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Causal association between blood metabolites and abdominal aortic calcification: A bidirectional Mendelian randomization study

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Abstract
Previous studies have reported correlations between metabolic factors and abdominal aortic calcification (AAC). However, the causal relationship between blood metabolites and AAC remains to be fully explored. We employed bidirectional two-sample Mendelian randomization (MR) to investigate the potential causal relationships between 486 blood metabolites and AAC. The inverse variance weighted method was primarily utilized for MR analysis, and the MR-Egger, weighted median, and Robust Adjusted Profile Score methods were used for supplementary analysis. Sensitivity analyses were conducted using Radial MR, MR-PRESSO, Cochran Q test, MR-Egger intercept, and leave-one-out analysis to evaluate the heterogeneity and pleiotropy. Furthermore, the Steiger test and linkage disequilibrium score regression were used to assess genetic correlation and directionality. Multivariable MR analysis was performed to evaluate the direct effect of metabolites on AAC. Through rigorous screening, we identified 6 metabolites with presumed causal effects on AAC: 4-methyl-2-oxopentanoate (effect size [ES] 0.46, 95% confidence interval [CI]: 0.10–0.82), erythrose (ES –0.35, 95% CI: –0.59 to –0.11), 10-undecenoate (11:1n1) (ES 0.14, 95% CI: 0.03–0.25), 1-myristoylglycerophosphocholine (ES 0.31, 95% CI: 0.11–0.50), glycerol 2-phosphate (ES 0.20, 95% CI: 0.04–0.37), and the unidentified metabolite X-11469 (ES 0.19, 95% CI: 0.08–0.30). Multivariable MR analysis revealed that genetically predicted erythrose, 10-undecenoate, 1-myristoylglycerophosphocholine, and X-11469 could directly affect AAC independent of other metabolites. Reverse MR analysis revealed an alteration in 12 blood metabolites due to AAC, including caffeine, 1,7-dimethylurate, arachidonic acid, and 1-arachidonoylglycerophosphocholine. This study provides evidence supporting a causal relationship between metabolites and AAC. These findings help elucidate the underlying biological mechanisms of AAC and may offer insights into screening, prevention, and treatment approaches.

Abbreviations: AAC = abdominal aortic calcification, BCAA = branched-chain amino acid, CI = confidence interval, CVD = cardiovascular disease, ES = effect size, GWAS = genome-wide association study, IV = instrumental variable, IVW = inverse variance weighted, LD = linkage disequilibrium, LOO = leave-one-out, LysoPC = lysophosphatidylcholine, MR = Mendelian randomization, MUFA = monounsaturated fatty acid, MVMR = multivariable Mendelian randomization, PLA2 = phospholipase A2, RAPS = Robust Adjusted Profile Score, RCT = randomized controlled trial, SNP = single nucleotide polymorphism, VSMC = vascular smooth muscle cell, WM = weighted median.

Keywords: abdominal aortic calcification, arachidonic acid, caffeine, Mendelian randomization, metabolites, vascular calcification

1. Introduction

Abdominal aortic calcification (AAC) is a pathological condition characterized by the deposition of hydroxyapatite crystals within the intima and media of the abdominal aorta.[1] This condition is particularly common among the elderly population.[2] Epidemiological studies have underscored AAC as an independent predictor for cardiovascular events and all-cause mortality.[3,4] The more severe aortic calcification, the greater its predicted impact,[5] while the circularity of calcification independently increases the risk.[6] From a pathophysiological perspective, aortic calcification results in arterial stiffness, which affects hemodynamics, notably the reduction of Windkessel

HY and WO contributed equally to this work.

The original studies from which these data were sourced obtained local ethical approval, and informed consent was obtained from all the participants.

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The datasets generated during and/or analyzed during the current study are publicly available.

No additional ethical approval was required for this study because all the data were available in publicly available databases.

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function.[7] This condition can cause an increase in left ventricle afterload,[8] diminished coronary artery perfusion,[9] and potential disturbance of delicate microcirculation in organs with low vascular resistance, such as the kidneys and brain.[10,11] Vascular calcification was previously regarded as a passive process; however, recent studies have indicated that it is an active, cell-mediated, and regulatable process, similar to physiological bone mineralization.[12] Given its generally irreversible nature, early detection and prevention of contributory factors for vascular calcification are imperative.

In recent years, advances in omics technologies, including metabolomics, have markedly enhanced our comprehension of disease pathogenesis.[13,14] Blood metabolites provide real-time information about biological processes within organisms, revealing the effects of internal biological states and external environmental factors on the body. Metabolomics provides a comprehensive understanding of the biological mechanisms of diseases by identifying modified metabolites and metabolic pathways. This helps to elucidate intricate biochemical processes and uncover underlying mechanisms, and plays a crucial role in identifying potential biomarkers. Prior observational and foundational studies have indicated a close correlation between metabolic factors and AAC. Uremic metabolites including tryptophan metabolites, trimethylamine N-oxide, and isocyanates, can accelerate vascular calcification through the activation of pro-calcification signaling pathways and the alteration of protein post-translational modifications.[15] Furthermore, cross-sectional studies have uncovered significant associations between certain serum metabolite levels, such as n-3 polyunsaturated fatty acids, and aortic calcification.[16] Metabolomic investigations employing the chronic kidney disease rat model have unearthed several metabolites and pathways involved in vascular calcification.[17] Nevertheless, a comprehensive and systematic exploration assessing the causal relationships between blood metabolites and AAC is still lacking.

Mendelian randomization (MR), an etiological research strategy that helps infer the causal relationship between exposures and outcomes, has gained prominence recently.[18] MR leverages the random allocation of genotypes from parents to offspring, which minimizes susceptibility to confounding factors, similar to the methodology employed in randomized controlled trials (RCTs). Thus, MR provides unbiased estimations, substantially reducing the likelihood of reverse causation and confounding.[19] In the absence of RCTs, MR is a pivotal alternative that providing persuasive evidence of the causal relationship between exposure and disease risk. Additionally, reverse MR analysis can shed light on the associations between disease risk and other traits, which can identify the biomarkers that cause the disease, are predictive of the disease, or have diagnostic potential.[20] Therefore, we conducted a large-scale bidirectional MR analysis to systematically explore the causality between blood metabolites and AAC.

2. Materials and methods

2.1. Study design

Our study employed an MR strategy to explore the causal relationship between blood metabolite levels and AAC severity. Our analysis utilized single nucleotide polymorphisms (SNPs) identified in a genome-wide association study (GWAS) as instrumental variables (IVs). The foundation of this study is based on 3 key MR assumptions: (1) IVs are strongly associated with the exposures of interest; (2) IVs are independent of confounding factors; and (3) IVs are not associated with outcome and affect outcome only via exposures.[21] All MR analyses were conducted using R software (version 4.3.1) with the assistance of the TwoSampleMR,[22] MR-PRESSO,[23] and Radial MR packages.[24] A summary of the study is presented in Figure 1.

2.2. GWAS data for blood metabolites and AAC

The GWAS data for blood metabolites in this MR study, spearheaded by Shin et al, included 7824 European participants and involved the investigation of approximately 2.1 million SNPs to study 486 blood metabolites. These data included 2 European cohorts, namely the TwinsUK and KORA studies, publicly accessible through the Metabolomics GWAS server (https://metabolomics.helmholtz-muenchen.de/gwas/).[25] Of the 486 metabolites, 309 were chemically identified and classified into 8 categories based on the Kyoto Encyclopedia of Genes and Genomes database, while the remaining 177 have not yet been classified. These categories include amino acids, carbohydrates, cofactors and vitamins, energy, lipids, nucleotides, peptides, and xenobiotics. Detailed names of the 486 metabolites are listed in Table S1, Supplemental Digital Content, http://links.lww.com/MD/N458. For each metabolite, the raw value was corrected by normalizing to the medians set to equal 1, and subsequently, a base 10 log transformation was applied.

We accessed the GWAS summary data of AAC from the NHGRI-EBI GWAS catalog under the accession number GCST90134614.[26] This dataset was established by Anurag et al using machine learning techniques to scan the DEXA imaging data from 38,264 participants in a UK Biobank subcohort. AAC severity was quantified using the Kauppila score system, which targets the lumbar spine region from vertebrae L1 to L4.[27] The total AAC score, ranging up to a maximum of 24, was calculated by adding the individual scores from L1 to L4. In this study population, 11.6% of patients exhibited significant calcification (score > 3).

2.3. Selection of genetic IVs

In our MR analysis, the selection of the IVs was based on 3 key assumptions. To address the first assumption, we aimed to obtain a robust number of metabolite-related SNPs for our analysis. To this end, we adopted a more inclusive significance threshold of $P < 1 \times 10^{-5}$. Additionally, we applied linkage disequilibrium (LD) criteria of $R^2 < 0.01$ and distance > 500 kb to clump the SNPs. This standard has been widely applied in prior studies.[28,29] The strength of each IV was assessed using the F-statistic, the formula for which is as follows:

$$R^2 = \frac{2\beta^2 MAF\,(1 - MAF)}{2\beta^2 MAF\,(1 - MAF) + 2N(Se\,(\beta))^2 MAF\,(1 - MAF)}$$

$$F = \frac{(N - k - 1)\,R^2}{k\,(1 - R^2)}$$

where β is the effect size for the genetic variant of interest, minor allele frequency for the genetic variant of interest, Se(β) is the standard error of the effect size for the genetic variant of interest, R^2 is the determinant coefficient of the regression equation that explains the degree of exposure, N is the sample size of the exposure, and k is the number of SNPs. SNPs with F-statistics < 10 and minor allele frequency < 0.01 were considered weak IVs and were excluded from subsequent analyses.[30] Moreover, palindromic SNPs for which the effective alleles were unclear were excluded from the study. We removed any SNPs directly associated with the outcome of the third assumption. At least 3 SNPs were assigned to each of the 486 metabolites.

2.4. Forward MR and sensitivity analysis

Our primary assessment of the causal effects of blood metabolites on AAC was based on the results of the random-effects inverse variance weighted (IVW) method. IVW provides the

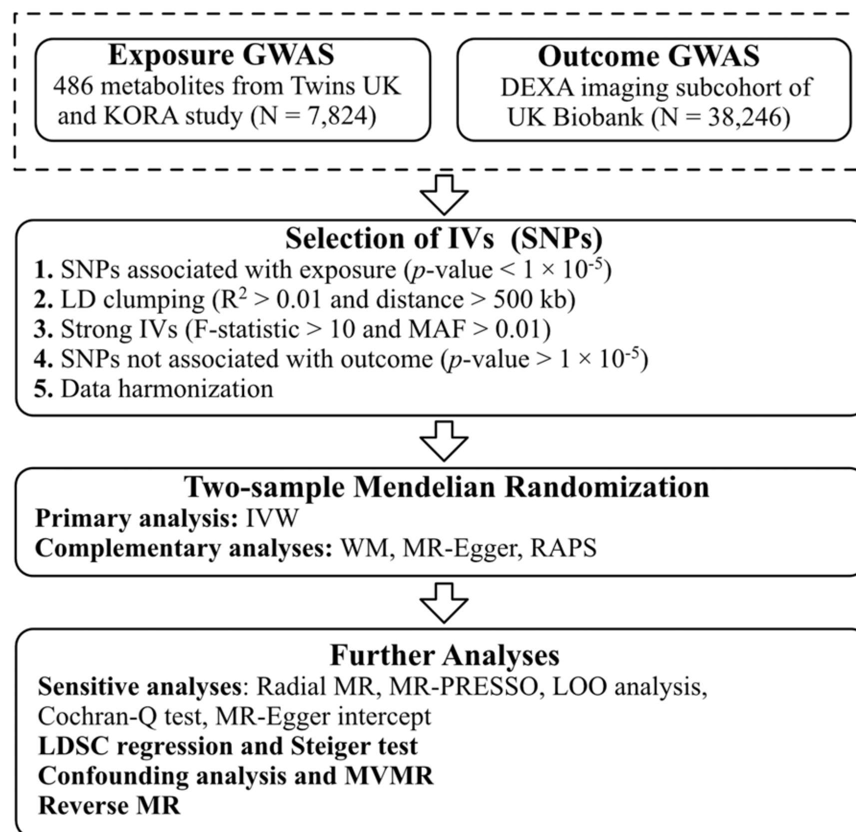

Figure 1. Overview of this Mendelian randomization (MR) analysis. GWAS, genome-wide association study; IVs, instrumental variables; IVW, inverse variance weighted; LD, linkage disequilibrium; LDSC, linkage disequilibrium score; LOO analysis, leave-one-out analysis; MR-PRESSO, MR-Pleiotropy RESidual sum and outlier; MVMR, multivariable Mendelian randomization analysis; RAPS, Robust Adjusted Profile Score; SNPs, single nucleotide polymorphisms; WM, weighted median.

most accurate analysis when there is no horizontal heterogeneity among SNPs.[31] To bolster the robustness of our results, we adopted additional MR methods, including the weighted median (WM), MR-Egger regression, and Robust Adjusted Profile Score (RAPS). The WM method excludes 50% of the IVs in the statistical analysis, making it more robust in the presence of some heterogeneity.[31] MR-Egger regression excels at detecting and delivering estimates in the presence of horizontal pleiotropy.[32] RAPS, a relatively novel technique, accounts for measurement errors in SNP-exposure effects, ensuring unbiased results even in the presence of numerous (e.g., hundreds of) weak instruments, and shows resistance to systematic and idiosyncratic pleiotropy.[33]

Sensitivity analyses were conducted to evaluate the impact of genetic variant heterogeneity, IV validity, potential pleiotropy, and outliers on the results. Radial MR was used to identify and remove heterogeneous SNPs.[24] After analysis, MR-PRESSO was used to reevaluate the presence of heterogeneous SNPs.[23] A Cochran *Q* test-derived *P*-value < .05 was used to indicate heterogeneity.[34] In addition, the MR-Egger intercept was employed to investigate directional pleiotropy and biases caused by invalid IVs.[32] To further confirm the reliability of our findings, we conducted a leave-one-out (LOO) analysis. This involved the sequential removal of individual SNPs from the MR analysis and reanalysis to measure their impact on the overall results.[32] The robustness of our findings was visually represented by forest plots.

To assess the statistical power of the estimates, we calculated the power with the mRnd online resource (https://shiny.cnsgenomics.com/mRnd/).[35] We set the Type I error rate to 0.05 and calculated the power using sample size, R^2 of IVs, effect size, and variance of the exposure and the outcome.

2.5. Genetic correlation and directionality assessment

Although we carefully excluded SNPs directly related to the outcome during the selection of IVs, it is possible that the remaining SNPs could influence the genetics of AAC through LD. This situation may distort the MR estimates. To address this issue, we utilized LD score regression, which uses chi-square statistics derived from GWAS summary data to estimate shared heritability between 2 traits.[36] This method effectively reduces the risk of confounding in our causal effect estimates.

In addition to addressing the confounding factors, we were aware of the possibility of bias due to reverse causality. To this end, we employed the Steiger test and reverse MR analyses. The Steiger test is instrumental in confirming the directionality of the causal relationship between 2 phenotypes, assuming the absence of pleiotropy.[37]

2.6. Confounding analysis and multivariable MR (MVMR)

Although we conducted sensitivity analyses to evaluate horizontal pleiotropy in our MR results, the possibility of SNPs being associated with confounding factors remained a concern. To address this issue, we utilized the Phenoscanner V2 website (http://www.phenoscanner.medschl.cam.ac.uk/) to investigate the potential correlations between SNPs and common AAC risk factors, such as diabetes,[38] body mass index,[39] hypertension,[40] smoking,[41] and low-density lipoprotein cholesterol.[42] SNPs associated with these confounders were excluded from our MR analysis.

Given that some IVs may be correlated with multiple exposure factors, it is challenging to assess the causal effect of a

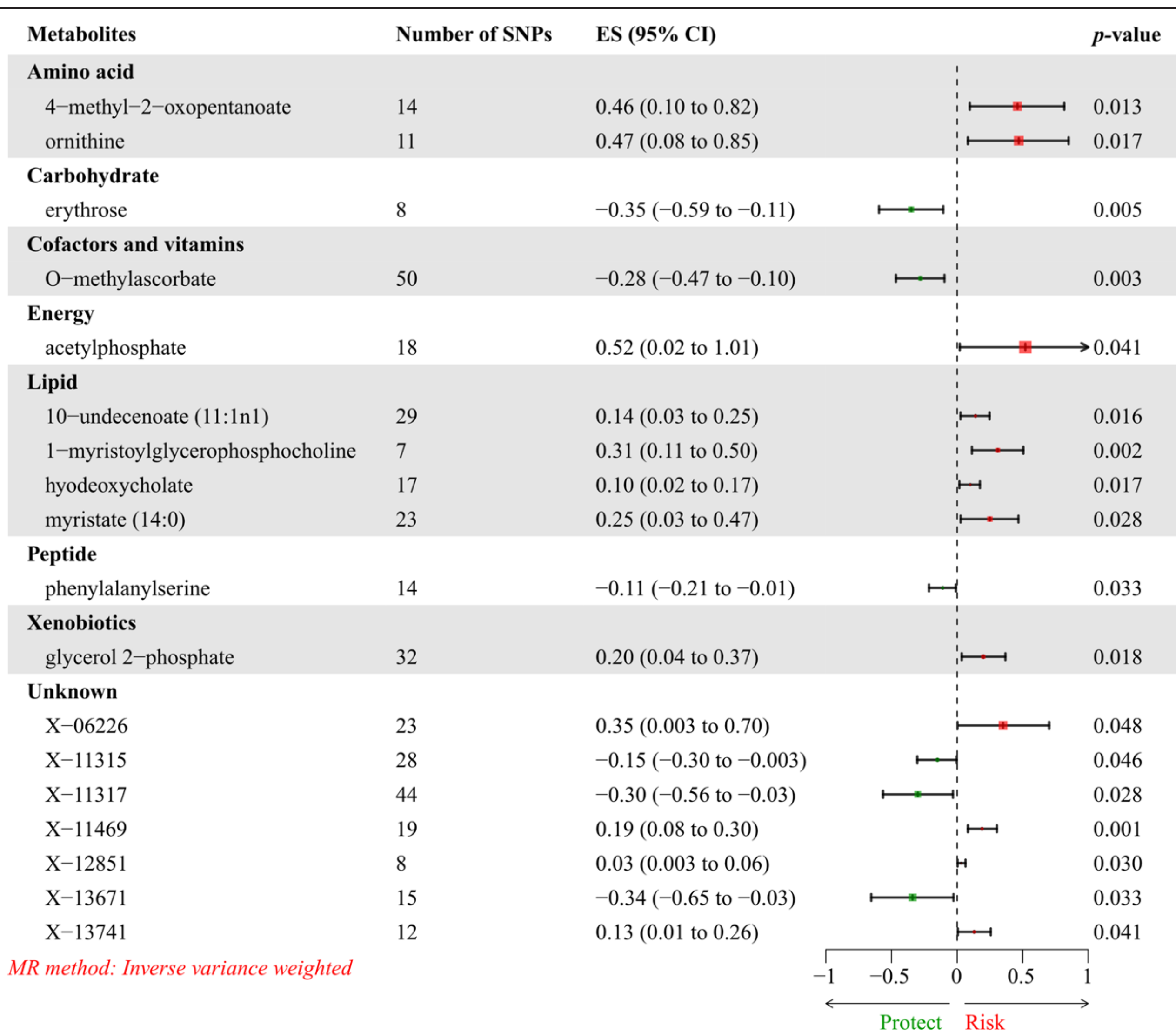

Figure 2. Forest plot for the causality of blood metabolites on abdominal aortic calcification derived from inverse variance weighted analysis. ES, effect size, representing the Kauppila score difference of abdominal aorta calcification per unit in metabolites; CI, confidence interval; SNPs, single nucleotide polymorphisms.

specific exposure on the outcome while excluding the effects of other exposures. Therefore, we conducted multivariable Mendelian randomization (MVMR) analysis on the identified metabolites.[43] MVMR is an MR method that allows the associations of SNPs with multiple phenotypes to be included in a single analysis, enabling estimation of the direct impact of each phenotype on the outcome.[44] We adopted the IVW and MR-PRESSO methods to ensure the precision and reliability of our findings.

2.7. Reverse MR analysis

Reverse MR analysis was performed to investigate the reverse causal relationship between AAC (as exposure) and blood metabolites (as results). The same parameters were used to extract the IVs, and 4 MR methods (IVW, WM, MR-Egger, and RAPS) were used to explore the potential causal relationships. After analysis, MR-PRESSO was used to evaluate the presence of heterogeneous SNPs. Cochran Q test ($P < .05$) was used to indicate heterogeneity. The MR-Egger intercept was employed to investigate directional pleiotropy and biases caused by invalid IVs. LOO analysis was conducted to confirm the reliability of our findings. The Steiger test was used to confirm directionality.

3. Results

3.1. Forward MR: causal effects of the blood metabolites on AAC

After filtering the SNPs using strict criteria, we extracted 486 metabolites from the metabolite GWAS dataset as exposures for the MR analysis. All included SNPs met the criterion of F-statistics > 10, indicating no potential weak IV bias. Detailed data on the IVs are presented in Table S2, Supplemental Digital Content, http://links.lww.com/MD/N458. After identifying and excluding outliers (Table S3, Supplemental Digital Content, http://links.lww.com/MD/N458) through radial MR analysis, the MR analysis was performed. Based on the significant ($P < .05$) estimates derived from the IVW method, as well as the consistent directions of estimates derived from the WM, MR-Egger, and RAPS methods, we preliminarily identified 18 blood metabolites with potential effects on AAC (Fig. 2). Among these, 11 metabolites have been chemically characterized as amino acids, carbohydrates, cofactors and vitamins, energy sources, lipids, peptides, and xenobiotics. Combined with the results of the complementary and sensitivity analyses, we identified 6 metabolites that met the strict screening criteria as candidates (Table 1): 4-methyl-2-oxopentanoate (effect size [ES] 0.46, 95% confidence interval [CI]: 0.10–0.82, $P = .013$),

Table 1

Mendelian randomization and sensitivity results for causality from blood metabolites on abdominal aortic calcification.

	MR analysis			Heterogeneity		Pleiotropy	
Metabolites	**Methods**	**ES (95% CI)**	***P***	***Q***	***P***	**Intercept**	***P***
Amino acid							
4-Methyl-2-oxopentanoate	IVW	0.46 (0.10–0.82)	.013	16.62	.22	−0.001	.95
	WM	0.63 (0.17–1.10)	.007				
	ME	0.50 (−0.78 to 1.77)	.460				
	RAPS	0.58 (0.23–0.93)	.001				
Ornithine	IVW	0.47 (0.08–0.85)	.017	10.36	.41	−0.006	.54
	WM	0.35 (−0.18 to 0.89)	.195				
	ME	0.85 (−0.40 to 2.10)	.215				
	RAPS	0.52 (0.07–0.97)	.023				
Carbohydrate							
Erythrose	IVW	−0.35 (−0.59 to 0.11)	.005	6.36	.50	−0.002	.76
	WM	−0.41 (−0.74 to 0.08)	.014				
	ME	−0.27 (−0.83 to 0.29)	.378				
	RAPS	−0.38 (−0.66 to 0.11)	.007				
Cofactors and vitamins							
O-Methylascorbate	IVW	−0.28 (−0.47 to 0.10)	.003	42.07	.75	0.004	.06
	WM	−0.56 (−0.83 to 0.28)	<.001				
	ME	−0.54 (−0.87 to 0.22)	.002				
	RAPS	−0.26 (−0.46 to 0.06)	.009				
Energy							
Acetylphosphate	IVW	0.52 (0.02–1.01)	.041	18.24	.37	−0.0003	.98
	WM	0.07 (−0.62 to 0.76)	.848				
	ME	0.55 (−1.51 to 2.60)	.610				
	RAPS	0.44 (−0.08 to 0.95)	.095				
Lipid							
10-Undecenoate (11:1n1)	IVW	0.14 (0.03–0.25)	.016	28.15	.46	0.003	.26
	WM	0.07 (−0.08 to 0.23)	.360				
	ME	0.07 (−0.08 to 0.23)	.373				
	RAPS	0.14 (0.01–0.27)	.031				
1-Myristoyl-glycero-phosphocholine	IVW	0.31 (0.11–0.50)	.002	4.46	.62	0.004	.55
	WM	0.26 (0.02–0.50)	.033				
	ME	0.23 (−0.10 to 0.55)	.231				
	RAPS	0.31 (0.08–0.54)	.007				
Hyodeoxycholate	IVW	0.10 (0.02–0.17)	.017	14.67	.55	0.001	.74
	WM	0.10 (−0.01 to 0.21)	.070				
	ME	0.08 (−0.06 to 0.21)	.290				
	RAPS	0.09 (0.003–0.17)	.043				
Myristate (14:0)	IVW	0.25 (0.03–0.47)	.028	22.00	.46	0.002	.71
	WM	0.37 (0.04–0.71)	.028				
	ME	0.18 (−0.25 to 0.61)	.429				
	RAPS	0.21 (−0.13 to 0.55)	.233				
Peptide							
Phenylalanylserine	IVW	−0.11 (−0.21 to 0.01)	.033	15.52	.28	−0.003	.60
	WM	−0.08 (−0.23 to 0.06)	.239				
	ME	−0.07 (−0.26 to 0.13)	.524				
	RAPS	−0.11 (−0.22 to 0.01)	.035				
Xenobiotics							
Glycerol 2-phosphate	IVW	0.20 (0.04–0.37)	.018	23.13	.84	0.002	.59
	WM	0.01 (−0.24 to 0.26)	.943				
	ME	0.12 (−0.21 to 0.46)	.482				
	RAPS	0.19 (0.01–0.38)	.037				
Unknown							
X-06226	IVW	0.35 (0.003–0.70)	.048	26.68	.22	0.004	.36
	WM	0.32 (−0.17 to 0.81)	.204				
	ME	0.04 (−0.69 to 0.77)	.909				
	RAPS	0.35 (−0.14 to 0.85)	.161				
X-11315	IVW	−0.15 (−0.30 to 0.003)	.046	20.01	.83	0.004	.24
	WM	−0.18 (−0.41 to 0.04)	.108				
	ME	−0.29 (−0.55 to 0.02)	.043				
	RAPS	−0.17 (−0.33 to 0.003)	.046				
X-11317	IVW	−0.30 (−0.56 to 0.03)	.028	58.54	.06	0.004	.23
	WM	−0.42 (−0.80 to 0.03)	.033				
	ME	−0.64 (−1.25 to 0.03)	.046				
	RAPS	−0.35 (−0.59 to 0.10)	.006				
X-11469	IVW	0.19 (0.08–0.30)	.001	21.83	.24	−0.003	.81
	WM	0.19 (0.03–0.35)	.017				
	ME	0.25 (−0.25 to 0.75)	.335				
	RAPS	0.21 (0.08–0.34)	.001				

(*Continued*)

Table 1
(*Continued*)

Metabolites	MR analysis			Heterogeneity		Pleiotropy	
	Methods	ES (95% CI)	*P*	*Q*	*P*	Intercept	*P*
X-12851	IVW	0.03 (0.003–0.06)	.030	2.96	.89	0.004	.41
	WM	0.03 (−0.004 to 0.07)	.080				
	ME	0.02 (−0.02 to 0.06)	.289				
	RAPS	0.03 (−0.003 to 0.07)	.070				
X-13671	IVW	−0.34 (−0.65 to 0.03)	.033	11.98	.61	−0.001	.93
	WM	−0.40 (−0.84 to 0.05)	.080				
	ME	−0.30 (−1.27 to 0.67)	.560				
	RAPS	−0.37 (−0.71 to 0.03)	.033				
X-13741	IVW	0.13 (0.01–0.26)	.041	12.88	.30	0.003	.84
	WM	0.14 (−0.03 to 0.30)	.107				
	ME	0.08 (−0.45 to 0.60)	.781				
	RAPS	0.15 (0.02–0.28)	.026				

CI = confidence interval, ES = effect size, IVW = inverse variance weighted, ME = MR-Egger, MR = Mendelian randomization, RAPS = Robust Adjusted Profile Score, WM = weighted median.

erythrose (ES −0.35, 95% CI: −0.59 to −0.11, $P = .005$), 10-undecenoate (11:1n1) (ES 0.14, 95% CI: 0.03–0.25, $P = .016$), 1-myristoylglycerophosphocholine (ES 0.31, 95% CI: 0.11–0.50, $P = .002$), glycerol 2-phosphate (ES 0.20, 95% CI: 0.04–0.37, $P = .018$), and X-11469 (ES 0.19, 95% CI: 0.08–0.30, $P = .001$). For all candidates, IVW and the other 3 complementary methods showed the same direction (Fig. 3). No outliers were detected in MR-PRESSO (Table S4, Supplemental Digital Content, http://links.lww.com/MD/N458), and neither Cochran *Q* test ($P > .05$) nor MR-Egger intercept test ($P > .05$) indicated significant heterogeneity or pleiotropy (Table 1). The results of LOO analysis confirmed that the estimated values were unaffected by the absence of each SNP (Figure S1, Supplemental Digital Content, http://links.lww.com/MD/N459).

Notably, the statistical power of most results was not optimal (Table S5, Supplemental Digital Content, http://links.lww.com/MD/N458). Given that our primary objective was to minimize the risk of Type I error to ensure more reliable positive findings, which inevitably led to a higher likelihood of a Type II error rate and a subsequent loss in statistical power, we considered these trade-offs acceptable.

3.2. Genetic correlation and directionality assessment

We assessed the SNP heritability of the 6 candidate metabolites by LD score regression analysis. Glycerol 2-phosphate ($h2 = 0.21$, $P = .008$) and 10-undecanoate (11:1n1) ($h2 = 0.26$, $P = 4.91 \times 10^{-5}$) exhibited significant genetic effects, while erythrose and X-11469 had positive heritability but were not statistically significant ($P > .05$) (Table S6, Supplemental Digital Content, http://links.lww.com/MD/N458). The h2-value could not be calculated for the remaining metabolites owing to low heritability or small sample size. Next, we explored the genetic relationship between AAC and 4 metabolites with positive heritability. There was no significant genetic correlation between the metabolites and AAC ($P > .05$) (Table S7, Supplemental Digital Content, http://links.lww.com/MD/N458), suggesting that shared genetics was unlikely to have a confounding effect on our MR analysis. The Steiger test confirmed that the causal relationships between the metabolites and AAC were not compromised by reverse causal effects ($P < .05$) (Table S8, Supplemental Digital Content, http://links.lww.com/MD/N458).

3.3. Confounding analysis and MVMR

To assess the potential influence of confounding factors on our study, we selected common risk factors for AAC including diabetes, body mass index, hypertension, smoking, and low-density lipoprotein cholesterol. We subsequently used the Phenoscanner website to determine whether the SNPs associated with the 6 candidate metabolites were independent of these risk factors. We found that erythroses were not associated with any confounding factor. For the other metabolites, we identified 19 SNPs that were associated with these risk factors (Table S9, Supplemental Digital Content, http://links.lww.com/MD/N458). After eliminating these SNPs, we performed an MR analysis, and all results were consistent with the initial findings, indicating that confounding factors did not bias our results.

After combining the SNPs, correcting direction, and removing LD, we performed MVMR analysis of the candidate metabolites and AAC. The outcomes of both MVMR IVW and MVMR MR-PRESSO analyses indicated that the genetically predicted erythrose, 10-undecanoate (11:1n1), 1-myristoylglycerophosphocholine, and X-11469, had independent causal effects on AAC (Fig. 4).

3.4. Reverse MR: causal effects of AAC on blood metabolites

In the reverse MR analysis, the *F*-statistics of the IVs for each metabolite were >10, indicating the absence of weak instrument bias in this analysis. We identified 12 blood metabolites that were associated with genetic susceptibility to AAC (Fig. 5). Nine metabolites were chemically characterized as lipids, nucleotides, peptides, and xenobiotics. There were no common metabolites between the results of forward and reverse MR. For all metabolites, the 3 complementary methods showed the same direction as the significant IVW estimates, and the results of MR-PRESSO, Cochran *Q* test, and MR-Egger intercept test revealed no heterogeneity or pleiotropy ($P > .05$) (Table S10, Supplemental Digital Content, http://links.lww.com/MD/N458). The Steiger test results confirmed the directionality ($P > .05$). However, after performing the LOO analysis, we found that the significance of the estimated values of all these metabolites was compromised by excluding a single SNP (Figure S2, Supplemental Digital Content, http://links.lww.com/MD/N459). We attributed this to the limited number of SNPs in our MR analysis.

4. Discussion

In this study, we integrated GWAS data from 2 European cohorts and employed rigorous MR methods to investigate the causal relationships between 486 blood metabolites and AAC. Our findings suggest that genetically predicted elevated levels of 4-methyl-2-oxopentanoate, 10-undecenoate (11:1n1), 1-myristoylglycerophoshocholine, X-11469, and the known

vascular calcification promoter glycerol 2-phosphate are associated with higher AAC scores,[45] while genetically predisposition to elevated erythrose levels are associated with lower AAC scores. Our MVMR analysis results suggest that erythrose, 10-undecenoate (11:1n1), 1-myristoylglycerophosphocholine, and X-11469 directly affect AAC independent of other metabolites. On the other direction, reverse MR results suggest that genetically proxied AAC scores might alter the levels of 12 blood metabolites.

4-Methyl-2-oxopentanoic acid, an abnormal metabolite of leucine, along with branched-chain amino acids (BCAA), has been reported for its adverse effects on various diseases. However, its relationship with vascular calcification has not been previously reported. A recent mediation analysis within a Japanese cohort revealed that 4-methyl-2-oxopentanoate and BCAA mediate more than 20% of the association between nonalcoholic fatty liver disease and the cardio-ankle vascular index.[46] High concentrations of 4-methyl-2-oxopentanoate are associated with insulin resistance and diabetes mellitus type 2 in humans and animals.[47,48] BCAA and its metabolic deficiencies have demonstrated associations with various cardiovascular diseases (CVD) and their risk factors in previous research studies.[49,50] A study has shown that BCAA can induce oxidative stress, inflammation, and the migration of human peripheral blood mononuclear cells.[51] Elevated BCAA can promote the progression of atherosclerosis by inducing HMGB1 translocation and further proinflammatory macrophage activation.[52] Another study highlighted that inhibiting BCAT1 to decrease BCAA catabolism can diminish the proinflammatory transcriptomic profile in macrophages, suggesting its therapeutic potential for inflammatory diseases.[53]

10-Undecylenoic acid (11:1n1) is a medium-chain monounsaturated fatty acid (MUFA). Recent studies have revealed the effects of different types of free fatty acids on vascular calcification. Palmitic acid, a saturated fatty acid, can induce vascular smooth muscle cell (VSMC) calcification in vitro and induce aortic calcification in rabbit models.[54,55] In contrast, substantial evidence from epidemiological studies and basic research consistently demonstrates that n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, inhibit vascular calcification.[16,56] The health effects of MUFA are subject to ongoing debate.[57] Oleic acid (18:1), the predominant blood MUFA, has been linked with the increase of coronary artery calcification, carotid plaque, and aortic valve calcification in the multi-ethnic study of atherosclerosis.[58] Prior study has revealed that low concentrations of 10-undecylenoic acid (11:1n1) significantly upregulate the expression of osteogenic markers and facilitate the nuclear translocation of NF-κB p65 in mouse osteoblasts,[59] indicating a potential mechanism through which it might affect vascular calcification. Given the role of NF-κB pathway activation and osteogenic differentiation of VSMCs in the development of vascular calcification,[60,61] it is hypothesized that 10-undecylenoic acid (11:1n1) may promote vascular calcification through the activation of the NF-κB pathway, thereby inducing osteogenic differentiation in VSMCs.

1-Myristoylglycerophosphocholine, known as lysophosphatidylcholine (lysoPC) (14:0), mainly produced from the breakdown of phosphatidylcholine. This process is mediated by secretory phospholipases A2 (PLA2).[62] As a bioactive lipid metabolite, lysoPC was found to act as a potent chemoattractant for monocytes,[63] T cells[64] as well as

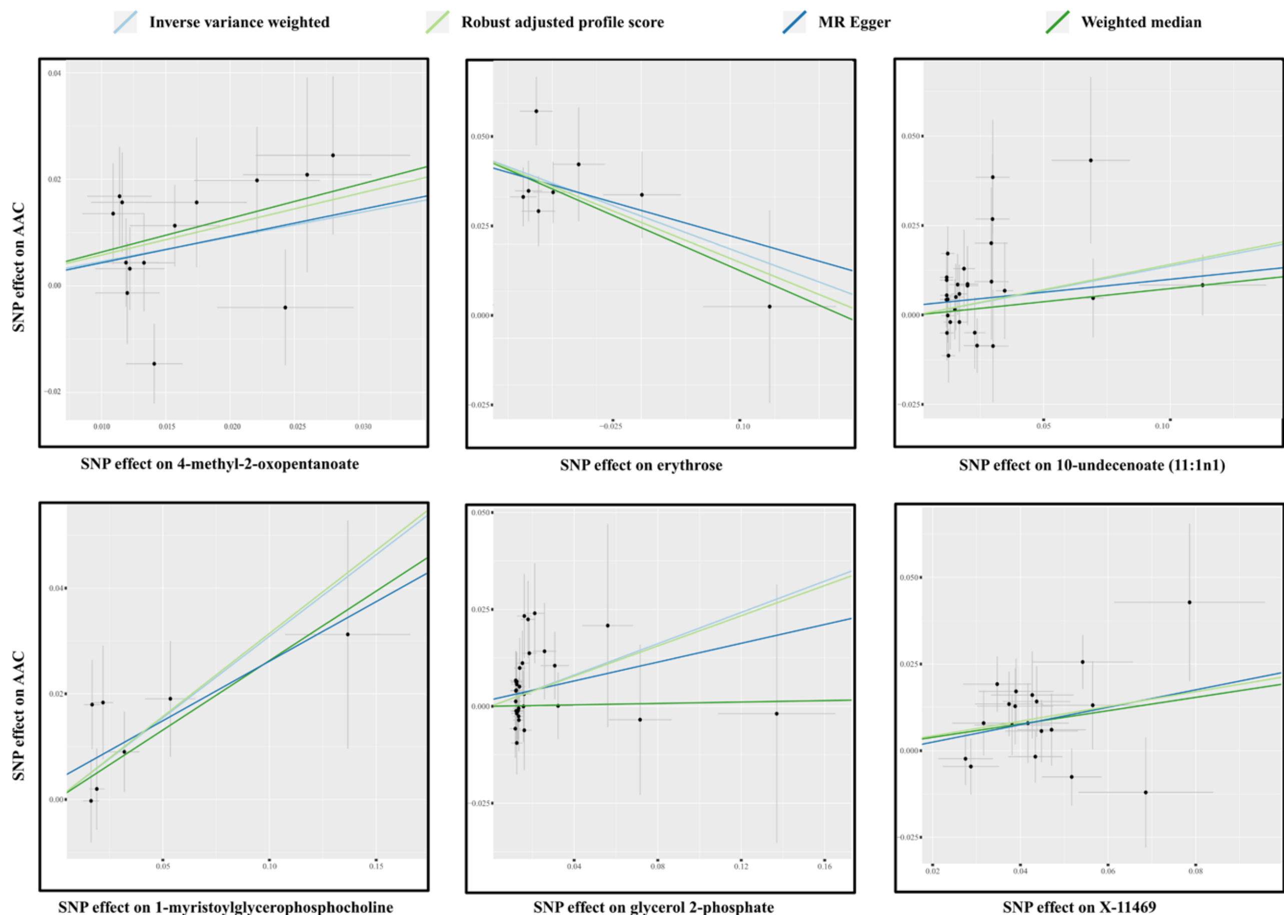

Figure 3. Scatterplot of significantly associated (IVW-derived $P < .05$) and directionally consistent estimates. AAC, abdominal aortic calcification; IVW, inverse variance weighted; SNP, single nucleotide polymorphism.

natural killer cells,[65] attracting them to sites of inflammation. Due to its complex bidirectional effects on vascular inflammation, lysoPC is an important homeostatic mediator of vascular inflammation.[66] LysoPC activates cytosolic calcium-independent PLA2, releasing arachidonic acid from aortic endothelial cells.[67,68] Interestingly, our reverse MR reveals a positive correlation between the genetic predisposition to AAC severity and the levels of arachidonate (20:4n6) and 1-arachidonoylglycerophosphocholine. Given the established role of PLA2 in vascular calcification,[69] these insights suggest lysoPC may accelerate AAC. It likely does so by stimulating PLA2 activity in VSMCs, promoting osteogenic differentiation.

Our study identified erythrose as a metabolite that protected against AAC. Erythrose is an endogenous metabolite formed by glucose in the pentose phosphate pathway. It also acts as a precursor for erythritol, a known biomarker for type 2 diabetes, central obesity, and CVD.[70] Despite its significance, detailed studies on erythrose's specific functions are scarce. Research involving in vitro studies and rodent models have indicates that erythritol can moderately reduce oxidative stress markers and clear free radicals.[71] This suggests that synthesizing erythritol might be a adaptive mechanism against oxidative stress caused by obesity and hyperglycemia. In a preliminary study with 24 diabetic patients, consuming 36 grams of erythritol daily for 4 weeks showed benefits. Specifically, it helped

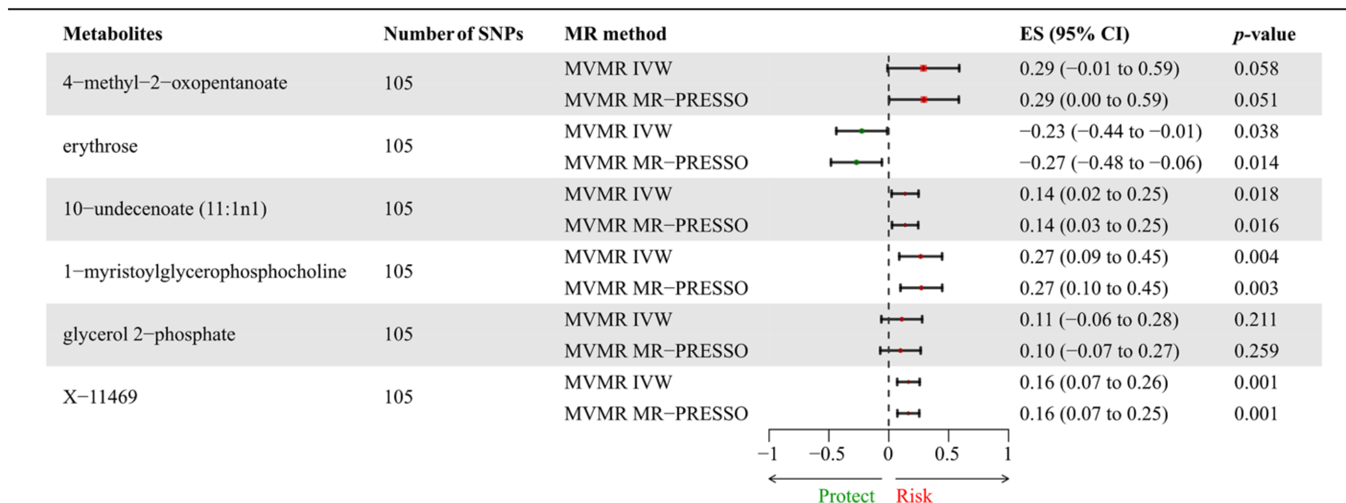

Figure 4. MVMR analysis of the final identified blood metabolites. ES, effect size, representing the Kauppila score difference of abdominal aorta calcification per unit in metabolites; CI, confidence interval; IVW, inverse variance weighted; MVMR, multivariable Mendelian randomization; MR-PRESSO, MR-Pleiotropy RESidual Sum and Outlier.

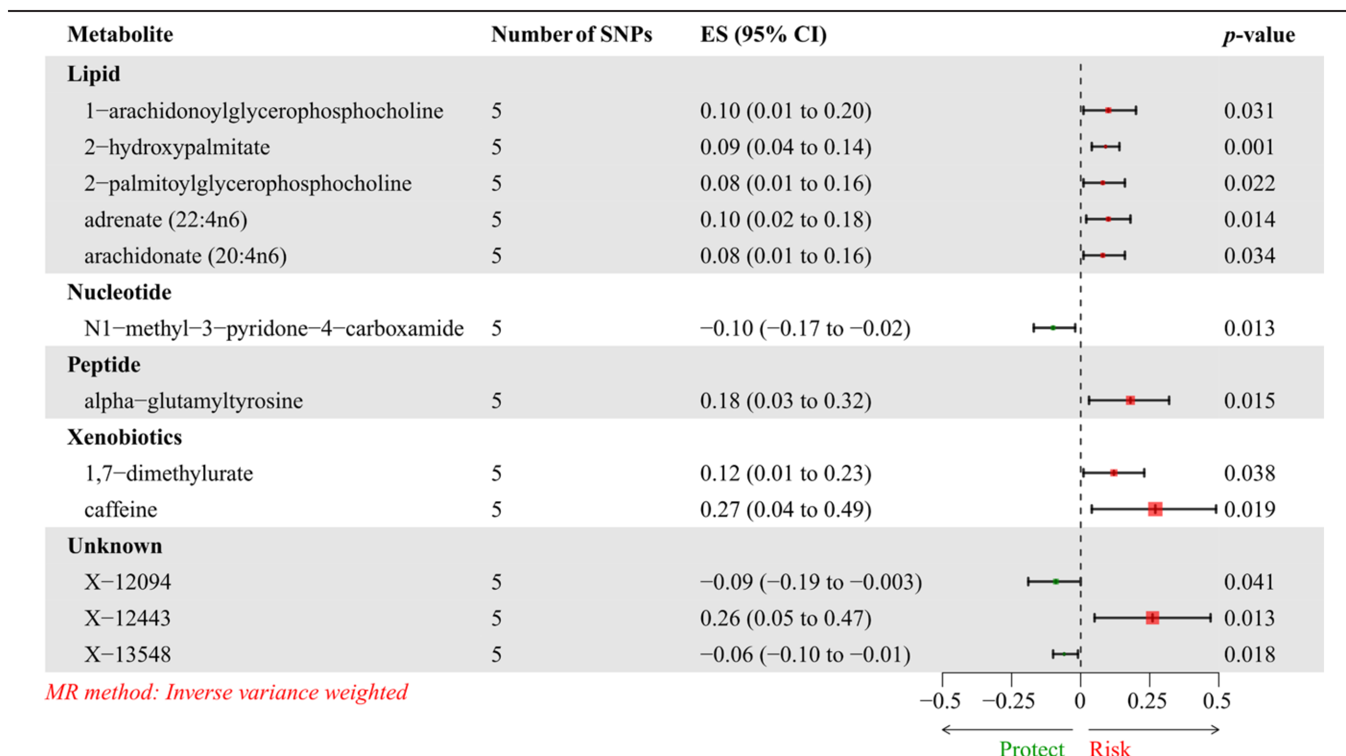

Figure 5. Forest plot for the causality of the severity of abdominal aortic calcification on blood metabolites derived from inverse variance weighted analysis. ES, effect size, representing the unit difference in blood metabolites per Kauppila score of abdominal aorta calcification; CI, confidence interval; SNPs, single nucleotide polymorphisms.

reduce arterial stiffness and enhance endothelial function.[72] Our findings support the idea that erythritol contributes to cardiovascular protection. They also imply that the pathway for synthesizing erythritol could offer a protective mechanism in AAC development.

The interplay between AAC and its metabolites was further substantiated using reverse MR analysis. Twelve metabolites were altered in response to AAC. These altered metabolites hold promise as potential biomarkers of AAC. A notable discovery was the link between high AAC scores and increased levels of caffeine and its metabolite, 1,7-dimethylurate. This finding aligns with a previous observational study. This study reported that heavy coffee intake was associated with higher AAC scores in patients with hypertension, diabetes, and CVD.[73] While in participants without hypertension, diabetes, and CVD, coffee consumption had no significant impact on AAC. This pattern was not observed in decaffeinated coffee. Furthermore, various animal studies and clinical trials have demonstrated a correlation between elevated caffeine consumption, reduced bone density, and an increased risk of fractures.[74] While liver and kidney metabolism may introduce confounding factors. Further research is essential to elucidate the influence of caffeine on vascular calcification. Another significant metabolite type elevated by AAC was arachidonate and its derivative 1-arachidonoylglycerophosphocholine. We noted that an exploratory lipidomic study showed that arachidonic acid and its derivatives lysoPC (20:4), PC (20:4), and TG (20:4) were significantly increased in patients with severe coronary artery calcification.[75] These findings underscore the potential role of these metabolites in vascular disease progression, highlighting the need for in-depth research into their biological mechanisms and cardiovascular impact.

This MR analysis has several strengths. One of the primary advantages of this study was the comprehensive inclusion of a wide range of blood metabolites, which allowed us to conduct a detailed exploration of potential causal pathways. Furthermore, the robustness of our findings is supported by the rigorous application of the MR methodology. Integrated analyses of multiple MR methods largely minimize interference from pleiotropy and heterogeneity. Rigorous and thorough sensitivity analyses enhanced the reliability and robustness of the results. Efforts to evaluate the genetic correlation and directionality between metabolites and AAC have enabled us to effectively avoid common pitfalls such as reverse causality and genetic correlation. This study had several limitations. First, our results did not survive the strict Bonferroni correction after adjusting for multiple comparisons. However, we employed a hypothesis-driven approach utilizing biological evidence to assess previously established epidemiological associations, without being constrained by the stringent statistical correction of *P*-values. The detection of recognized promoters of vascular calcification within our result also substantiates the validity of our approach. Second, the study used GWAS data from European populations, which may have limited the general applicability of our findings to other populations. Third, another limitation is related to the accuracy and statistical power of MR estimation, which partly depends on the sample size. In future studies, expanding the sample size will be beneficial in validating our results. It is also important to acknowledge that, although MR analysis provides valuable etiological insights, applying these findings in clinical settings should be based on further validation through RCTs and basic research.

5. Conclusion

This bidirectional MR study revealed an intricate interplay between blood metabolites and AAC. These identified metabolites help elucidate the underlying biological mechanisms of AAC and may serve as biomarkers for clinical screening and prevention strategies for high-risk populations. However, further basic and clinical studies are required to validate these results.

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