite ratios. Notes: The specific name of the Exposure ID is in Supplementary table 1.

To identify the risk factors more strongly associated with CAD, we focused on the most rigorous IVW analysis, which identified 86 blood metabolites and metabolite ratios that may have a causal relationship with CAD ($P < 0.05$). After correction based on the false discovery rate (FDR) method ($P < 0.1$)[32,33], four known blood metabolites (beta-hydroxyisovaleroylcarnitine, 1-palmitoyl-2-arachidonoyl, 1-stearoyl-2-docosahexaenoyl, linoleoyl-arachidonoyl-glycerol) and two metabolite ratios (spermidine to N-acetylputrescine ratio, benzoate to linoleoyl-arachidonoyl-glycerol ratio) were found to have a significant causal relationship with CAD ($P < 0.05$). All six exposures were known (Table 1, Fig. 3). The results were further visualized using scatterplots (Fig. 4–9).

In the IVW analysis, beta-hydroxyisovaleroylcarnitine (OR 1.06, 95% CI 1.027–1.094, FDR 0.07), 1-palmitoyl-2-arachidonoyl (OR 1.07, 95% CI 1.029–1.110, FDR 0.09), 1-stearoyl-2-docosahexaenoyl (OR 1.07, 95% CI 1.034–1.113, FDR 0.07), and linoleoyl-arachidonoyl-glycerol (OR 1.07, 95% CI 1.036–1.105, FDR 0.05) showed significant and causal associations with CAD. Additionally, the spermidine to N-acetylputrescine ratio (OR 0.94, 95% CI 0.903–0.972, FDR 0.09) and benzoate to linoleoyl-arachidonoyl-glycerol ratio (OR 0.87, 95% CI 0.879–0.962, FDR 0.07), two metabolite ratios, were also significantly and causally associated with CAD.

Sensitivity analysis

Although the IVW approach is effective in inferring causal relationships, it is susceptible to weak instrumental bias. Therefore, we conducted sensitivity and multicausality analyses to assess the robustness of the causal relationships. No significant outlier SNPs were found through the MR-PRESSO outlier test. Neither the MR-Egger intercept test nor the MR-PRESSO Global test indicated the presence of considerable pleiotropy ($p > 0.05$). The Cochran Q test showed low heterogeneity between SNPs from the IVW and MR-Egger methods. The "leave-one-out" method and analysis of individual SNP effects demonstrated that the MR analysis was responsible, and individual SNPs did not influence the results. The funnel plot showed no horizontal multidirectionality or heterogeneity in our MR study. These results suggest that the causal effect of beta-hydroxyisovaleroylcarnitine (OR 1.06, 95% CI 1.027–1.094) on CAD is likely reliable. Additionally, a reverse MR analysis found no causal relationship between CAD (exposure) and beta-hydroxyisovaleroylcarnitine (OR 1.06, 95% CI 1.027–1.094) (outcome) (Supplementary Table 4, 5, 6).

Replication analysis

To validate our results, we performed MR analysis on additional GWAS data for CAD (bbj-a-159: 29,319 cases and 183,134 controls; ebi-a-GCST005194: 34,541 cases and 261,984 controls) and found a potential causal relationship between beta-hydroxyisovaleroylcarnitine (OR 1.06, 95% CI 1.027–1.094) and CAD (Supplementary Table 3). A reverse MR analysis also showed no causal relationship between CAD (exposure) and beta-hydroxyisovaleroylcarnitine (OR 1.06, 95% CI 1.027–1.094) (outcome) (Supplementary Table 4).

Metabolic pathway analysis

Metabolic pathway analysis revealed that the "arginine and proline metabolism" pathway (Fig. 10A) may be associated with CAD occurrence ($P = 0.004$). Additionally, the majority of metabolites and pathways associated with CAD were related to "amino acid metabolism" (Fig. 10B), involving 13 metabolites. (Supplementary Table 7).

Discussion

The unbiased evaluation of the causal association between 1400 metabolites and CAD was conducted through a Mendelian randomization (MR) study using three CAD-related GWAS datasets. The study utilized genetic variants as instruments to identify 224 metabolites nominally associated with CAD. To avoid false positives, the results obtained through the IVW method were further investigated by looking at FDR values. Six metabolites that showed strong associations with CAD ($P < 0.1$) were identified. Validation with three different GWAS datasets confirmed beta-hydroxyisovaleroylcarnitine as having the most reliable causal relationship with CAD. Furthermore, pathway enrichment analysis revealed the "arginine and proline metabolism" pathway as the main metabolic pathway associated with CAD. Most of the identified metabolites and pathways were related to amino acid metabolism, which is closely linked to CAD.

In recent years, the impact of family genetic history on CAD has gained increasing attention. GWAS studies have identified more than 60 common variants highly associated with CAD, including familial hypercholesterolemia (FH)[36], hyperlipidemia[37], and other disorders that significantly increase the risk of CAD[38]. Clinical risk factors for CAD, such as plasma total homocysteine (tHcy)[39] and lipoproteins[40], have also been identified.

Exposure ID	Outcome	nSNP	P	OR (95%CI)	FDR
GCST90199802	CAD	28	< 0.001	1.060 (1.027, 1.095)	0.0748
GCST90200054	CAD	15	0.001	1.069 (1.029, 1.11)	0.0998
GCST90200065	CAD	27	< 0.001	1.073 (1.034, 1.113)	0.0748
GCST90200109	CAD	27	< 0.001	1.07 (1.036, 1.105)	0.0502
GCST90200797	CAD	16	0.001	0.937 (0.903, 0.972)	0.0998
GCST90200988	CAD	11	< 0.001	0.92 (0.879, 0.962)	0.0748

Table 1. IVW results for four blood metabolites and two metabolite ratios. Notes: The specific name of the Exposure ID is in Supplementary Table 1.

exposure	nsnp	method	pval	OR(95% CI)
GCST90199802	28	MR Egger	0.185	1.050 (0.979 to 1.125)
Beta-hydroxyisovaleroylcarnitin	28	Weighted median	0.414	1.019 (0.974 to 1.067)
	28	Inverse variance weighted	**<0.001**	1.060 (1.027 to 1.095)
	28	Simple mode	**0.042**	1.125 (1.010 to 1.254)
	28	Weighted mode	0.495	1.020 (0.964 to 1.080)
GCST90200054	15	MR Egger	0.165	1.052 (0.983 to 1.126)
1-palmitoyl-2-arachidonoyl-	15	Weighted median	**<0.001**	1.056 (1.027 to 1.087)
GPE (16:0/20:4)	15	Inverse variance weighted	**<0.001**	1.069 (1.029 to 1.110)
	15	Simple mode	**0.012**	1.081 (1.025 to 1.140)
	15	Weighted mode	**<0.001**	1.057 (1.030 to 1.084)
GCST90200065	27	MR Egger	0.734	1.013 (0.940 to 1.093)
1-stearoyl-2-docosahexaenoyl-	27	Weighted median	**0.001**	1.072 (1.028 to 1.118)
GPE (18:0/22:6)	27	Inverse variance weighted	**<0.001**	1.073 (1.034 to 1.113)
	27	Simple mode	0.290	1.043 (0.966 to 1.127)
	27	Weighted mode	**0.004**	1.073 (1.027 to 1.121)
GCST90200109	27	MR Egger	0.288	1.037 (0.971 to 1.109)
Linoleoyl-arachidonoyl-	27	Weighted median	**0.013**	1.053 (1.011 to 1.096)
glycerol (18:2/20:4)	27	Inverse variance weighted	**<0.001**	1.070 (1.036 to 1.105)
	27	Simple mode	0.524	1.025 (0.951 to 1.104)
	27	Weighted mode	**0.021**	1.048 (1.010 to 1.087)
GCST90200797	16	MR Egger	**0.003**	0.890 (0.835 to 0.949)
Spermidine to N-	16	Weighted median	**0.002**	0.929 (0.887 to 0.974)
acetylputrescine ratio	16	Inverse variance weighted	**<0.001**	0.937 (0.903 to 0.972)
	16	Simple mode	0.791	0.985 (0.882 to 1.100)
	16	Weighted mode	**<0.001**	0.899 (0.858 to 0.943)
GCST90200988	11	MR Egger	0.621	0.972 (0.870 to 1.085)
Benzoate to linoleoyl-arachidonoyl-	11	Weighted median	**0.010**	0.937 (0.891 to 0.984)
glycerol (18:2 to 20:4) [1] ratio	11	Inverse variance weighted	**<0.001**	0.920 (0.879 to 0.962)
	11	Simple mode	0.106	0.926 (0.851 to 1.008)
	11	Weighted mode	**0.037**	0.939 (0.892 to 0.989)

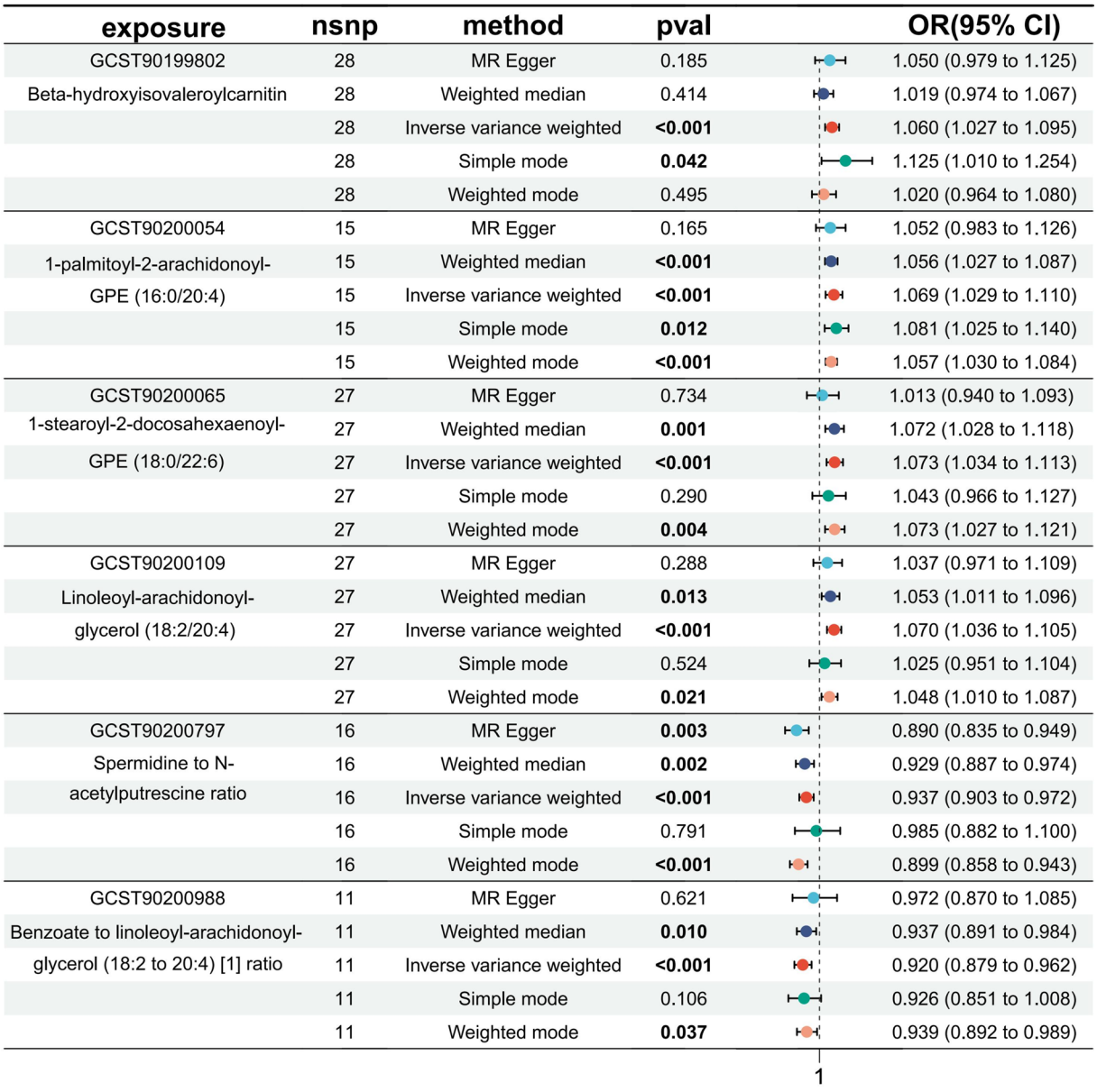

Figure 3. MR results for 4 blood metabolites and 2 metabolite ratios. Notes: The specific name of the Exposure ID is in Supplementary Table 1.

However, the association of hydroxyisovaleroylcarnitine with CAD has not been previously reported. Hydroxyisovaleroylcarnitine and beta-hydroxyisovaleroylcarnitine refer to the same substance but have different nomenclatures[41]. Previous studies have primarily focused on the association of hydroxyisovalerylcarnitine with 3-methylcrotonyl-coenzyme carboxylase deficiency[42,43], a rare metabolic disorder, and its diagnostic significance. However, its role in CAD remains unknown. Interestingly, hydroxyisovaleroylcarnitine levels can reflect leucine intake[41], and abnormal leucine catabolism can impact protein homeostasis, energy balance, and signaling pathways, which are closely related to CAD mechanisms[44].

Other conditions, such as inflammatory bowel disease (IBD), have been associated with elevated hydroxyisovalerylcarnitine levels[45,46]. Patients with IBD are more susceptible to CAD-related diseases due to systemic inflammation[47,48], hypercoagulability, and impaired coronary microvascular and left ventricular function[49]. The underlying mechanisms of how IBD affects CAD development are not fully understood but may involve gut microbiome dysfunction[50]. Considering the close association between metabolic markers and the gut flora, discovering effective metabolites can elucidate the mechanisms between these diseases. Moreover, diet has been increasingly recognized as a factor that affects CAD outcomes[51]. Ketone body metabolism, as an essential metabolic modality, has demonstrated its impact on cardiovascular-related diseases. Ketone bodies can potentially affect fatty acid and glucose utilization in healthy myocardium and may influence functional recovery after ischemic episodes[52]. Hydroxyisovaleroylcarnitine has begun to be used as a promising biomarker for evaluating adverse clinical outcomes in heart failure management[53]. Studies have shown that elevated levels of this

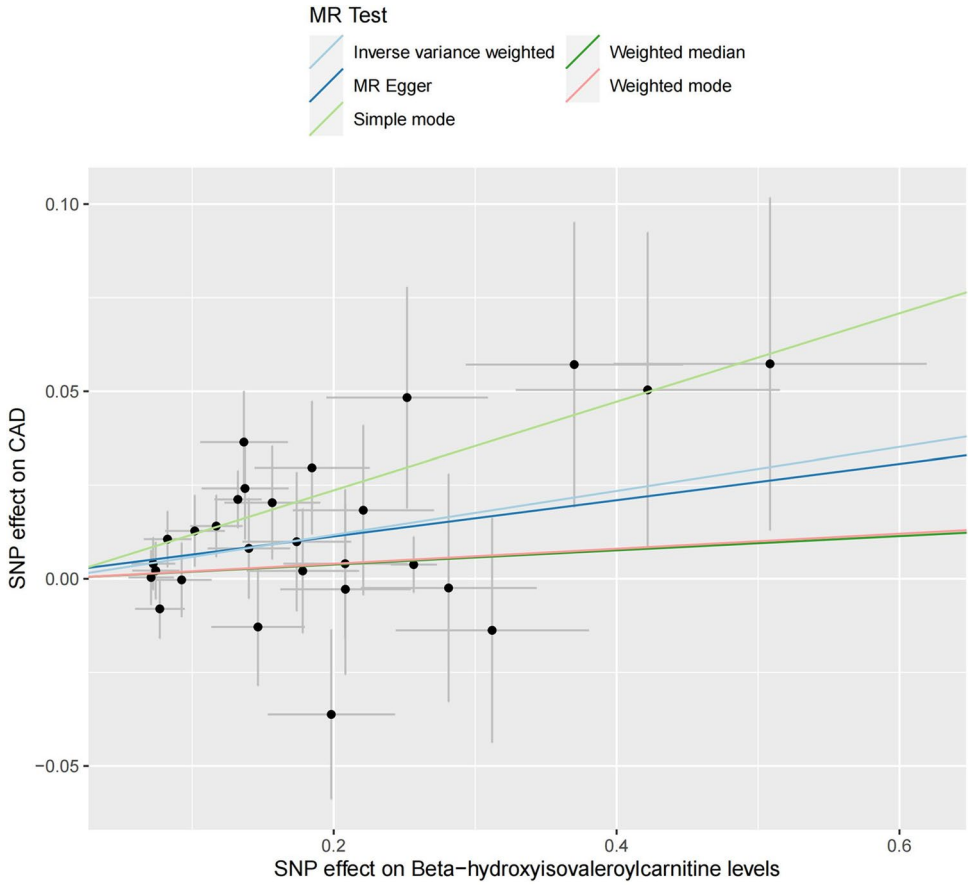

Figure 4. Scatterplot of causal association between beta-hydroxyisovaleroylcarnitine levels and CAD.

metabolite are associated with an unfavorable prognosis[54], particularly in the context of sildenafil-based therapeutic interventions for heart failure.

The MR analysis in this study identified specific metabolites, some of which have been reported in previous studies. For example, 1-palmitoyl-2-arachidonic acid, a phosphatidylcholine and major metabolite of glycerophospholipids, has a well-established role in atherosclerotic disease. It has been found that oxidized phospholipids promote atherosclerotic inflammation[55], and antibodies to 1-palmitoyl-2-arachidonoyl have been used as autoimmune markers for cardiovascular disease diagnosis[56]. On the one hand, the accumulation of 1-palmitoyl-2-arachidonoyl can contribute to atherogenesis by activating endothelial cells and inducing vascular barrier disorders[56]. On the other hand, clinical observations have found elevated levels of 1-palmitoyl-2-arachidonoyl in smokers, hypertensive individuals, and patients with myocardial infarction, which suggest that there is a close relationship between 1-palmitoyl-2-arachidonoyl and cardiovascular disease[57]. The role of 1-palmitoyl-2-arachidonoyl in coronary artery disease (CAD) warrants further attention and exploration.

In this study, metabolic pathway analysis revealed that the pathways "arginine and proline metabolism," "glutathione metabolism," and "amino acid metabolism" were primarily associated with CAD. Arginine and proline metabolism have been identified as key metabolic pathways during the systemic immune and low-grade inflammatory states of CAD[58]. Asymmetric dimethylarginine, in particular, is thought to be associated with common cardiovascular factors. Elevated levels of dimethylarginine have been linked to endothelial dysfunction and the occurrence of adverse events in CAD patients[59]. Studies on the improvement of cardiovascular disease through L-arginine supplementation have yielded positive results. For instance, Rodionov et al. found that high arginine supplementation prevented left ventricular dilatation and preserved contractile capacity in a CAD model[60]. On one hand, L-arginine has been shown to prevent atherosclerosis in coronary arteries in hypercholesterolemic rabbits[61]. On the other hand, oral L-arginine reduces monocyte adhesion to endothelial cells and improves endothelial function in stable CAD patients[62,63]. Nutrients like L-arginine also play an essential role in preventing and halting the progression of heart failure and arrhythmia through supplementation[64].

Progress has also been made in the exploration of proline in cardiovascular disease. High cholesterol and LDL serve as risk factors for CAD. Karvonen et al. found a correlation between proline and serum cholesterol and LDL levels, and administration of proline resulted in elevated levels of both[65]. Proline has been shown to promote atherosclerosis. The CAD-associated junctional protein, with a proline-rich region located between endothelial cells, plays a crucial role in CAD[66]. In CAD, the substitution of serine with proline in ADAMTS7 was found to promote atherogenesis by increasing vascular endothelial cell migration and angiogenesis[67]. Proline also protects against myocardial infarction injury through oxidative modulation, improves post-infarction

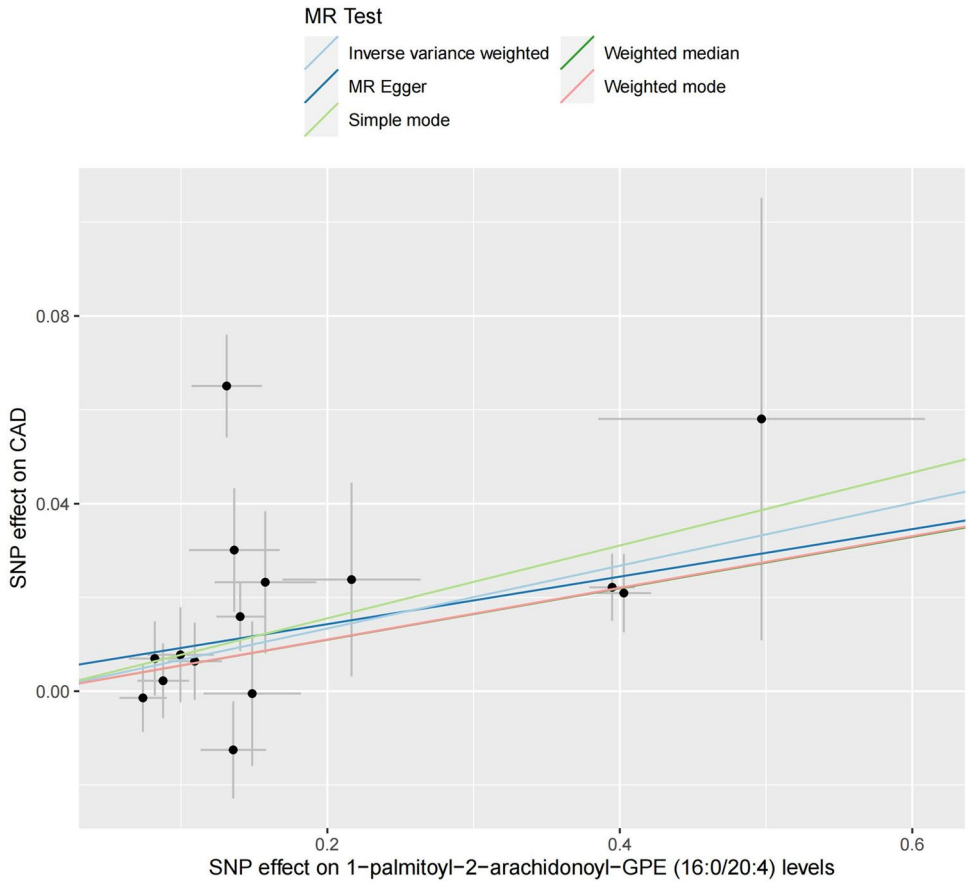

Figure 5. Scatterplot of causal association between 1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4) levels and CAD.

myocardial remodeling, and attenuates cardiomyocyte apoptosis[68]. GWAS has identified that proline/serine-rich coiled-coil 1 (PSRC1) encodes a protein associated with lipid levels and coronary artery disease[69]. PSRC1 prevents the development of atherosclerosis and enhances plaque stability by regulating cholesterol transport and inflammation in macrophages and in the livers of apoE mice[70]. These findings suggest that arginine and proline metabolism may play an essential role in the biological mechanisms of CAD.

Limitation

The study faced several challenges in elucidating intricate CAD subtypes due to categorical constraints in raw data, necessitating a holistic CAD analysis approach that precluded nuanced classification. This limitation highlights the importance of larger datasets to refine the precision of the 1,091 plasma metabolites and 309 metabolite ratios identified through GWASs.

Mendelian randomization emerged as a powerful tool to uncover causal relationships between blood metabolites and CAD. However, empirical validation of these findings through subsequent studies is crucial to strengthen our understanding. The fidelity of MR analyses depends heavily on the accurate interpretation of exposure-related instrumental variables, emphasizing the need for sample size expansion to precisely assess genetic impacts on metabolite profiles.

Our study identified numerous metabolites associated with CAD risk, but a deeper exploration into their mechanistic roles in CAD pathogenesis requires further research. Given the complexity of our data with 1,400 exposures, we employed the FDR method for multiple testing, aiming to balance the need to minimize false positives while still capturing meaningful positive metabolites. Notably, the choice of FDR is not standardized, and our use of a broader range may have limitations but also potentially increased the significance of our positive findings. It is noteworthy that in the meticulous process of screening instrumental variables, a stringent criterion of 1×10^{-8} is conventionally employed to safeguard the robust correlation and specificity of the selected SNPs, thereby mitigating the potential confounding effects of horizontal gene pleiotropy on the outcomes. Nevertheless, confronted with the constraint of sample size, we adopted a more lenient threshold of 1×10^{-5} to encompass a broader spectrum of SNPs as instrumental variables. Anticipating future studies with enlarged sample sizes or enhanced statistical prowess, we intend to reassess the implications of varying p-value thresholds on MR results, aiming to further substantiate and consolidate our current findings.

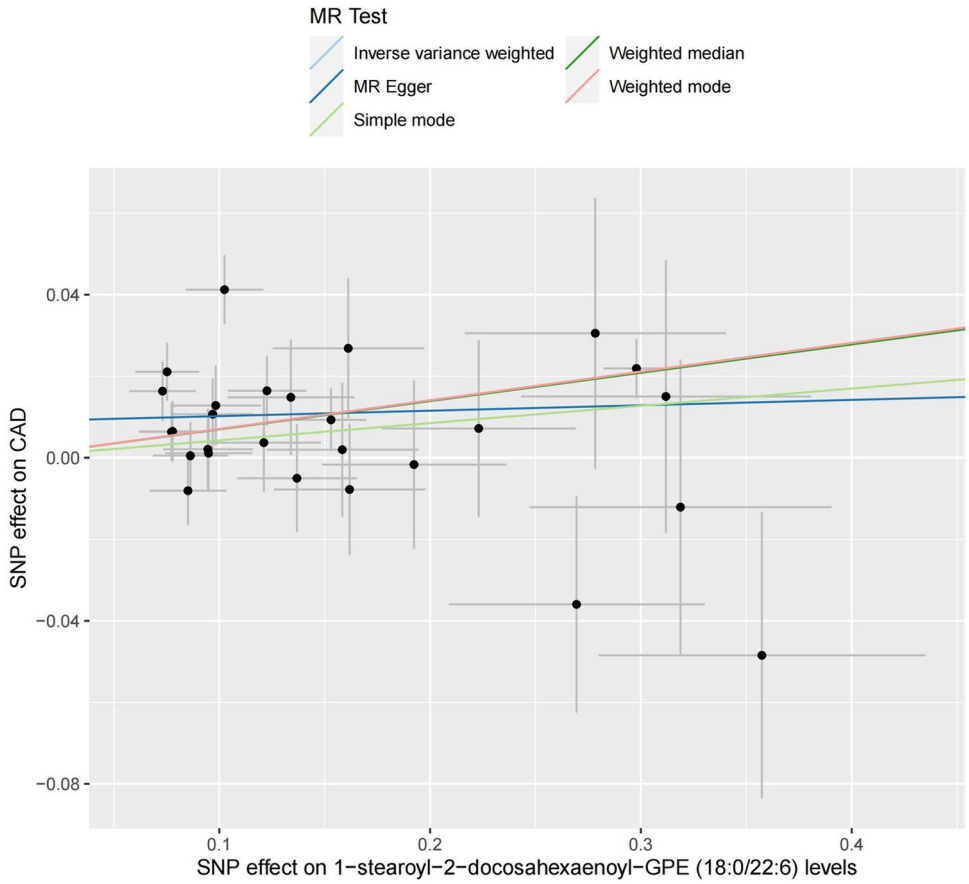

Figure 6. Scatterplot of causal association between 1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6) levels and CAD.

Conclusion

In conclusion, we found multiple putative metabolites with causal effects on CAD by doing MR analysis within the spectrum of accessible plasma metabolites. Among these, beta-hydroxyisovaleroylcarnitine was confirmed in multiple datasets. These potential metabolites require additional experimental research to confirm their status as biomarkers and clarify the underlying mechanisms.

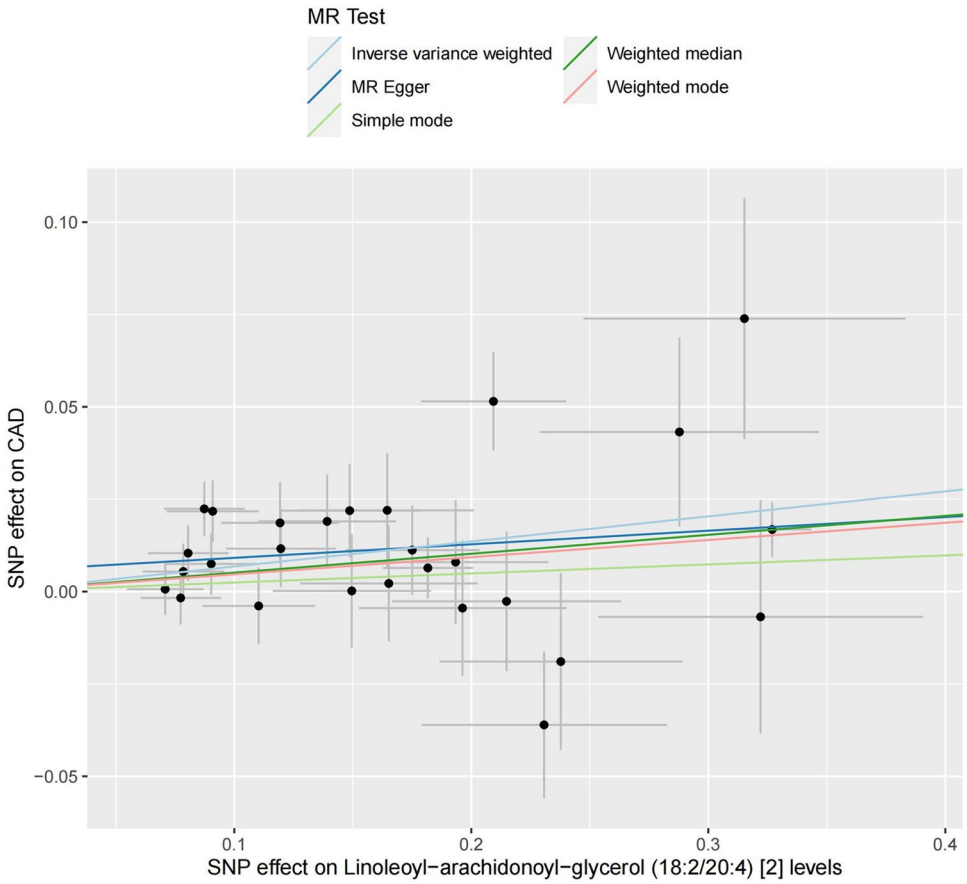

Figure 7. Scatterplot of causal association between linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2] levels and CAD.

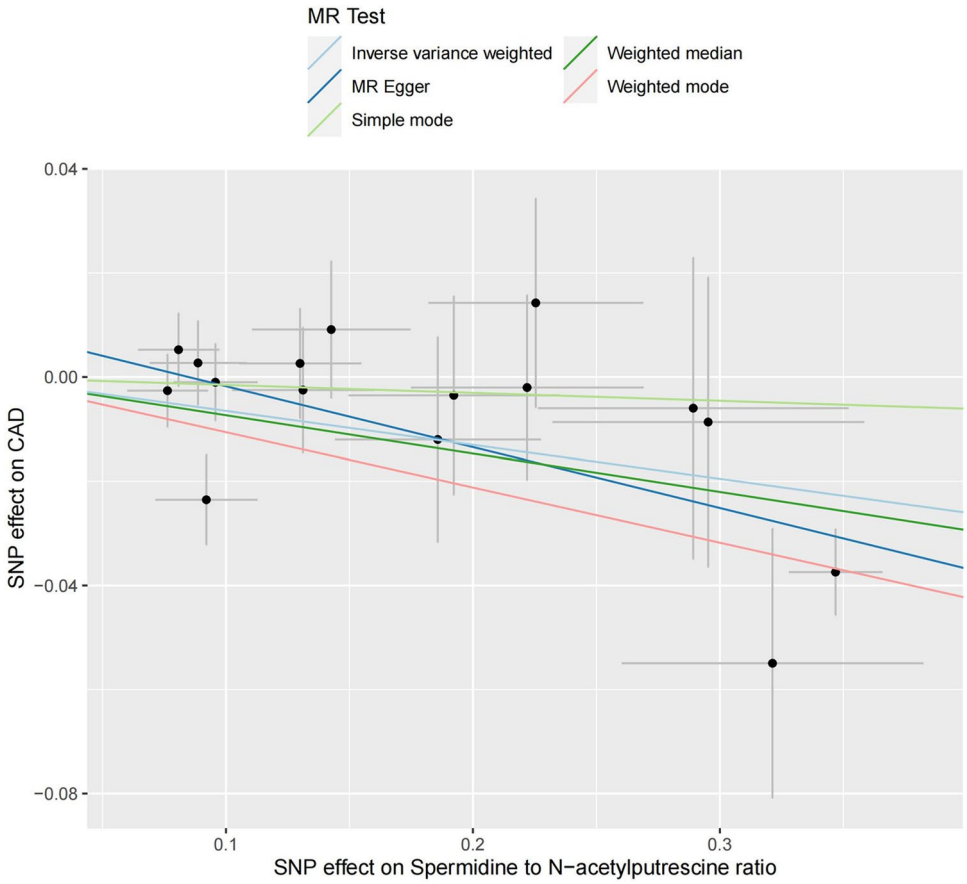

Figure 8. Scatterplot of causal association between spermidine to N-acetylputrescine ratio and CAD.

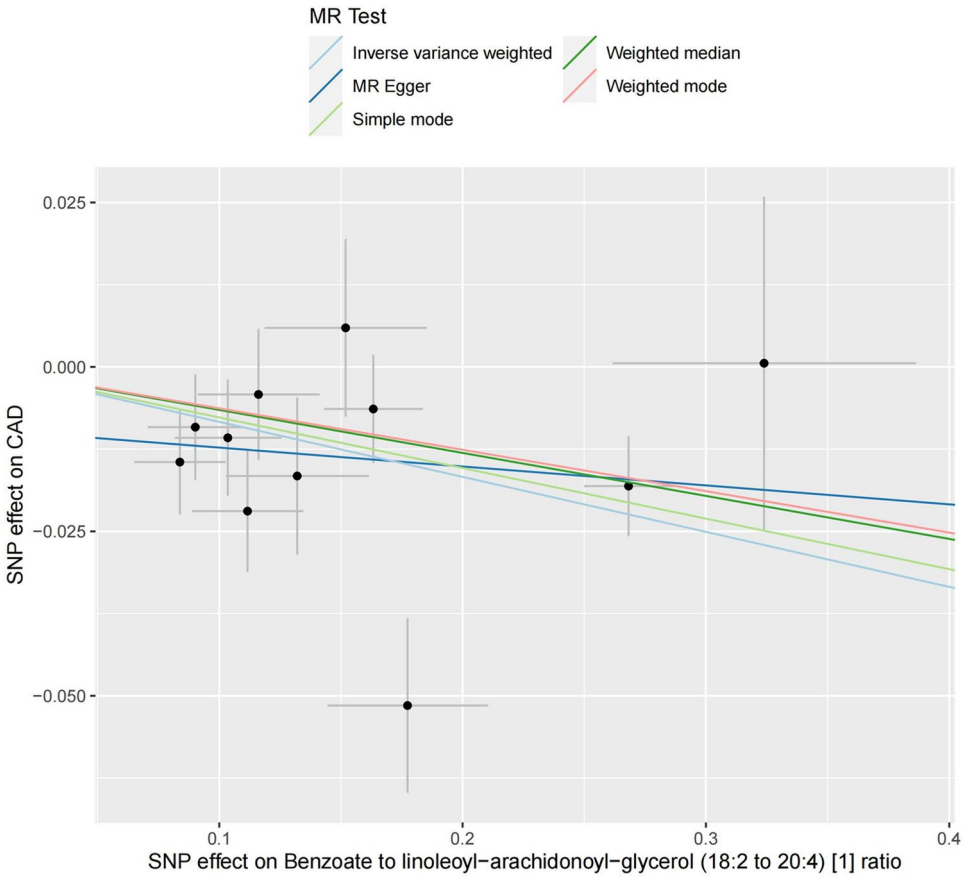

Figure 9. Scatterplot of causal association between benzoate to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio and CAD.

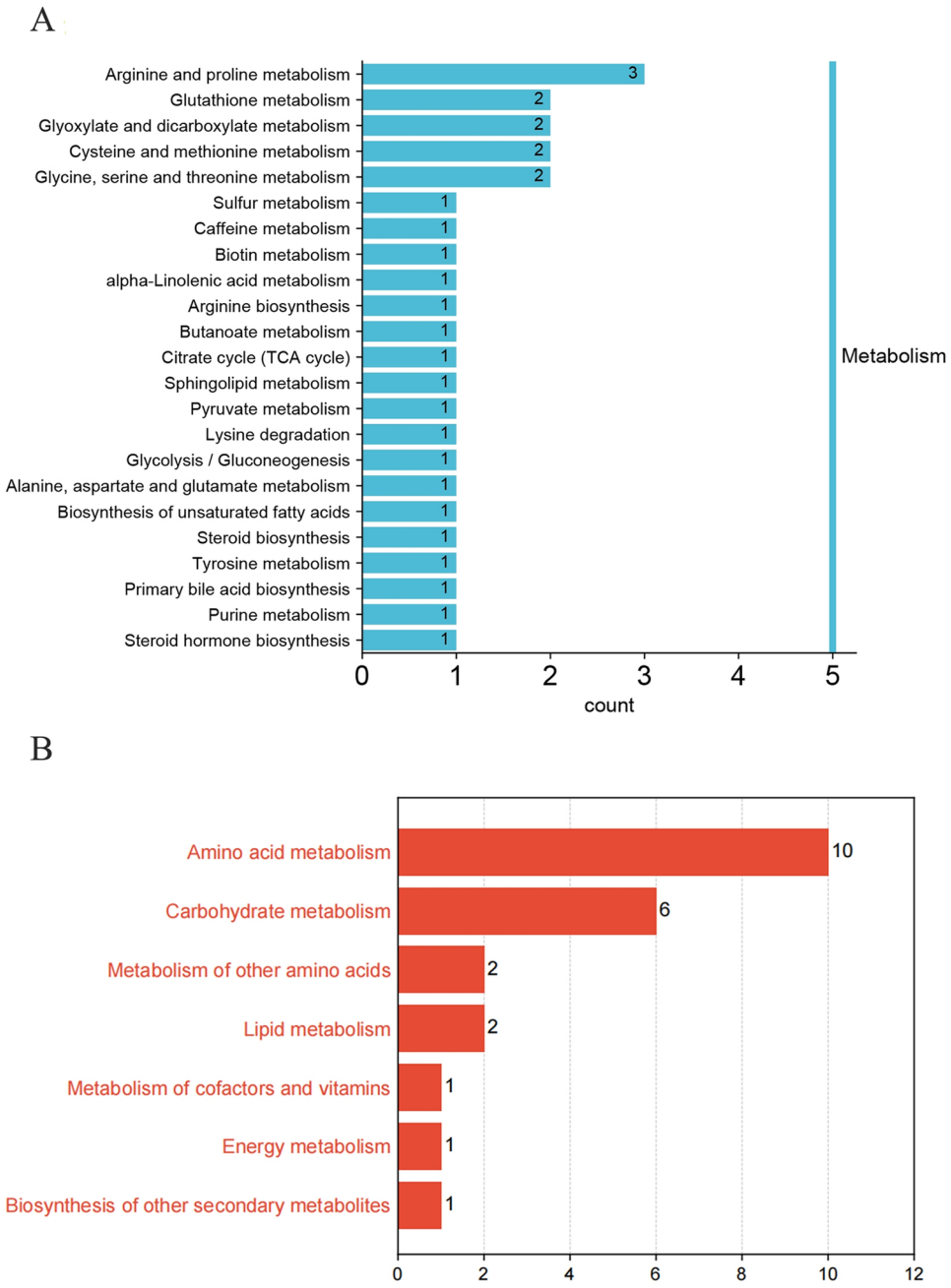

Figure 10. Secondary and tertiary metabolic pathways from enrichment analysis.

Data availability

The data required for our analysis were obtained from publicly available data. Data were obtained from IEU OpenGwas (https://gwas.mrcieu.ac.uk/).

Code availability

All code and software are open source and free of charge. If you need it, you can contact Kai Yang or Yongmei Liu to get.

Received: 27 February 2024; Accepted: 9 August 2024
Published online: 16 August 2024

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Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-69879-2.

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