Role of metabolites in mediating the effect of lipidomes on rheumatoid arthritis

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Research Article

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Abstract

Background

Evidence from observational studies and clinical trials suggests that lipidomes are associated with an increased risk of rheumatoid arthritis (RA). However, considering inherent confounding factors and the challenge of reverse causality in observational studies, the direct causal relationship between lipidomes and RA remains unknown. Therefore, we conducted mendelian randomization (MR) as well as mediation analysis to elucidate the causal relationship among lipidomes, RA, and metabolites as a mediator.

Methods

The bidirectional MR analysis was performed to evaluate the relationship of lipidomes and RA, with a focus on the role of metabolites. Instrumental variables (IVs) were used as the central methodological technique, supplemented by MR-Egger, weighted median, simple mode, as well as weighted mode methods.

Results

Findings from this study revealed that six lipidomes had a positive correlation with RA, while one showed a negative association. Furthermore, mediation MR analysis results revealed that undecenoylcarnitine (C11:1) served as a mediator for the effect of sterol ester (27:1/20:4) on RA and the mediation effect was calculated to be 7.98%.

Conclusions

Our study demonstrated the genetic causal effect of lipidomes on RA, emphasizing the potential mediating role of undecenoylcarnitine (C11:1) and providing insights for the clinical intervention of RA.

Background

As a chronic, systemic, inflammatory autoimmune disease, rheumatoid arthritis (RA) affects both joints and extra-articular organs. It is a widely distributed disease worldwide, with a prevalence ranging from approximately 0.5–2%, depending on sex, age, and the patient collective studied [1–5]. While the cause of RA is unknown, genetic, environmental, immune, and metabolic factors have all been shown to contribute to its development [6]. Metabolism and immune responses are believed to be involved in the pathogenesis of RA [7–8]. Lipidome metabolism is a critical component of cellular metabolism and effective immune responses. It has been reported that patients with RA may experience disorders of lipidome metabolism [9–10]. A particularly significant fact is that these disorders have been observed before the onset of symptoms [11]. Therefore, aspects of lipidome metabolism could potentially

contribute to the development of RA and associated comorbidities, making them potential therapeutic targets. Certainly, the presence of lipidome disorders as a concomitant symptom of RA cannot be ruled out. However, due to limitations in sample size and the interference of confounding factors, the conclusions drawn from observational and correlational studies still exhibit deviations. Thus, further research is required to establish a causal relationship between lipidomes and RA.

Mendelian randomization (MR) is a data analysis approach used in epidemiological studies to evaluate the inference of etiological factors. It used genetic variants were used as instrumental variables (IVs) to assess their causal relationship between the exposure factors and the outcomes. The strength of MR lies in the fact that alleles adhere to the principle of random allocation, which allows it to avoid the influences of confounding factors alongside reverse causality that occur in previous epidemiological studies [12–16]. Therefore, we adopted MR to identify the causal relationship between RA and lipidomes.

Methods

Study design

Two-sample MR, a method estimating causal effects of risk factors on diseases using only genome-wide association studies (GWAS) summary statistics, was used to evaluate the causal relationship between lipidomes and RA [13]. To ensure the effectiveness of the analysis, three core assumptions must be met: (1) IV1- There should be a strong correlation between single nucleotide polymorphisms (SNPs) and exposure; (2) IV2- SNPs and confounding factors should be independent; (3) IV3- SNPs should only affect outcomes through exposure factors. Following this, we delve into the role of metabolites as mediating factors in the relationship between RA and lipidomes. The illustration of the study design was displayed on Fig. 1.

Data sources

GWAS summary statistics for RA were obtained from the IEU open GWAS project (ebi-a-GCST90038685), which involved 484,598 participants and 9,587,836 SNPs [8]. The univariate GWAS summary statistics for lipidomes uses in this study were acquired from the GWAS catalog (accession codes: GCST90277238-GCST90277416). For the mediator, we also relied on the NHGRI-EBI GWAS Catalog (accession codes: GCST90199621-GCST902010209).

Selection for genetic variation

To screen suitable genetic IVs that satisfied three core assumptions, we applied a series of restrictive conditions on the IVs. Firstly, we included SNPs that reached a threshold of genome-wide significance ($p < 1e\text{-}05$). Secondly, we set a threshold for removing linkage disequilibrium within a 10000kb range, which was $r^2 < 0.01$. Palindrome SNPs with allele frequencies close to 0.5 were removed using the Two-sample MR R package, as these palindrome SNPs could lead to ambiguity in coordinating alleles between the exposed and resulting datasets. Finally, to evaluate whether the included SNPs were influenced by weak

IVs, we calculated the variance explanatory ratio of individual SNPs and then calculated the F statistic value (F > 10). If the SNP's F statistic was less than 10, it indicated the possibility of weak instrumental bias, which could be eliminated to avoid affecting the results [12, 18].

Statistical, pleiotropy, and heterogeneity analyses

MR analysis was conducted using R 4.3.1 software (http://www.Rproject.org)[19]. To investigate the causal relationship between liposome metabolism and RA, inverse variance weighting (IVW) [20], MR Egger [21], weighted median [12], simple mode [12, 13], as well as weighted mode methods [12] were performed using the “Mendelian Randomization” package. We adopted Cochran’s Q statistical test to detect and quantify the heterogeneity within the IVs, and implemented a "leave one out" approach to explore the potential impact of individual SNPs on this causal association [13]. The MR Egger intercept was mainly adopted to assess possible horizontal pleiotropic effects between genetic variation and other confounding factors [14]. Additionally, the MR-PRESSO method was used to identify and exclude outliers that could significantly impact the estimation results [12–13, 22].

Results

Lipidomes and metabolites associated with RA

Lipidomes and metabolites linked to RA must meet three conditions: (1) The p value of the results generated by the IVW method need to be less than0.05. (2) The p value of the results of pleiotropy should be more than 0.05. (3) The results of IVW, MR Egger, weighted median, simple mode, as well as weighted mode should be consistent. To identify the lipidome with a causal effect on RA, we conducted a two-sample MR analysis, using the IVW method as the primary analysis. Based on a p value of less than 0.05, we observed that RA-Phosphatidylcholine (16:0_16:1) levels had a protective effect (OR = 0.998651, 95% CI: 0.997359–0.999944, p = 0.040944). Conversely, six lipidomes were correlated with an increased risk of RA: Sterol ester (27:1/20:5) levels (OR = 1.001193, 95% CI: 1.000392–1.001995, p = 0.003523), Phosphatidylinositol (16:0_20:4) levels (OR = 1.001385, 95% CI: 1.000403–1.002368, p = 0.005706), Phosphatidylcholine (18:0_20:5) levels (OR = 1.000982, 95% CI: 1.00019–1.001775, p = 0.01506), Sterol ester (27:1/20:4) levels (OR = 1.000618, 95% CI: 1.000074–1.001162, p = 0.025884), Phosphatidylethanolamine (O-18:2_18:2) levels (OR = 1.001637, 95% CI: 1.000161–1.003116, p = 0.029699), and Phosphatidylcholine (18:0_20:4) levels (OR = 1.00054, 95% CI: 1.000014–1.001095, p = 0.044519). It suggests that these lipidomes may contribute to the development of RA (Fig. 2). On the other hand, our analysis indicated that RA had no causal effect on seven lipidomes, including sterol ester (27:1/20:4) levels (p = 0.929274), Sterol ester (27:1/20:5) levels (p = 0.522395), Phosphatidylcholine (16:0_16:1) levels (p = 0.270819), Phosphatidylcholine (18:0_20:4) levels (p = 0.583645), Phosphatidylcholine (18:0_20:5) levels (p = 0.387426), Phosphatidylethanolamine (O-18:2_18:2) levels (p = 0.915225), and Phosphatidylinositol (16:0_20:4) levels (p = 0.625424). Based on the results of MR analysis, we identified metabolites associated with RA. After filtering, we found nine metabolites related to RA: 4-cholesten-3-one levels (OR = 0.998633, 95% CI: 0.99763–0.99963622, p = 0.00758648), O-sulfo-l-

tyrosine levels (OR = 0.998529701, 95% CI: 0.99743–0.99963, p = 0.008841), 3-hydroxyhexanoylcarnitine (1) levels (OR = 1.001878, 95% CI: 1.000653–1.003105, p = 0.002654), Undecenoylcarnitine (C11:1) levels (OR = 1.001144, 95% CI: 1.000305–1.001984, p = 0.007555), Succinate levels (OR = 0.998097, 95% CI: 0.996742–0.999453, p = 0.005963), Cystathionine levels (OR = 1.00182, 95% CI: 1.000722–1.00292, p = 0.001158), X-22771 levels (OR = 1.00249251, 95% CI: 1.000822924–1.004164882, p = 0.003419523), X-24306 levels (OR = 0.998356, 95% CI: 0.997156881–0.999557125, p = 0.007312674), and X-25343 levels (OR = 1.001909, 95% CI: 1.00060358–1.003215337, p = 0.004138974) (Fig. 3).

Undecenoylcarnitine (C11:1) as a mediator in the relationship between lipidomes and RA

The results of MR further revealed a causal effect of genetically predicted sterol ester (27:1/20:4) levels (OR = 1.044049, 95% CI: 1.001409–1.088504, p = 0.042745) on undecenoylcarnitine (C11:1) levels (GCST90200236), as illustrated by the IVW method (Fig. 4A). Considering our previous findings that established connections between "lipidome → RA" and "lipidome → undecenoylcarnitine (C11:1)," we hypothesized that undecenoylcarnitine (C11:1) might potentially mediate the relationship between lipidomes and RA. The sensitivity analysis was implemented to strengthen this conclusion.

Undecenoylcarnitine (C11:1) on as a potential risk factor for on RA

In our investigation of the effects of metabolites on RA, we found that undecenoylcarnitine (C11:1) had a causal relationship with RA according to the IVW method (OR = 1.001144, 95% CI: 1.000304538–1.001984428, p = 0.007554917), suggesting that undecenoylcarnitine (C11:1) might act as a risk factor in the pathogenesis of RA (Fig. 4A). To validate these findings, a series of sensitivity analyses involving the MR-Egger, weighted mode, simple mode, as well as weighted median methods were conducted.

Undecenoylcarnitine (C11:1) as a mediator in the causal relationship between the sterol ester (27:1/20:4) and RA

Using the mediation MR analysis method, we found that undecenoylcarnitine (C11:1) could act as a mediator in the causal relationship between sterol ester (27:1/20:4) and RA (b = 4.93e-05, 95% CI: -0.00175, 0.00185). The mediation effect was calculated to be 7.98% (Fig. 4B).

Discussion

Existing studies have revealed that the lipidomic profile in the synovial fluid of patients with RA is severely disrupted. The degree of disorder is closely related to the extent of synovitis observed on ultrasonography [23]. However, comprehensive analyses have not been undertaken to corroborate the causal relationship between lipidomes and RA. In this mediation MR study, a causal relationship was identified between seven lipidomes and RA. The results of the mediation MR analysis indicate that

undecenoylcarnitine (C11:1) could act as a mediator in the causal relationship between sterol ester and RA, and The mediation effect was calculated to be 7.98%. This mediation MR study underscored the association of lipidomes with RA, underscoring the role of undecenoylcarnitine (C11:1) as a mediator.

Many studies have reported altered lipidomic profiles in patients with RA. The main changes include the followings: (1) Reduced levels of serum total cholesterol and triglycerides in untreated RA patients [24–25]; (2) Increased levels of the aforementioned lipidomes in treated RA patients [26–27]; (3) HDL lacking antioxidant capacity in patients with RA [28–29]. Recent insights suggest that lipidomes play a crucial role as components of immune cell membranes, facilitating appropriate cell signaling in response to antigens or other cellular ligands [30–32]. While research has established a strong relationship between lipidomes and RA, the exact causality remains elusive. Meanwhile, patients with RA, like those with other chronic inflammatory diseases, also experience alterations in metabolism, which may contribute to higher morbidity and mortality rates [33]. Therefore, mediation MR analysis was used to comprehensively analyze the causal relationship between lipidomes and RA, with a particular emphasis on the role of metabolites in this relationship.

We have identified a positive association between RA and the following lipid species: sterol ester (27:1/20:5), phosphatidylinositol (16:0_20:4), phosphatidylcholine (18:0_20:5), sterol ester (27:1/20:4), phosphatidylethanolamine (O-18:2_18:2), and phosphatidylcholine (18:0_20:4). In addition, phosphatidylcholine (16:0_16:1) shows a negative causal relationship with RA. Phospholipids are important components of cell membranes and organelle membranes, essential for maintaining normal membrane fluidity and function. The phospholipid signaling system is a significant cellular pathway involved in regulating processes such as cell growth, division, survival, and communication [34]. It is plausible that dysregulation within this system may contribute to the development of RA.

It's worth noting that undecenoylcarnitine (C11:1) may serve as a link between sterol ester (27:1/20:4) and RA. Moreover, more and more metabolites have been identified to be related with the pathogenesis of diseases [35–38]. For instance, lactic acid, as a byproduct of glycolysis, acts as a signaling molecule in chronic inflammatory and cancerous tissues [36]. Metabolic disorders are associated with the development of RA [37–38]. Glycolysis, the arachidonic acid, butyric acid, and tryptophan metabolic pathways have garnered significant interest and have been extensively studied for their involvement in RA. Disruptions in these metabolic pathways can directly or indirectly contribute to inflammation, immune responses, and the development of atherosclerosis in RA patients [39–40]. However, it should be noted that certain studies have reported inconsistent results, possibly due to the heterogeneity of RA patients and the limited number of samples available. For example, Zhou and Srivastava found that branched-chain amino acids are downregulated in the synovial fluid of RA patients but upregulated in the joint tissues of CIA rats [41–42]. Nevertheless, despite these findings, there have been currently no studies that thoroughly investigate the relationship between RA and lipidome metabolism. Our research findings suggest that undecenoylcarnitine (C11:1) plays a regulatory role in the influence of sterol ester (27:1/20:4) on RA, further providing theoretical supports for the treatment as well as the prevention of RA.

Although our research had certain advantages, like a large sample size, the removal of confounding factors, and the clarification of causal relationships, it still had inherent limitations. One significant shortcoming was the lack of animal and clinical experiments to further investigate the molecular mechanisms of liposomes and metabolites in the development of RA. Therefore, future endeavors should focus on refining these experiments and striving to elucidate the involved mechanisms. It is important to note that there were still inevitable deficiencies in our study. Specifically, our findings were based on theoretical assumptions and have not been substantiated through rigorous clinical or animal experimentation. As a result, the precise molecular mechanisms underlying our observations remain uncertain. Further investigations involving cellular, animal, and clinical experiments are required to shed light on these mechanisms.

Conclusions

Our mediation MR research indicated underlying causal relationships among lipidomes, metabolites, and RA. Specifically, the undecenoylcarnitine(C11:1) pathway mediated a regulatory effect of the lipidomes on RA, providing new insights into the potential clarification of the pathogenesis of RA.

Abbreviations

RA Rheumatoid arthritis

MR Mendelian randomization

IVs Instrumental variables

GWAS Genome-wide association studies

SNPs Single nucleotide polymorphisms

IVW Inverse variance weighting

Declarations

Acknowledgements

The GWAS summary data were obtained from the online public platform (https://gwas.mrcieu.ac.uk/). The analyses of GWAS summary data were performed under application R version 4.3.2.

Authors' contributions

Study design and funding: Chengjiang Wu; data collection and analysis: Xiaojie Cai.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available.

Ethics approval and consent to participate

The GWAS summary data used in this study were all from the online public platform (https://gwas.mrcieu.ac.uk/). The study protocols were approved by respective local ethics committees, and participants have provided written informed consent.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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Figures

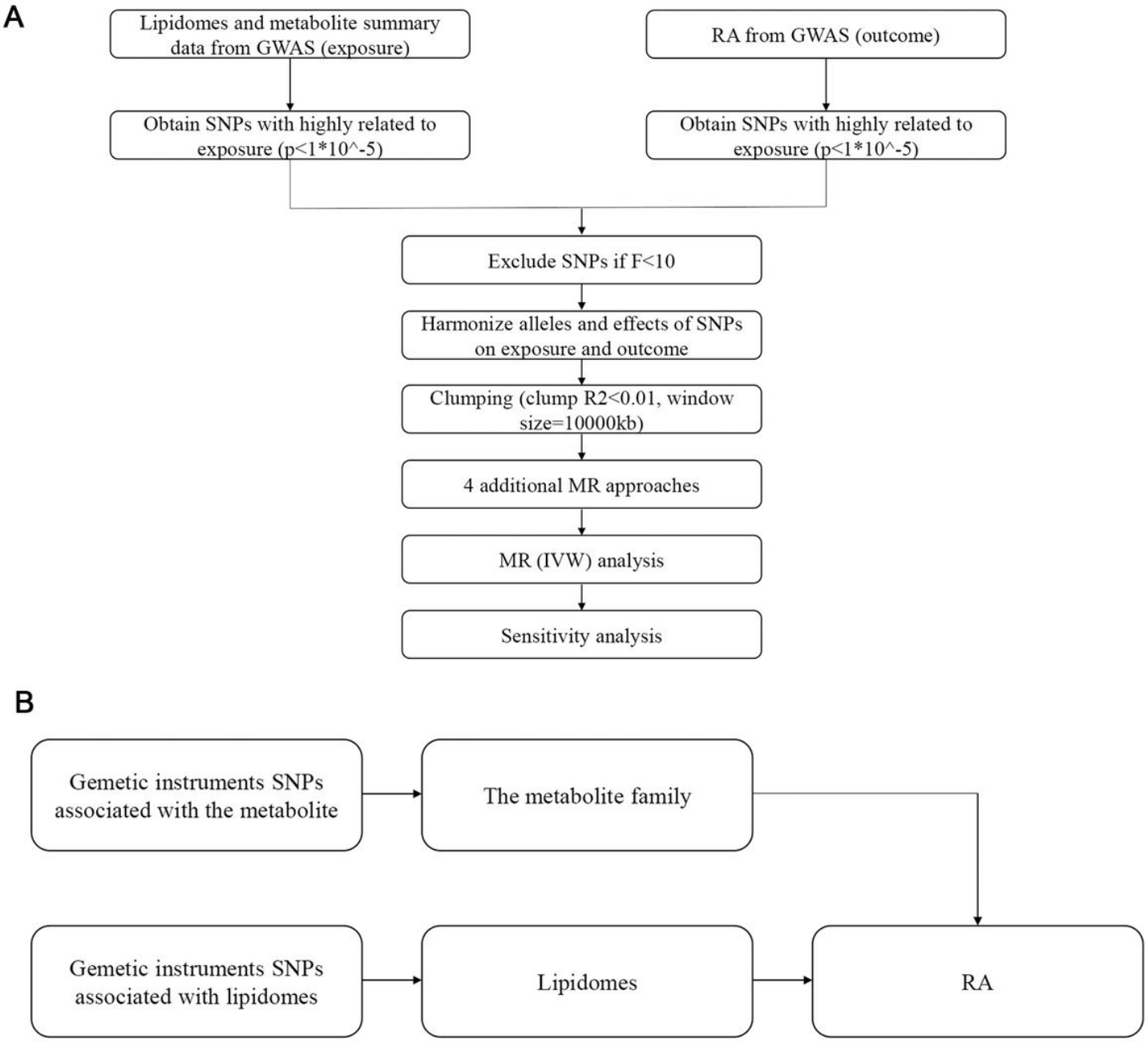


Figure 1

(A) Illustration of the study design and workflow. (B) Two-step Mendelian randomization assessment detailing the impact of lipidomes on rheumatoid arthritis (RA) through metabolites.

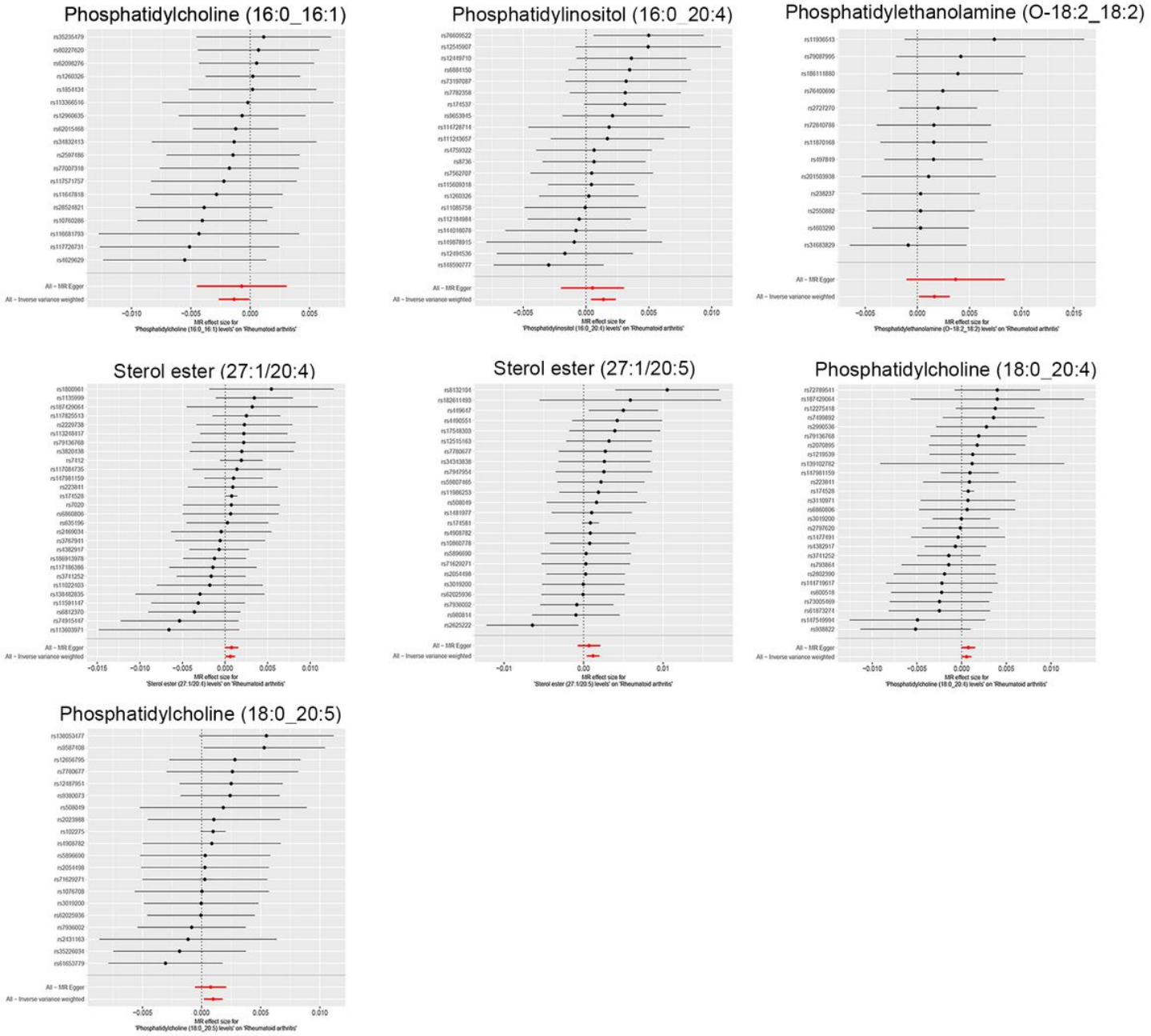


Figure 2

Forest plot representing the causal relationship between lipidomes and RA.

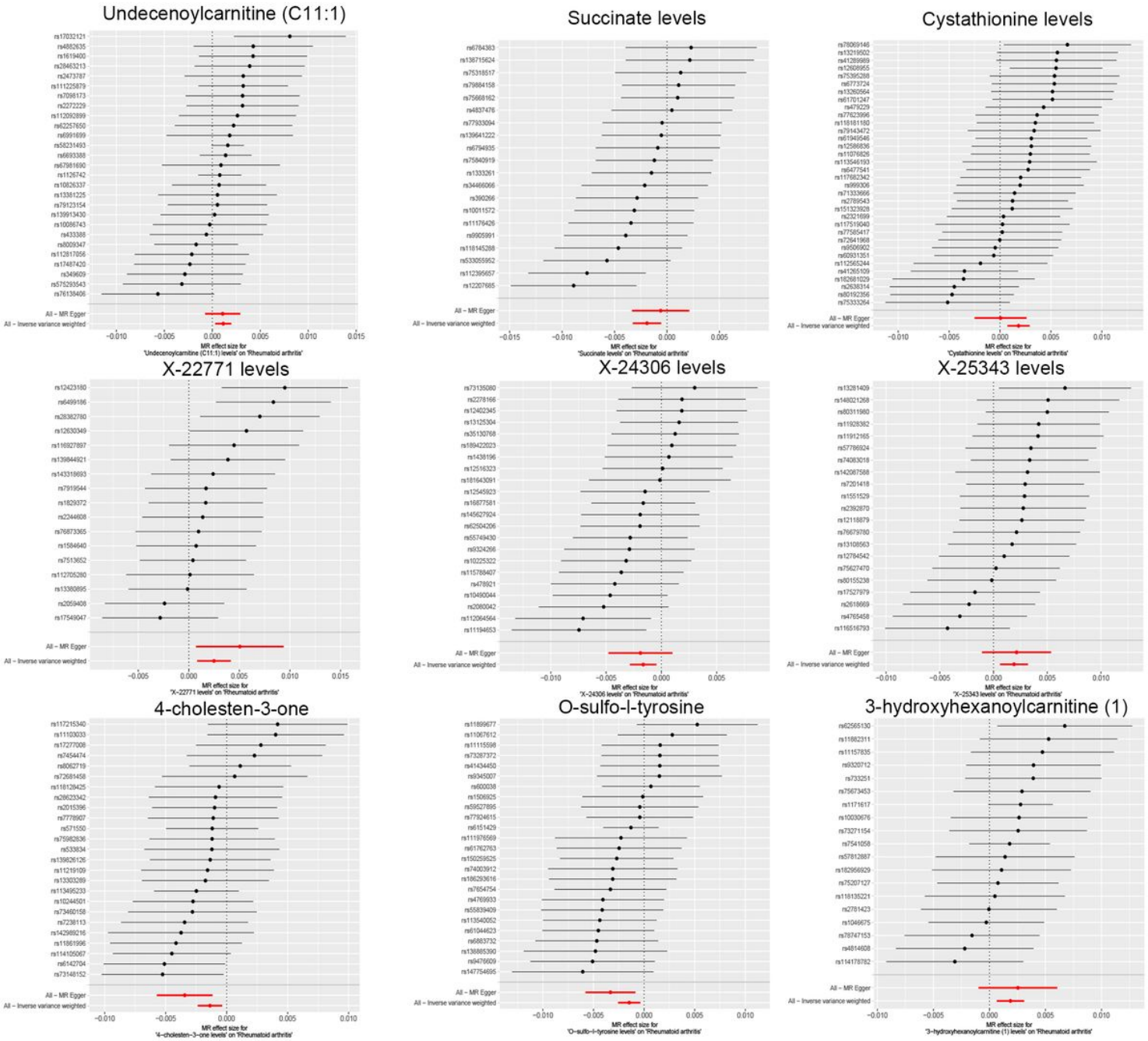
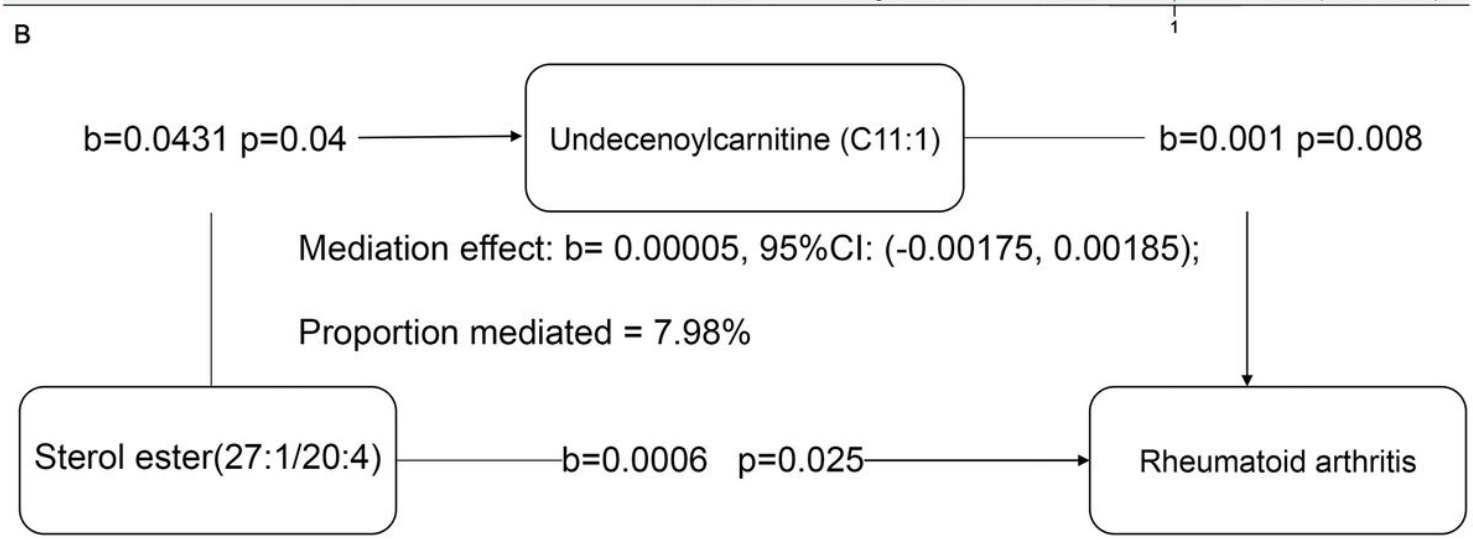


Figure 3

Forest plot highlighting the causal link between the metabolites and RA.

A

exposure	outcome	nsnp	method	pval	OR(95% CI)
Sterol ester (27:1/20:4) levels	Undecenoylcarnitine (C11:1) levels	28	MR Egger	0.354	1.030 (0.968 to 1.096)
		28	Weighted median	0.189	1.033 (0.984 to 1.083)
		28	Inverse variance weighted	**0.043**	1.044 (1.001 to 1.089)
		28	Simple mode	0.152	1.126 (0.962 to 1.318)
		28	Weighted mode	0.154	1.036 (0.988 to 1.086)
Undecenoylcarnitine (C11:1) levels	Rheumatoid arthritis	27	MR Egger	0.258	1.001 (0.999 to 1.003)
		27	Weighted median	**0.019**	1.001 (1.000 to 1.003)
		27	Inverse variance weighted	**0.008**	1.001 (1.000 to 1.002)
		27	Simple mode	0.157	1.002 (0.999 to 1.004)
		27	Weighted mode	0.058	1.001 (1.000 to 1.003)
Sterol ester (27:1/20:4) levels	Rheumatoid arthritis	28	MR Egger	0.073	1.001 (1.000 to 1.002)
		28	Weighted median	**0.023**	1.001 (1.000 to 1.001)
		28	Inverse variance weighted	**0.026**	1.001 (1.000 to 1.001)
		28	Simple mode	0.206	1.001 (0.999 to 1.003)
		28	Weighted mode	**0.028**	1.001 (1.000 to 1.001)



B

Figure 4

(A) Forest plot representing the causal relationship among sterol ester (27:1/20:4), undecenoylcarnitine(C11:1) and RA. (B) Depiction of the role of undecenoylcarnitine(C11:1)in mediating the causal effect of sterol ester (27:1/20:4) on RA.