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Research article

Casual relationships between circulating metabolites and rheumatoid arthritis: A mendelian randomization study

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ABSTRACT

Background: Blood metabolites serve as pivotal indicators in identifying and predicting the course of rheumatoid arthritis (RA). However, empirical substantiation of a direct causal link between these serum biomarkers and the development of RA is still lacking comprehensive support.
Method: In pursuit of a thorough exploration of the causal links between circulating blood metabolites and RA, we deployed a two-sample Mendelian randomization (MR) approach during our initial investigative phase. This method was utilized to examine the potential connections between 249 distinct circulating metabolites and the prevalence of RA. In the validation phase, we conducted replication analyses with a new metabolic dataset consisting of 123 metabolites. Furthermore, we employed the Mendelian randomization based on Bayesian model averaging (MR-BMA) technique to pinpoint key metabolic characteristics that have significant causal implications.
Results: In our primary analysis, we found that acetate, acetoacetate and pyruvate exhibited a consistent protective causal association with rheumatoid arthritis, while lactate demonstrated a positive correlation with rheumatoid arthritis risk. It is also noteworthy that a substantial subset of traits related to both saturated and unsaturated fatty acids showed causal influences. Subsequent secondary analyses substantiated these observations, revealing that traits associated with the average number of methylene groups in a fatty acid chain exhibited protective effects. Ultimately, our MR-BMA analyses unveiled that the ratio of polyunsaturated fatty acids (PUFAs) to total fatty acids assumes a paramount role in increasing the susceptibility to rheumatoid arthritis.
Conclusions: By employing systemic MR analyses, our study has successfully generated an all-encompassing atlas elucidating the intricate connections between circulating metabolites and the susceptibility to rheumatoid arthritis. Our results indicate the high unsaturation degree is a dominant risk factors correlated with rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA), an enduring condition of the immune system, is distinguished by inflammation and discomfort in the

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joints, impacting roughly one in every hundred individuals worldwide [1]. Rheumatoid arthritis can result in permanent cartilage and bone damage, ultimately impairing patients' quality of life in the absence of intervention. While the precise etiology of RA remains elusive, genetic and environmental factors are believed to contribute significantly to its onset and progression [2]. In individuals afflicted with RA, the composition of the gut microbiota and the associated metabolic profiles exhibit significant alterations, with a complex interplay between these two entities [3]. Patients with RA frequently exhibit metabolic abnormalities, including insulin resistance and dyslipidemia, which can contribute to an elevated susceptibility to cardiovascular complications and mortality [4]. The metabolic perturbations detected in RA patients are hypothesized to be linked to underlying pathogenic processes, potentially elucidating the intricate interplay between genetic predispositions and environmental triggers in the etiology of inflammatory conditions [5].

Metabolomic strategies have led to notable advancements, particularly in pinpointing biomarkers that delineate clinical subsets, assess risk factors, and forecast therapeutic outcomes for those afflicted with rheumatoid arthritis. Carlson et al. have reported the identification of 30 metabolites, including putative biomarkers for RA, such as various phospholipids, diol, docosahexaenoic acid methyl ester and linolenic acid [6]. Margarida et al. developed a multivariate diagnostic model that incorporated several metabolites and demographic factors to improve the accuracy of rheumatoid arthritis diagnosis. Specifically, the predictive model incorporated demographic variables such as age and gender, alongside metabolic markers including the serum concentrations of alanine, succinate, and creatine phosphate. The model achieved a high level of diagnostic accuracy, with an area under the curve (AUC) of 84.5 % [7]. Ilona et al. have recently reported that plasma GlycA and GlycB hold promise as potential biomarkers for assessing treatment efficacy in patients with rheumatoid arthritis. These findings suggest that measuring GlycA and GlycB levels could provide clinicians with a valuable tool for monitoring RA disease progression and response to treatment [8]. Several promising metabolite biomarkers for the

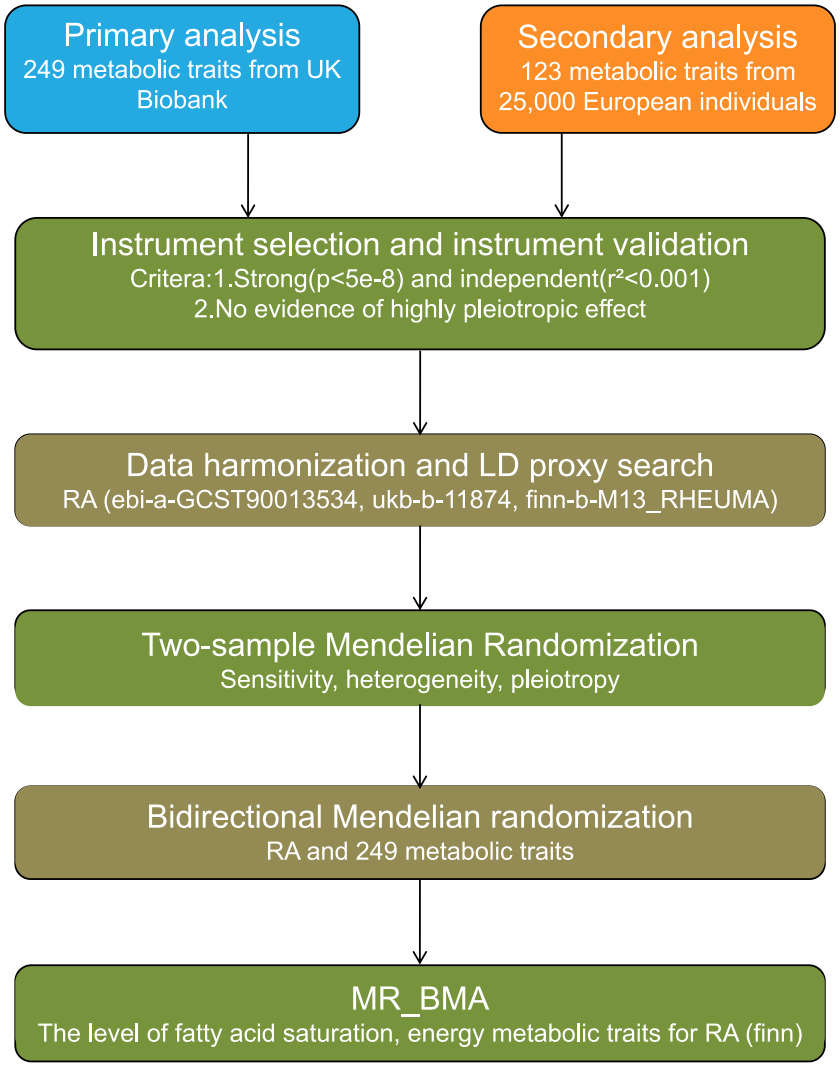

Fig. 1. Schematic illustration of the study design. LD linkage disequilibrium, RA rheumatoid arthritis, MR_BMA Mendelian randomization based on Bayesian model averaging.

diagnosis of RA have been identified by Hemi et al. These biomarkers include acyl carnitines, aspartyl-phenylalanine, and pipecolic acid [9]. However, it is important to note that metabolite biomarkers may be influenced by confounding factors such as diet, medication use, comorbidities, smoking, and exercise [10]. Therefore, it is crucial to conduct systematic epidemiological evaluations to demonstrate the causal relationship of these metabolites on rheumatoid arthritis.

Mendelian randomization (MR) is an emerging statistical technique, designed to disentangle the causal nexus between exposure and outcome by leveraging genetic instrumental variables (IVs) that are associated with the exposure of interest [11]. The method leverages genetic variants as instrumental variables, thereby circumventing inherent limitations of conventional observational studies and furnishing more robust evidence for causal inference [12]. Recently, the implementation of large-scale genome-wide association study (GWAS) analyses has unveiled a promising approach for scrutinizing the genetic landscape of rheumatoid arthritis and identifying potential causal metabolites [13]. Although several MR studies have described causal relationships between certain metabolic risk factors, including total cholesterol levels [14,15], hexadecanedioate [16], monounsaturated fatty acids (MUFAs) [17], omega-3 fatty acids [18] and rheumatoid arthritis, the current body of research still presents a dearth of comprehensive data correlating blood metabolites with the risk of rheumatoid arthritis. Here, we performed a comprehensiveMR analysis to assess the causal effects of hundreds of circulating metabolites on the risk of rheumatoid arthritis in multiple European GWAS cohorts. Our results provide compelling evidence that the high degree of unsaturation is a significant risk factor of developing rheumatoid arthritis.

2. Method

2.1. Design of the study

Our study assessed the causal impact of circulating metabolites on the risk of developing RA using two-sample MR and Mendelian randomization based on Bayesian model averaging (MR-BMA) [19]. Fig. 1 presents a schematic overview of our study design, delineating the analytical framework. The primary and secondary analyses were conducted utilizing two distinct metabolic datasets. First, we procured the GWAS summary datasets of 249 circulating metabolites from the UK Biobank. In order to further confirm causality, we investigated 123 circulating metabolites [20].We obtained the RA GWAS datasets from the UK Biobank, FinnGen Consortium Round 5, and a recent publication by Eunji [21]. Our study examined potential bidirectional associations between RA and metabolic traits using two-sample MR analyses. Considering the substantial correlation among metabolites within the same subcategory, the MR-BMA approach was employed to prioritize the key metabolites. We restricted our analyses to individuals of European ancestry.

2.2. Metabolic profile for primary analyses

We used 249 circulating metabolites from the Nightingale Health Metabolic Biomarkers Phase 1 release study in UK Biobank (June 2019–April 2020) for the primary analysis (Table 1). A random sample of 115,078 participants was selected for this study. NMR high-throughput metabolic profiling was conducted using nonfasting baseline plasma collected in an EDTA solution. A total of two NMR spectra were recorded: one for proteins and lipids within lipoprotein particles and a second for low-molecular weight metabolites. 168 human metabolites were measured in absolute concentrations, while 81 were presented as ratios in the NMR profiling (https://biobank.ndph.ox.ac.uk/ukb/label.cgi?id=220) [22].

The comprehensive summary statistics, incorporating over 12.3 million SNPs and adjusted through the BOLT-LMM [23] to account for demographic composition as well as covariates such as age, gender, fasting status, and genetic array data, are accessible within the IEU open GWAS project.

2.3. Metabolic profile for secondary analyses

We used summary datasets of 123 blood metabolites quantified with high-throughput NMR technology by Kuttunen [20] for validation (Table 1). This study encompassed 24,925 European ancestry individuals from 10 cohorts, analyzing over 12,133,295 SNPs with adjustments for age, sex, post-prandial timing, and the initial ten principal components within a fixed-effect meta-analytic

Table 1
Detailed information of included data sources.

Traits	Sample Size	Year	Population	PubMed ID	Web source
249 Circulating metabolites	115,078	2020	European	NA	https://www.ukbiobank.ac.uk/
123 Circulating metabolites	24,925	2016	European	27005778	http://www.computationalmedicine.fi/data/NMR_GWAS/
Diagnoses - secondary ICD10: M06.99 Rheumatoid arthritis	463,010	2018	European	NA	https://gwas.mrcieu.ac.uk/datasets/ukb-b-11874/
Rheumatoid arthritis	58,284	2020	European	33310728	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90013534/
Rheumatoid arthritis (M13_RHEUMA)	153,457	2021	European	NA	https://gwas.mrcieu.ac.uk/datasets/finn-b-M13_RHEUMA/

framework, with comprehensive summary statistics accessible through the IEU open GWAS project.

2.4. The IV selection

In the selection of Single nucleotide polymorphisms (SNPs) associated with metabolite markers within the GWAS datasets, a stringent significance threshold of $p < 5 \times 10^{-8}$ was applied. To exclude SNPs in linkage disequilibrium (LD), an LD clumping process was executed. The LD was ascertained by an R2 value exceeding 0.001 or by the proximity of SNPs within a 10,000 kilobase (kb) range, aligned with the 1000 Genomes Project's reference data for European ancestries. The presence of weak instruments was tested by computing mean F-statistics.

2.5. Data sources of rheumatoid arthritis

In this study, we utilized three distinct rheumatoid arthritis summary statistics datasets obtained from UK Biobank, FinnGen Round 5, and Eunji's datasets (Table 1). Specifically, the RA information in FinnGen R5 are concisely summarized in (https://r5.risteys.finngen.fi/phenocode/M13_RHEUMA) as "Rheumatoid arthritis," accompanied by the corresponding ICD numbers (ICD-10:M05/M06, ICD-9:7140A/7140B, ICD-8:712 [1-3]). The GWAS data in this particular dataset comprises of 6236 cases and 147,221 controls. The summary statistics for UK Biobank datasets (5201 cases and 457,732 controls, ICD-10 M06) can be accessed via the open GWAS project under accession number ukb-b-9125. In addition, the summary statistics for Eunji's datasets (14,361 cases and 43,923 controls) are available for download from the open GWAS project under accession number ebi-a-GCST90013534 In FinnGen R5 dataset, SAIGE software (https://github.com/weizhouUMICH/SAIGE/tree/finngen_r5_jk) [24] was deployed to examine 16,962,023 SNPs, incorporating adjustments for gender, age, ten principal components, and the genotyping batch. Meanwhile, for the UK Biobank dataset, the fastGWA-GLMM [25] was applied, taking into account covariates such as age, gender, the interaction between age and gender, and the top twenty principal components. In Eunji's datasets, SNPTEST software [26] based on a probabilistic dosage model was employed with adjustments for 5 principal components (PCs).

2.6. Mendelian randomization

A two-sample MR was used to investigate metabolites' causal effect on RA. We utilized the inverse-variance weighted (IVW) method as the principal analytical strategy for estimating causal effects. When the number of instrumental variables (IVs) was less than or equal to three, we used a fixed-effect IVW; otherwise, we employed a random-effect IVW. IVW analyses used I2, H-statistics and Cochran's Q values to estimate heterogeneity [19]. Furthermore, to ascertain the robustness of our findings, we performed sensitivity analyses employing the MR-Egger regression and the weighted median approach. The potential pleiotropy effects were detected by MR-Egger method according to the p-value of intercept. The weighted median approach was employed in instances where the MR analysis was influenced by a significant proportion of invalid instrumental variables contributing to half of the analysis weight [27]. We calculated F-statistics using the approximation method introduced by HongWu et al. [28]. We defined IVs with F-statistics exceeding a threshold of 10 as valid for our analysis, ensuring a strong first-stage regression. To investigate the potential bidirectional effects between metabolic traits and RA, we conducted bidirectional MR analyses, with RA as the exposure and metabolic traits as the outcome variables.

2.7. MR-BMA

Owing to the pronounced interrelationships among diverse metabolic traits, which often involve a substantial number of shared genetic variants, it is crucial to adjust for the influence of "measured pleiotropy" to ensure accurate analysis. To this end, we conducted a follow-up analysis to assess the causal effects of metabolic biomarkers on RA using MR-BMA. In the realm of high-dimensional data analysis, the MR-BMA method stands out as a superior alternative to multivariable MR methods, offering distinct advantages in handling complex datasets. This sophisticated approach facilitates the precise pinpointing of metabolic biomarkers with substantial implications for rheumatoid arthritis. Specifically, We amalgamated SNPs linked to the ensemble of chosen biomarkers and engaged rigorous clumping procedures to sift out those in LD, following the same criteria as used for the instrumental variable (IV) selection. To prioritize the traits based on their significance, we employed the posterior probability (PP) and the marginal inclusion probability (MIP), ranking them in descending order of importance. Furthermore, we determined the model-averaged causal estimate (MACE) to encapsulate the overarching influence of each metabolic trait on the outcomes. Employing Q statistics alongside Cook's distance, we discerned and flagged aberrant instruments as outliers. Post-exclusion of these SNP outliers, we repeated the processes, yielding optimal estimates inclusive of posterior probabilities, marginal inclusion probabilities, causal estimates, and the MACE.

2.8. Statistical analyses

The statistical significance of the MR results was determined based on a 2-sided p value threshold of <0.05. In order to mitigate the potential loss of valid exposures resulting from multiple testing corrections, we refrained from implementing such corrections, given the lack of independence among many of the traits. And we selected unsaturation and energy metabolite related traits as the input for MR-BMA. All analyses were conducted using the R platform (version 4.0.2), with statistical analyses and data visualizations performed using the "TwoSampleMR" (0.5.5) and "ggplot2" (3.4.1) packages [29]. The R-code used for MR-BMA was deposited in github

(https://github.com/verena-zuber/demo_ AMD). Please note that the use of a 2-sided p value threshold of <0.05 without multiple testing corrections may increase the risk of false positives.

3. Result

3.1. Primary analysis

In our primary analysis, a total of 249 metabolites were used for univariable MR. Our findings indicate that energy metabolites exhibit significant associations when using various MR methods. Besides lactate (odds ratio [OR] 1.005, 95 % confidence interval [CI] 1.002–1.008, p = 0.0003) is positive correlated with RA, pyruvate (OR 0.849, 95 % CI 0.731–0.986, p = 0.032), glucose (OR 0.795, 95 % CI 0.636–0.994, p = 0.044), acetoacetate (OR 0.660, 95 % CI 0.492–0.886, p = 0.006) and acetate (OR 0.440, 95 % CI 0.205–0.945, p = 0.035) demonstrated a strong negative casual association with the risk of RA (Fig. 2, Supplementary Table S1). Notably, glycine demonstrated universally inverse associations with the risk of RA (OR 0.931, 95 % CI 0.881–0.984, p = 0.012) (Supplementary Fig. S1) among all three MR methods, which can reduce FLS proliferation and inflammatory infiltration in RA by inducing ferroptosis [30].

Furthermore, a number of fatty acid saturation traits were positively or negatively correlated with RA risk (Fig. 3, Supplementary Table S1). The ratios of polyunsaturated fatty acids (PUFAs) to total fatty acids (OR 1.133, 95 % CI 1.022–1.257, p = 0.018), omega−3 fatty acids to total fatty acids (OR 1.066, 95 % CI 1.003–1.134, p = 0.040), docosahexaenoic acid to total fatty acids (OR 1.111, 95 % CI 1.019–1.213, p = 0.017) and the degree of unsaturation (OR 1.096, 95 % CI 1.033–1.162, p = 0.002) were positively associated with RA. Conversely, the ratios of MUFAs to total fatty acids (OR 0.889, 95 % CI 0.804–0.981, p = 0.02), MUFAs (OR 0.867, 95 % CI 0.778–0.966, p = 0.01) and linoleic acid to total fatty acids (OR 0.885, 95 % CI 0.787–0.994, p = 0.04) were indicative of negative associations using weighted median analysis.

Supplementary Figs. S1–S10 offer a visual tapestry through heatmaps, illustrating the causal estimates for a spectrum of metabolic traits in relation to rheumatoid arthritis. Accompanying these are Supplementary Tables S1–S5, which delve into the numerical underpinnings of these associations, detailing the effect sizes, the per-instrument causal influences, assessments of heterogeneity, and the outcomes of pleiotropic testing. Supplementary Table S6 provided detailed information on the utilized SNPs.

Reverse MR is used to assess whether 249 metabolic biomarkers could be causally affected by RA. Our findings revealed positive associations between RA and multiple traits related to total lipids, the ratio of docosahexaenoic acid to total fatty acids, and triglycerides, particularly in Glycoprotein acetyls (p = 2.2e-4). It is worth noting the significant negative relationship between RA and histidine across different cohorts when using various MR methods (p = 6.5e-06) (Supplementary Table S17).

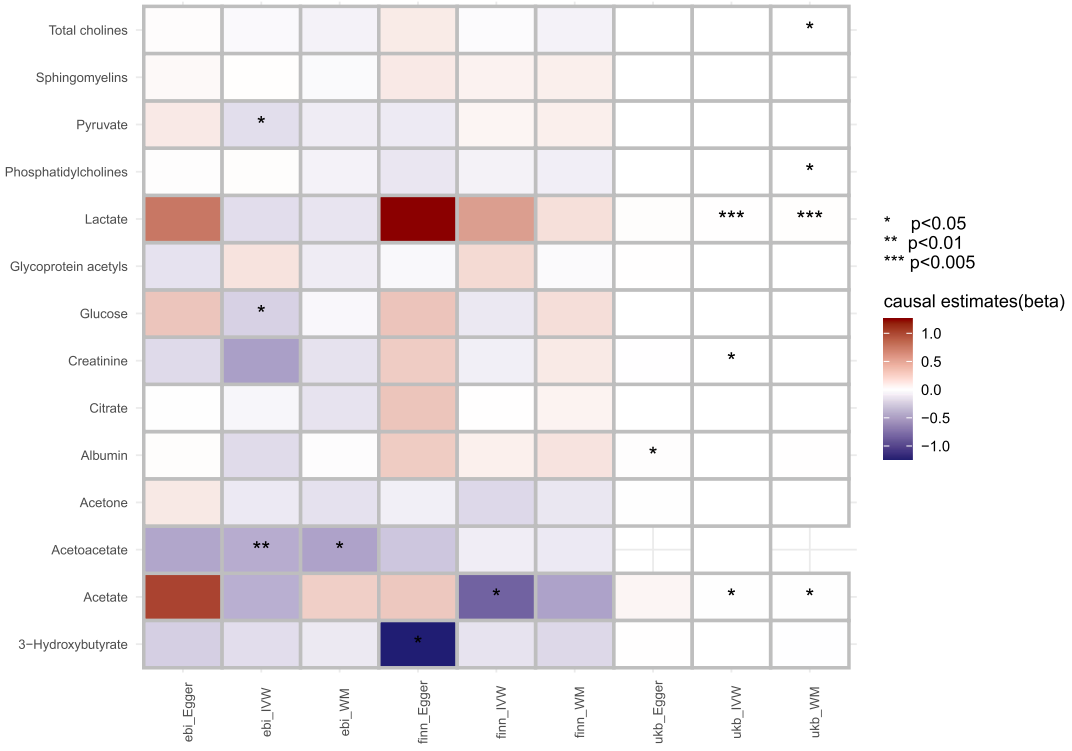

Fig. 2. Heatmap showing the causal estimates of energy metabolic traits on rheumatoid arthritis in the primary analyses with IVW, MR-Egger, and weighted median methods. MR, Mendelian randomization; IVW, inverse-variance weighted; WM, weighted median; Egger, MR-Egger; ebi, ebi-a-GCST90013534; finn, FinnGen Release 5; ukb, UK Biobank.

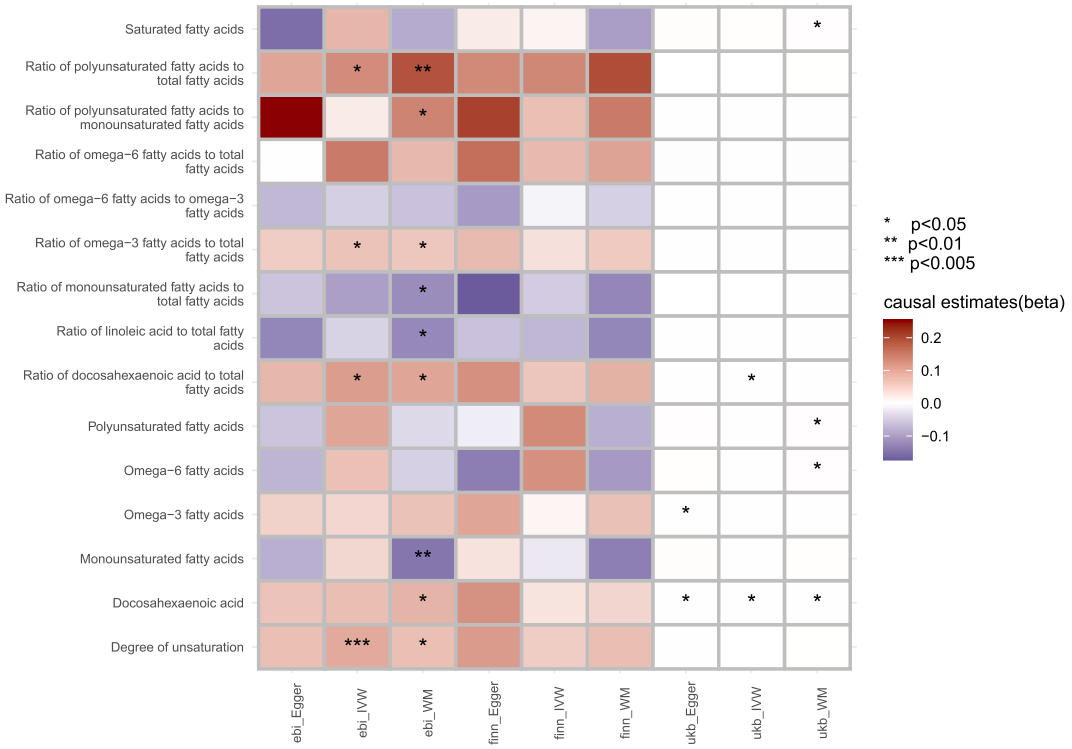

Fig. 3. Heatmap showing the causal estimates of traits related with fatty acid (un)saturation in met-d on rheumatoid arthritis in the primary analyses with IVW, MR-Egger, and weighted median methods. MR, Mendelian randomization; IVW, inverse-variance weighted; WM, weighted median; Egger, MR-Egger; ebi, ebi-a-GCST90013534; finn, FinnGen Release 5; ukb, UK Biobank.

3.2. Secondary analysis

We performed MR analyses using 123 metabolic biomarkers and observed that RA risk is inversely associated with traits related to energy metabolites, such as acetoacetate (OR 0.688, 95 % CI 0.524–0.903, p = 0.007) (Supplementary Table S7). Interestingly, we also found that traits indicating a low degree of unsaturation, such as the average number of methylene groups per double bond (OR 0.919, 95 % CI 0.860–0.982, p = 0.012) and the average number of methylene groups in a fatty acid chain (OR 0.905, 95 % CI 0.826–0.991, p = 0.031) were protective, while traits suggesting increased fatty acid saturation, such as the average number of double bonds in a fatty acid chain (OR 1.088, 95 % CI 1.011–1.171, p = 0.024), the ratio of bisallylic groups to total fatty acids (OR 1.083, 95 % CI 1.018–1.151, p = 0.011), and the ratio of bisallylic groups to double bonds (OR 1.080, 95 % CI 1.018–1.147, p = 0.010) (Fig. 4, Supplementary Table S7), demonstrated a positive correlation with the risk of RA. Details of the estimates, information of used SNPs, pleiotropic and heterogeneity testing are presented in Supplementary Tables S7–12.

3.3. MR-BMA analysis to identify leading traits on RA

We deployed the MR-BMA methodology to the realms of energy metabolism and the spectrum of (un)saturated fatty acids, leveraging the SNPs identified in our preliminary analyses. For the pivotal application of MR-BMA, we turned to the FinnGen R5 dataset, chosen for its extensive compilation of case and control subjects, thereby fortifying the robustness of our outcomes.

For the (un)saturated fatty acid category, we identified 71 SNPs for RA (Supplementary Table 13). After correcting for outliers, 2 SNPs (rs28752924, ATXN2 gene) was removed. We observed the ratio of PUFAs to total fatty acids and the ratio of omega-6 fatty acids to total fatty acids are the leading factors of RA (Table 2, Supplementary Table S15).

For the energy metabolic category, we identified 88 SNPs for RA (Supplementary Table 14). In the following analysis, a single SNP in NFATC1 gene was removed as an outlier. Acetate and glucose showed protective effects on RA risk (Table 2, Supplementary Table S16).

4. Discussion

In the present study, we utilized MR methodology to conduct a comprehensive analysis of metabolic measurements, which yielded compelling evidence of a robust causal relationship between metabolites and rheumatoid arthritis. Notably, we observed a negative correlation between a low degree of unsaturation and RA. Our MR-BMA analysis identified acetate and glucose as protective factors

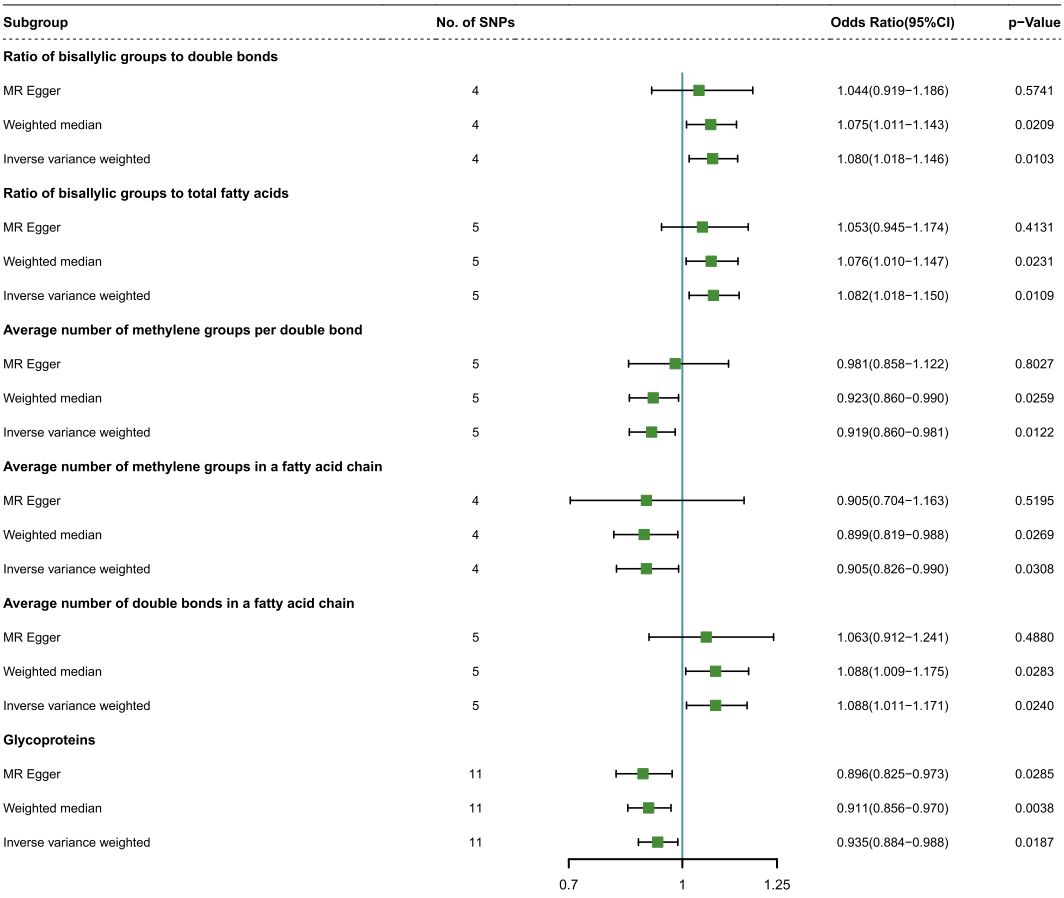

Fig. 4. Forest plots showing the causal estimates of seven metabolic traits that are significantly associated with RA in the secondary analyses with IVW, MR-Egger, and weighted median methods. MR, Mendelian randomization; IVW, inverse-variance weighted, No., number; SNP, single-nucleotide polymorphism; CI, confidence interval.

Table 2
Ranking of the metabolic traits for rheumatoid arthritis using MR-BMA.

Risk factor or model	Ranking by MIP	MIP	MACE	PP	Causal estimates	p-value
Unsaturated fatty acids						
Model averaging using 71 SNPs						
Ratio of saturated fatty acids to total fatty acids	1	0.137	−0.041	0.04	0.021	0.393
Ratio of linoleic acid to total fatty acids	2	0.088	0.005	0.056	0.069	0.832
Ratio of docosahexaenoic acid to total fatty acids	3	0.084	0.005	0.056	0.069	0.802
Degree of unsaturation	4	0.079	0.007	0.04	0.021	0.739
Ratio of polyunsaturated fatty acids to total fatty acids	5	0.079	0.008	0.04	0.021	0.778
Model averaging using 69 SNPs (except rs28752924, ATXN2)						
Ratio of polyunsaturated fatty acids to total fatty acids	1	0.139	0.02	0.085	0.15	0.081
Ratio of omega-6 fatty acids to total fatty acids	2	0.118	0.015	0.069	0.128	0.089
Ratio of polyunsaturated fatty acids to monounsaturated fatty acids	3	0.117	0.014	0.07	0.13	0.090
Ratio of monounsaturated fatty acids to total fatty acids	4	0.106	−0.011	0.064	−0.122	0.095
Monounsaturated fatty acids	5	0.091	−0.009	0.052	−0.104	0.107
Energy metabolic						
Model averaging using 88 SNPs						
Acetate	1	0.675	−0.376	0.369	−0.561	0.005
Glucose	2	0.194	−0.059	0.071	−0.331	0.044
Model averaging using 87 SNPs (except NFATC1)						
Acetate	1	0.737	−0.393	0.413	−0.535	0.004
Glucose	2	0.132	−0.032	0.042	−0.271	0.077

MIP marginal inclusion probability, MR-BMA Mendelian randomization based on Bayesian model averaging, PP, posterior probability.

against the risk of RA. Hence, our results underscore the significance of metabolic factors in the pathogenesis and prevention of RA, and provide novel insights for future investigations.

4.1. Energy metabolites and rheumatoid arthritis

Energy metabolites, such as pyruvate, acetate, acetoacetate, butyrate, and glucose, play crucial roles in various metabolic processes, including the tricarboxylic acid cycle, fatty acid synthesis, and glycolysis. Given the promotion of energy generation through anaerobic glycolytic metabolism in rheumatoid arthritis (RA) patients, resulting from enhanced anaerobic catabolism and weakened aerobic oxidation, it is plausible that energy metabolites may exert an influence on the progression of RA by providing energy support.

Besides glucose, pyruvate, acetate, acetoacetate and butyrate belong to short-chain fatty acids(SCFAs). Numerous studies have demonstrated the independent therapeutic potential of SCFAs as an adjunctive treatment for rheumatoid arthritis. The immune system is capable of engaging in a bidirectional dialogue with the indigenous gut microbiota, facilitated predominantly by their metabolic byproducts. In particular, short-chain fatty acids (SCFAs) emerge as pivotal mediators in this intricate crosstalk. Notably, SCFAs, encompassing acetate, propionate, and butyrate, have demonstrated their ability to modulate the maturation and activity of virtually all immune cell subsets within the gut's immune cell repertoire [31]. An earlier research demonstrated that the addition of a diverse array of prebiotic ingredients to the diet resulted in an augmentation of Treg cell populations, an elevation in Th1/Th17 ratios, and an alleviation of symptoms associated with RA, likely due to the promotion of SCFAs production [32]. In an independent study, pretreatment with butyrate, acetate, and propionate in a murine model of collagen-induced arthritis (CIA) was associated with elevated regulatory B cell counts and attenuated arthritic manifestations, implicating a possible prophylactic function [33]. Moreover, in a collagen-induced arthritis (CIA) mouse model, butyrate supplementation significantly reduced the incidence of arthritis, ameliorated symptoms, and mitigated bone erosion [34]. SCFAs have the potential to exert a substantial impact on arthritis suppression through the regulation of Th17 cells [35].

SCFAs have also been used as a potential therapy for RA in animal trials. For instance, the ethyl acetate extract derived from the Tibetan medicine Rhamnella gilgitica demonstrated significant amelioration of type II collagen-induced arthritis in rats by modulating the JAK-STAT signaling pathway [36]. Additionally, administration of Prevotella histicola in humanized mice resulted in protection against arthritis through expansion of Allobaculum and enhancement of butyrate production [37].

4.2. (Un)saturated fatty acids and the risk of rheumatoid arthritis

Although numerous studies have demonstrated PUFAs' beneficial effects on health [38], our findings indicate a different relationship between the degree of unsaturation and rheumatoid arthritis in Europeans.Specifically, MUFAs was negatively correlated with RA, which is consistent with previous research by Lingling Sun [17]. The Mediterranean diet, renowned for its abundance of monounsaturated fats, has demonstrated a capacity to mitigate the progression of RA [39]. In a case-control analysis involving 145 RA patients and 188 controls, Linos et al. demonstrated an inverse and independent association between olive oil consumption and the development of RA [40]. Moreover, a cross-sectional analysis involving 37 individuals with RA demonstrated a significant inverse association between dietary intake of MUFAs and the risk of developing RA [41].The protective influence of MUFAs is presumed to stem from their anti-inflammatory attributes, with oleic acid, an n-9 MUFA, showing comparable anti-inflammatory efficacy to n-3 PUFAs present in fish oils [42].Taken together, these results suggest that increasing the intake of monounsaturated fatty acids through dietary supplementation may represent a potential and cost-effective therapeutic strategy for RA.

Unexpectedly, our results revealed a positive correlation between PUFAs, particularly omega-3 fatty acids, and RA, which is consistent with the findings of Gaizhi Zhu's study [18]. Clinical investigations have provided evidence of the potential of omega-3 in modulating disease activity in inflamed and tender joints [43]. Nonetheless, in a southern European demographic, no noteworthy correlations were detected between omega-3 PUFAs and rheumatoid arthritis (RA) [44], and supplementation with omega-3 PUFAs failed to manifest substantial benefits in a cohort of Korean RA patients [45]. The primary mechanism by which omega-3 PUFAs exert their effects is through the regulation of Th1 to Th2 polarization, modulating immune responses [46]. Recent research has highlighted the pathogenic role of CXCR3+Th2 cells in synovial inflammation associated with RA [47]. IL-10-expressing Th2 cells have been implicated in antibody production in RA [48], and elevated antibody levels increase the risk of RA development [49]. Thus, elevated omega-3 levels can potentially contribute to Th2-mediated RA.

Ultimately, our bidirectional MR analysis uncovered an inverse relationship between RA susceptibility and specific metabolic markers, most notably the ratio of docosahexaenoic acid to total fatty acids, while no consistent linkages were observed with other fatty acid traits, whether saturated or unsaturated. Significantly, these findings present the initial evidence of potential bidirectional causal associations between metabolic traits (such as triglycerides in IDL and free cholesterol to total lipids ratio in large VLDL) and suggest the existence of a vicious cycle associated with higher docosahexaenoic acid levels and an increased risk of RA. Accordingly, reducing the intake of docosahexaenoic acid could emerge as an economically viable strategy for the initial prevention of rheumatoid arthritis.

4.3. Interconnected metabolic network associated with rheumatoid arthritis

In the pathogenesis and progression of RA, pro-inflammatory effects and the abnormal proliferation of immune cells impose substantial metabolic demands on T cells. As a result, blood metabolites may play a crucial role in understanding RA. The inflamed joints in RA are characterized by heightened metabolic activity and elevated energy requirements. Glucose and fatty acids serve as the

primary energy sources for T cells. Glucose is metabolized to pyruvate through glycolysis, which under hypoxic conditions is further converted to lactate by lactate dehydrogenase (LDH). Accumulation of lactate in the inflamed joints of RA patients has been reported, and it is known to exacerbate inflammatory responses [50]. Under aerobic conditions, pyruvate is transformed into acetate via the routes of reactive oxygen species (ROS) and ketoglutarate dehydrogenase (KDH), and subsequently into acetyl-CoA by acyl-CoA synthetase short-chain family member 2 (ACSS), entering the tricarboxylic acid (TCA) cycle for energy metabolism [51]. Metabolites from the aforementioned processes are negatively correlated with RA, suggesting that aerobic metabolic pathways are conducive to anti-inflammatory effects. Notably, ROS have been demonstrated to be associated with anti-inflammatory immune responses in RA, activating regulatory T cells (Tregs) and inhibiting inflammation [52]. Additionally, acetoacetate and butyrate are engaged in ketogenesis to address situations of glucose and energy scarcity. Monounsaturated fatty acids (MUFA) can be produced through a lipid synthesis process based on acetyl-CoA, while linoleic acid, an essential fatty acid, is exogenously acquired and can be metabolized into PUFA. These fatty acids collectively participate in energy metabolism through β-oxidation [53] (Fig. 5). With the exception of PUFA, all other processes are negatively correlated with RA, indicating that an ample supply of energy substrates and effective oxidative metabolism in the blood are beneficial for anti-inflammatory effects, thereby alleviating RA.

4.4. Sensitivity analyses

No statistically significant pleiotropic effects were identified within the examined exposures, even amidst considerable heterogeneity observed among the metabolic traits. This variability is not unexpected, given the utilization of around 50 instrumental variables (IVs) for each trait in the two-sample MR analysis. Heterogeneity observed suggests that different SNPs have varying causal effects, which is consistent with the involvement of various enzymes associated with lipid metabolic pathways. To estimate causality, we employed the random-effects model within the IVW method.

4.5. Limitations

Firstly, the utilization of summary-level data on blood metabolites in this Mendelian randomization (MR) study may limit the ability to fully capture the complex etiology of rheumatoid arthritis (RA), which is primarily driven by autoimmune disorders. Therefore, further investigations analyzing changes in metabolites specifically in synovial fluid are warranted to uncover additional biomarkers and drug targets for RA.

Secondly, it is crucial to note that the study predominantly enrolled individuals of European ancestry, mitigating population stratification bias but potentially limiting the generalizability of the findings to diverse ethnic populations. Future studies conducted in non-European populations are necessary to validate and extend the applicability of these results.

Lastly, there is a possibility of participant overlap between the GWAS used in this MR study, which could introduce weak instrument bias. Although the F-statistics provided no indication of instrument bias in this analysis, further MR studies using non-

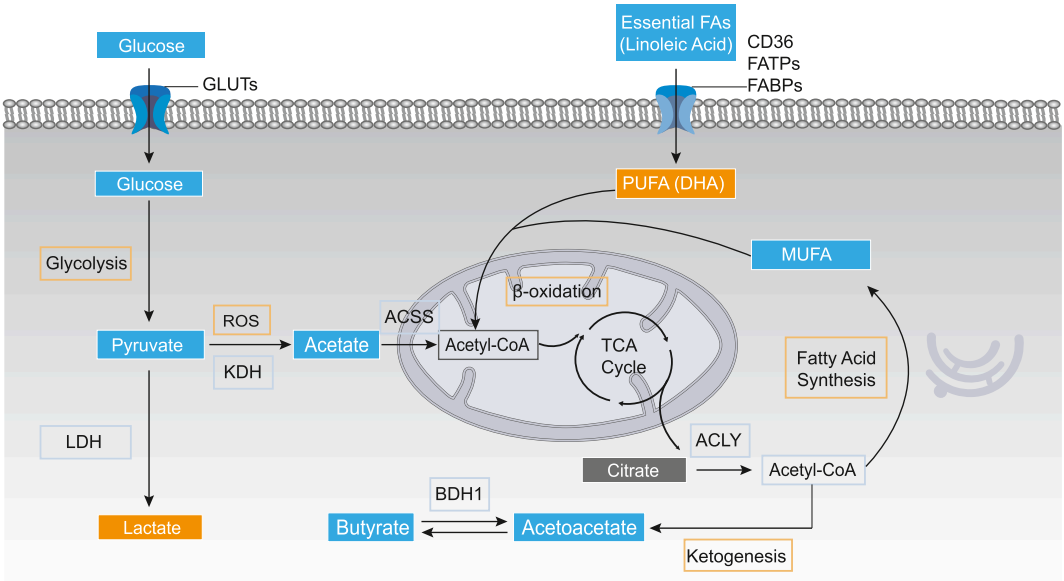

Fig. 5. Diagrammtic of interconnection of all the metabolic alterations related to RA. Blue squares represent metabolites with a negative causal association with RA and orange squares indicate metabolites with a positive causal association with RA. GLUTs, glucose transporters; LDH, Lactate dehydrogenase; ROS, Reactive oxygen species; KDH, Keto acid dehydrogenase; ACSS, Acyl-CoA Synthetase Short Chain Family; TCA, tricarboxylic acid cycle; CoA, coenzyme A; BDH1, 3-Hydroxybutyrate Dehydrogenase 1; FATPs, Fatty Acid Transport Proteins; FABPs, Fatty Acid-Binding Proteins; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acid; DHA, Docosahexaenoic Acid.

overlapping cohorts are essential for a more robust elucidation of the contribution of blood metabolites to the pathogenesis of RA.

5. Conclusion

Our extensive MR study has brought to light that acetate, along with a reduction in fatty acid unsaturation, exerts causal effects that mitigate the risk of RA. Consequently, the supplementation of diets with monounsaturated fatty acids, exemplified by oleic acid, could be considered a promising preventative approach for European populations at risk of RA.

Data availability statement

Access to the GWAS dataset's summary statistics for metabolic traits is facilitated through the IEU open GWAS project (https://gwas.mrcieu.ac.uk/datasets/) or the MR-Base (https://www. mrbase.org/), utilizing the accession IDs met-d and met-c. For the RA datasets, summary-level results are procurable from FinnGen Round 5 (https://r5.finngen.fi/). The UK Biobank datasets yield their summary statistics through the open GWAS project, accessible via the designated accession numbers ukb-b-9125 (https://gwas.mrcieu.ac.uk/datasets/ukb-b-9125/). Similarly, Eunji's datasets are available through the open GWAS project, identified by the accession numbers ebi-a-GCST90013534 (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90013534/).

Ethics statement

Ethical oversight and endorsement for this investigation were exempted, given the utilization of data from a public repository. Affirmative consent had been secured from all participants whose data were included in the public database study.

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CRediT authorship contribution statement

Gaole An: Visualization, Methodology, Formal analysis. **Chenghui Zhao:** Writing – original draft, Resources, Investigation, Data curation. **Xiaoye Chen:** Validation, Investigation. **Weidong Wang:** Writing – review & editing, Resources, Project administration, Conceptualization. **Yuwang Bi:** Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33085.

References

[1] M.C. Hochberg, T.D. Spector, Epidemiology of rheumatoid arthritis: update, Epidemiol. Rev. 12 (1990) 247–252.
[2] D.L. Scott, F. Wolfe, T.W. Huizinga, Rheumatoid arthritis, Lancet 376 (2010) 1094–1108.
[3] D. Yu, J. Du, X. Pu, L. Zheng, S. Chen, N. Wang, J. Li, S. Chen, S. Pan, B. Shen, The gut microbiome and metabolites are altered and interrelated in patients with rheumatoid arthritis, Front. Cell. Infect. Microbiol. 11 (2021) 763507.
[4] G. Kerekes, M.T. Nurmohamed, M.A. Gonzalez-Gay, I. Seres, G. Paragh, Z. Kardos, Z. Barath, L. Tamasi, P. Soltesz, Z. Szekanecz, Rheumatoid arthritis and metabolic syndrome, Nat. Rev. Rheumatol. 10 (2014) 691–696.
[5] V.C. Romao, J.E. Fonseca, Etiology and risk factors for rheumatoid arthritis: a state-of-the-art review, Front. Med. 8 (2021) 689698.
[6] A.K. Carlson, R.A. Rawle, C.W. Wallace, E. Adams, M.C. Greenwood, B. Bothner, R.K. June, Global metabolomic profiling of human synovial fluid for rheumatoid arthritis biomarkers, Clin. Exp. Rheumatol. 37 (2019) 393–399.
[7] M. Souto-Carneiro, L. Toth, R. Behnisch, K. Urbach, K.D. Klika, R.A. Carvalho, H.M. Lorenz, Differences in the serum metabolome and lipidome identify potential biomarkers for seronegative rheumatoid arthritis versus psoriatic arthritis, Ann. Rheum. Dis. 79 (2020) 499–506.
[8] I. Dudka, A. Chachaj, A. Sebastian, W. Tanski, H. Stenlund, G. Grobner, A. Szuba, Metabolomic profiling reveals plasma GlycA and GlycB as a potential biomarkers for treatment efficiency in rheumatoid arthritis, J Pharmaceut Biomed 197 (2021).

[9] H.M. Luan, W.J. Gu, H. Li, Z. Wang, L. Lu, M.Y. Ke, J.W. Lu, W.J. Chen, Z.Z. Lan, Y.L. Xiao, J.Y. Xu, Y. Zhang, Z.W. Cai, S.J. Liu, W.Y. Zhang, Serum metabolomic and lipidomic profiling identifies diagnostic biomarkers for seropositive and seronegative rheumatoid arthritis patients, J. Transl. Med. 19 (2021).
[10] R. Coras, J.D. Murillo-Saich, M. Guma, Circulating pro-and anti-inflammatory metabolites and its potential role in rheumatoid arthritis pathogenesis, Cells 9 (2020) 827.
[11] C.A. Emdin, A.V. Khera, S. Kathiresan, Mendelian Randomization, vol 318, Jama-J Am Med Assoc, 2017, pp. 1925–1926.
[12] E. Sanderson, G.D. Smith, F. Windmeijer, J. Bowden, Corrigendum to: an examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings, Int. J. Epidemiol. 49 (2020) 1057.
[13] K. Ishigaki, S. Sakaue, C. Terao, Y. Luo, K. Sonehara, K. Yamaguchi, T. Amariuta, C.L. Too, V.A. Laufer, I.C. Scott, S. Viatte, M. Takahashi, K. Ohmura, A. Murasawa, M. Hashimoto, H. Ito, M. Hammoudeh, S.A. Emadi, B.K. Masri, H. Halabi, H. Badsha, I.W. Uthman, X. Wu, L. Lin, T. Li, D. Plant, A. Barton, G. Orozco, S.M.M. Verstappen, J. Bowes, A.J. MacGregor, S. Honda, M. Koido, K. Tomizuka, Y. Kamatani, H. Tanaka, E. Tanaka, A. Suzuki, Y. Maeda, K. Yamamoto, S. Miyawaki, G. Xie, J. Zhang, C.I. Amos, E. Keystone, G. Wolbink, I. van der Horst-Bruinsma, J. Cui, K.P. Liao, R.J. Carroll, H.S. Lee, S.Y. Bang, K. A. Siminovitch, N. de Vries, L. Alfredsson, S. Rantapaa-Dahlqvist, E.W. Karlson, S.C. Bae, R.P. Kimberly, J.C. Edberg, X. Mariette, T. Huizinga, P. Dieude, M. Schneider, M. Kerick, J.C. Denny, P. BioBank Japan, K. Matsuda, K. Matsuo, T. Mimori, F. Matsuda, K. Fujio, Y. Tanaka, A. Kumanogoh, M. Traylor, C. M. Lewis, S. Eyre, H. Xu, R. Saxena, T. Arayssi, Y. Kochi, K. Ikari, M. Harigai, P.K. Gregersen, K. Yamamoto, S. Louis Bridges Jr., L. Padyukov, J. Martin, L. Klareskog, Y. Okada, S. Raychaudhuri, Multi-ancestry genome-wide association analyses identify novel genetic mechanisms in rheumatoid arthritis, Nat. Genet. 54 (2022) 1640–1651.
[14] G. Zhang, Y. Cai, J. Liang, J. Zhang, Z. Jing, L. Lv, R. Zhang, J. Song, X. Dang, Q. Song, Causal relationships between rheumatism and dyslipidemia: a two-sample Mendelian randomization study, Front. Endocrinol. 13 (2022).
[15] M. Kasher, S.S. Cherny, G. Livshits, C.I.W. Group, Exploring potential shared genetic influences between rheumatoid arthritis and blood lipid levels, Atherosclerosis 363 (2022) 48–56.
[16] X.H. Yu, R.R. Cao, Y.Q. Yang, S.F. Lei, Identification of causal metabolites related to multiple autoimmune diseases, Hum. Mol. Genet. 31 (2022) 604–613.
[17] L. Sun, J. Zhu, S. Mi, Y. Li, T. Wang, Y. Li, Causal association of monounsaturated fatty acids with rheumatoid arthritis but not osteoarthritis: a two-sample Mendelian randomization study, Nutrition 91–92 (2021) 111363.
[18] G. Zhu, S. Zhou, Y. Xu, R. Gao, H. Li, B. Zhai, X. Liu, Y. He, X. Wang, G. Han, W. Su, R. Wang, Mendelian randomization study on the causal effects of omega-3 fatty acids on rheumatoid arthritis, Clin. Rheumatol. 41 (2022) 1305–1312.
[19] V. Zuber, J.M. Colijn, C. Klaver, S. Burgess, Selecting likely causal risk factors from high-throughput experiments using multivariable Mendelian randomization, Nat. Commun. 11 (2020) 29.
[20] J. Kettunen, A. Demirkan, P. Wurtz, H.H. Draisma, T. Haller, R. Rawal, A. Vaarhorst, A.J. Kangas, L.P. Lyytikainen, M. Pirinen, R. Pool, A.P. Sarin, P. Soininen, T. Tukiainen, Q. Wang, M. Tiainen, T. Tynkkynen, N. Amin, T. Zeller, M. Beekman, J. Deelen, K.W. van Dijk, T. Esko, J.J. Hottenga, E.M. van Leeuwen, T. Lehtimaki, E. Mihailov, R.J. Rose, A.J. de Craen, C. Gieger, M. Kahonen, M. Perola, S. Blankenberg, M.J. Savolainen, A. Verhoeven, J. Viikari, G. Willemsen, D.I. Boomsma, C.M. van Duijn, J. Eriksson, A. Jula, M.R. Jarvelin, J. Kaprio, A. Metspalu, O. Raitakari, V. Salomaa, P.E. Slagboom, M. Waldenberger, S. Ripatti, M. Ala-Korpela, Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA, Nat. Commun. 7 (2016) 11122.
[21] E. Ha, S.C. Bae, K. Kim, Large-scale meta-analysis across East Asian and European populations updated genetic architecture and variant-driven biology of rheumatoid arthritis, identifying 11 novel susceptibility loci, Ann. Rheum. Dis. 80 (2021) 558–565.
[22] J. Mi, L. Jiang, Z. Liu, X. Wu, N. Zhao, Y. Wang, X. Bai, Identification of blood metabolites linked to the risk of cholelithiasis: a comprehensive Mendelian randomization study, Hepatol Int 16 (2022) 1484–1493.
[23] P.R. Loh, G. Tucker, B.K. Bulik-Sullivan, B.J. Vilhjalmsson, H.K. Finucane, R.M. Salem, D.I. Chasman, P.M. Ridker, B.M. Neale, B. Berger, N. Patterson, A. L. Price, Efficient Bayesian mixed-model analysis increases association power in large cohorts, Nat. Genet. 47 (2015) 284–290.
[24] W. Zhou, J.B. Nielsen, L.G. Fritsche, R. Dey, M.E. Gabrielsen, B.N. Wolford, J. LeFaive, P. VandeHaar, S.A. Gagliano, A. Gifford, L.A. Bastarache, W.Q. Wei, J. C. Denny, M. Lin, K. Hveem, H.M. Kang, G.R. Abecasis, C.J. Willer, S. Lee, Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies, Nat. Genet. 50 (2018) 1335–1341.
[25] L. Jiang, Z. Zheng, H. Fang, J. Yang, A generalized linear mixed model association tool for biobank-scale data, Nat. Genet. 53 (2021) 1616–1621.
[26] J. Marchini, B. Howie, S. Myers, G. McVean, P. Donnelly, A new multipoint method for genome-wide association studies by imputation of genotypes, Nat. Genet. 39 (2007) 906–913.
[27] M. Verbanck, C.Y. Chen, B. Neale, R. Do, Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases, Nat. Genet. 50 (2018) 693–698.
[28] H. Wu, H. Wang, D. Liu, Z. Liu, W. Zhang, Mendelian randomization analyses of associations between breast cancer and bone mineral density, Sci. Rep. 13 (2023) 1721.
[29] G. Hemani, J. Zheng, B. Elsworth, K.H. Wade, V. Haberland, D. Baird, C. Laurin, S. Burgess, J. Bowden, R. Langdon, V.Y. Tan, J. Yarmolinsky, H.A. Shihab, N. J. Timpson, D.M. Evans, C. Relton, R.M. Martin, G. Davey Smith, T.R. Gaunt, P.C. Haycock, The MR-Base platform supports systematic causal inference across the human phenome, Elife 7 (2018).
[30] H. Ling, M. Li, C. Yang, S. Sun, W. Zhang, L. Zhao, N. Xu, J. Zhang, Y. Shen, X. Zhang, C. Liu, L. Lu, J. Wang, Glycine increased ferroptosis via SAM-mediated GPX4 promoter methylation in rheumatoid arthritis, Rheumatology 61 (2022) 4521–4534.
[31] F. Golpour, M. Abbasi-Alaei, F. Babaei, M. Mirzababaei, S. Parvardeh, G. Mohammadi, M. Nassiri-Asl, Short chain fatty acids, a possible treatment option for autoimmune diseases, Biomed. Pharmacother. 163 (2023) 114763.
[32] J. Hager, H. Bang, M. Hagen, M. Frech, P. Trager, M.V. Sokolova, U. Steffen, K. Tascilar, K. Sarter, G. Schett, J. Rech, M.M. Zaiss, The role of dietary fiber in rheumatoid arthritis patients: a feasibility study, Nutrients 11 (2019).
[33] Y. Yao, X. Cai, Y. Zheng, M. Zhang, W. Fei, D. Sun, M. Zhao, Y. Ye, C. Zheng, Short-chain fatty acids regulate B cells differentiation via the FFA2 receptor to alleviate rheumatoid arthritis, Br. J. Pharmacol. 179 (2022) 4315–4329.
[34] J. He, Y. Chu, J. Li, Q. Meng, Y. Liu, J. Jin, Y. Wang, J. Wang, B. Huang, L. Shi, X. Shi, J. Tian, Y. Zhufeng, R. Feng, W. Xiao, Y. Gan, J. Guo, C. Shao, Y. Su, F. Hu, X. Sun, J. Yu, Y. Kang, Z. Li, Intestinal butyrate-metabolizing species contribute to autoantibody production and bone erosion in rheumatoid arthritis, Sci. Adv. 8 (2022) eabm1511.
[35] H. Evans-Marin, R. Rogier, S.B. Koralov, J. Manasson, D. Roeleveld, P.M. van der Kraan, J.U. Scher, M.I. Koenders, S. Abdollahi-Roodsaz, Microbiota-dependent involvement of Th17 cells in murine models of inflammatory arthritis, Arthritis Rheumatol. 70 (2018) 1971–1983.
[36] J. Su, Q. Li, J. Liu, H. Wang, X. Li, D. Wuntrang, C. Liu, Q. Zhao, Meng X. RuyuYao, Y. Zhang, Ethyl acetate extract of Tibetan medicine Rhamnella gilgitica ameliorated type II collagen-induced arthritis in rats via regulating JAK-STAT signaling pathway, Ethnopharmacol 267 (2021) 113514.
[37] B. Balakrishnan, D. Luckey, R. Bodhke, J. Chen, E. Marietta, P. Jeraldo, J. Murray, V. Taneja, Prevotella histicola protects from arthritis by expansion of Allobaculum and augmenting butyrate production in humanized mice, Front. Immunol. 12 (2021) 609644.
[38] F. Shahidi, P. Ambigaipalan, Omega-3 polyunsaturated fatty acids and their health benefits, Annu. Rev. Food Sci. Technol. 9 (2018) 345–381.
[39] Y. Matsumoto, Y. Sugioka, M. Tada, T. Okano, K. Mamoto, K. Inui, D. Habu, T. Koike, Monounsaturated fatty acids might be key factors in the Mediterranean diet that suppress rheumatoid arthritis disease activity: the TOMORROW study, Clin. Nutr. 37 (2018) 675–680.
[40] A. Linos, V.G. Kaklamani, E. Kaklamani, Y. Koumantaki, E. Giziaki, S. Papazoglou, C.S. Mantzoros, Dietary factors in relation to rheumatoid arthritis: a role for olive oil and cooked vegetables? Am. J. Clin. Nutr. 70 (1999) 1077–1082.
[41] H. Hayashi, K. Satoi, N. Sato-Mito, T. Kaburagi, H. Yoshino, M. Higaki, K. Nishimoto, K. Sato, Nutritional status in relation to adipokines and oxidative stress is associated with disease activity in patients with rheumatoid arthritis, Nutrition 28 (2012) 1109–1114.
[42] M.J. James, R.A. Gibson, M.A. Neumann, L.G. Cleland, Effect of dietary supplementation with n-9 eicosatrienoic acid on leukotriene B4 synthesis in rats: a novel approach to inhibition of eicosanoid synthesis, J. Exp. Med. 178 (1993) 2261–2265.
[43] I. Kostoglou-Athanassiou, L. Athanassiou, P. Athanassiou, The effect of omega-3 fatty acids on rheumatoid arthritis, Mediterr J Rheumatol 31 (2020) 190–194.

[44] P. de Pablo, D. Romaguera, H.L. Fisk, P.C. Calder, A.M. Quirke, A.J. Cartwright, S. Panico, A. Mattiello, D. Gavrila, C. Navarro, C. Sacerdote, P. Vineis, R. Tumino, W.E. Ollier, D.S. Michaud, E. Riboli, P.J. Venables, B.A. Fisher, High erythrocyte levels of the n-6 polyunsaturated fatty acid linoleic acid are associated with lower risk of subsequent rheumatoid arthritis in a southern European nested case-control study, Ann. Rheum. Dis. 77 (2018) 981–987.
[45] Y. Park, A. Lee, S.C. Shim, J.H. Lee, J.Y. Choe, H. Ahn, C.B. Choi, Y.K. Sung, S.C. Bae, Effect of n-3 polyunsaturated fatty acid supplementation in patients with rheumatoid arthritis: a 16-week randomized, double-blind, placebo-controlled, parallel-design multicenter study in Korea, J. Nutr. Biochem. 24 (2013) 1367–1372.
[46] P. Zhang, R. Smith, R.S. Chapkin, D.N. McMurray, Dietary (n-3) polyunsaturated fatty acids modulate murine Th1/Th2 balance toward the Th2 pole by suppression of Th1 development, J. Nutr. 135 (2005) 1745–1751.
[47] J. Aldridge, A.-K.H. Ekwall, L. Mark, B. Bergström, K. Andersson, I. Gjertsson, A.-C. Lundell, A. Rudin, T helper cells in synovial fluid of patients with rheumatoid arthritis primarily have a Th1 and a CXCR3+ Th2 phenotype, Arthritis Res. Ther. 22 (2020) 1–11.
[48] J. Wang, L. Ma, S. Yang, S. Wang, X. Wei, S. Song, IL-10-Expressing Th2 cells contribute to the elevated antibody production in rheumatoid arthritis, Inflammation 39 (2016) 1017–1024.
[49] C.Y. Wu, H.Y. Yang, J.H. Lai, Anti-citrullinated protein antibodies in patients with rheumatoid arthritis: biological effects and mechanisms of immunopathogenesis, Int. J. Mol. Sci. 21 (2020).
[50] M.M. Hanlon, M. Canavan, B.E. Barker, U. Fearon, Metabolites as drivers and targets in rheumatoid arthritis, Clin. Exp. Immunol. 208 (2021) 167–180.
[51] X. Liu, D.E. Cooper, A.A. Cluntun, M.O. Warmoes, S. Zhao, M.A. Reid, J. Liu, P.J. Lund, M. Lopes, B.A. Garcia, K.E. Wellen, D.G. Kirsch, J.W. Locasale, Acetate production from glucose and coupling to mitochondrial metabolism in mammals, Cell 175 (2018) 502–513 e513.
[52] C.M. Weyand, J.J. Goronzy, Immunometabolism in early and late stages of rheumatoid arthritis, Nat. Rev. Rheumatol. 13 (2017) 291–301.
[53] Y.G. Jeon, Y.Y. Kim, G. Lee, J.B. Kim, Physiological and pathological roles of lipogenesis, Nat. Metab. 5 (2023) 735–759.