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Original Article

Immune-metabolic crosstalk in rheumatoid arthritis: mediation effects of plasma metabolites on inflammatory cytokines

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Abstract: Background: Rheumatoid arthritis (RA) is a chronic inflammatory condition defined by synovitis and progressive joint damage. The connection between inflammatory cytokines, molecules, and RA is still poorly understood despite the information combining both immune dysregulation and metabolic reprogramming to its pathophysiology. Methods: A two-sample Mendelian randomization (MR) analysis was carried out using summary statistics from the largest-scale genome-wide association study (GWAS) to examine the link between 91 inflammatory cytokines and RA risk. Metabolite pathways were identified through mediation analysis. Multiple MR Methods including inverse variance weighting (IVW) were used for causal estimation and the hypothesis was verified by sensitivity analysis. Results: The analysis results showed that there was a causal association between six inflammatory cytokines and RA. Among them, HGF and IL-10RA increased the risk of RA, while CXCL9, EN-RAGE, IFN-γ and CCL8 showed protective effects. These associations were validated using multiple MR methodologies, including inverse-variance weighting, MR-Egger regression, and leave-one-out sensitivity analyses. Further mediation MR analysis revealed seven metabolite-mediated pathways of inflammatory cytokines and RA. Specifically, the protective effect of CXCL9 was achieved in part by reducing plasma eicosapentaenoic acid (EPA; 20:5n3) levels (mediating effect = -0.007; proportion mediated = 8.10%). The protective effect of EN-RAGE was mediated by a decrease in the level of salicyluric glucuronide (mediating effect = -0.015; 12.70%). IFN-γ reduced the risk of RA through two independent metabolic pathways: reducing the level of 1-arachidonoyl-glycerol-3-phosphocholine (20:4n6) levels (mediating effect = -0.005; 4.67%), and increasing the ratio of glycine-to-pyridoxal (mediating effect = -0.020; 19.60%). In contrast, IL-10RA increases the risk of RA by increasing 3,7-dimethylurate (mediating effect = 0.011; 12.50%) and decreasing N, N-dimethylalanine levels (mediating effect = 0.010; 11.70%). The protective effect of CCL8 was mediated by the reduction of specific sphingomyelin species (d17:2/16:0, d18:2/15:0) (mediating effect = -0.012; 12.30%). Conclusions: The results of this study not only confirmed the causal role of specific inflammatory cytokines in the pathogenesis of RA from a genetic perspective, but more importantly, for the first time, systematically revealed the specific plasma metabolite mediating pathways that connect these inflammatory cytokines to RA. These findings more specifically link the immune dysregulation of RA to metabolic remodeling, especially the protective role of IFN-γ through glycine/pyridoxal balance, and the pathway of IL-10RA through purine metabolites to affect risk, providing novel and mechanistic insights into the immunometabolic network of RA. We believe that these specific intermediate metabolites are expected to be potential candidate targets for future targeted therapies or intervention strategies, aiming to precisely regulate the interaction between inflammation and metabolism.

Keywords: Rheumatoid arthritis, inflammation, inflammatory cytokines, metabolites, Mendelian randomization

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by persistent synovitis and progressive joint destruction [1]. In addition to common joint symptoms such as pain, stiffness, swelling, and deformity, RA can lead to systemic complications involving

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the skin, heart, and blood vessels [2, 3], with increasing severity with age [4]. There is growing evidence that the occurrence and progression of RA involves a complex relationship between immune, genetic, and environmental factors [5]. Pro-inflammatory cytokines such as tumor necrosis factor, interleukin-1, and interleukin-6 have been shown to contribute to chronic inflammation and joint damage [6, 7]. In response, damaged tissue undergo metabolic reprogramming, which worsens joint damage and causes widespread effects [8]. A considerable proportion of patients exhibit poor treatment response, which underscores the need to consider novel therapeutic targets, despite biological agents targeted these cytokines [9]. Metabolites are increasingly recognized as possible therapeutic targets and donors to the development of disease as middlemen of biochemical pathways [10]. Although inflammatory cytokines are known to have an effect on metabolite levels, the exact mechanism by which cytokines control RA through metabolic compounds remains unclear. Therefore, elucidating the causal interactions between inflammatory mediators, metabolites, and RA is crucial for improving early detection, prevention, and treatment strategies.

Single-nucleotide polymorphisms (SNPs) as genetic instrumental variables (IVs) are used by Mendelian randomization (MR), a potent method of genetic epidemiology, to establish causal relationships between exposures and outcomes. Compared with observational studies, MR minimizes confounding through the random allocation of genetic variants [11]. Here, we applied a two-sample MR framework to evaluate the effects of 91 circulating inflammatory cytokines on RA risk. Our cytokine selection was informed by a recent proteome-wide GWAS [12], whose broad coverage improves our ability to detect novel risk factors. By integrating metabolomic data, we also explored potential mediating mechanisms, offering a clearer view of how inflammation and metabolism jointly influence RA. We expect these findings to shed light on key immunometabolic pathways in RA and to inform future therapeutic development.

Materials and methods

Study design

The research process is illustrated in the flowchart figure (**Figure 1**). We first obtained publicly available GWAS summary data for circulating inflammatory cytokines, plasma metabolites and RA. Subsequently, two-sample MR analyses were applied to assess the causal relationships between circulating inflammatory cytokines, plasma metabolites and RA. Finally, we used mediated MR (two-step MR) analysis to explore the causal relationship between specific inflammatory factors and RA.

GWAS statistics source

Details of all GWAS summary statistics utilized in this study are provided in **Table 1**. Genetic associations for 91 circulating inflammatory cytokines were obtained from a meta-analysis of 11 cohorts (N = 14,824 individuals of European ancestry), profiled using the Olink Target Inflammation panel (accession numbers: GCST90274758 to GCST90274848) [12]. Summary statistics for 1091 plasma metabolites and 309 metabolite ratios from a total of 8,299 European participants can be accessed from the GWAS database, with the catalog identifiers ranging from GCST90199621 to GCST90201020 [13]. The RA GWAS data included 14,361 cases and 43,923 controls of European descent, sourced from the GWAS database (accession: ebi-a-GCST90013534).

Selection of genetic IVs

Estimating causal effects with genetic variation relies on three key assumptions: (1) Relevance: IVs representing genetic variation are strongly associated with exposure; (2) Independence: genetic variation is not affected by potential confounders; and (3) Exclusivity: genetic variation affects the outcome only through exposure factors and should not directly affect the outcome itself. To ensure the accuracy and reliability of the results, SNPs were rigorously screened. First, a significance threshold with $P < 1 \times 10^{-5}$ was used to detect a sufficient number of IVs associated with exposure factors [14]. Second, SNPs exhibiting linkage disequilibrium were filtered out with criteria of $R^2 = 0.001$ and Kb = 10,000. Then, the F-statistic was calculated for each SNP to estimate its sample overlap effect and weak instrumental bias. Weakly IVs with F-statistic values less than 10 were excluded.

MR analyses and sensitivity analyses

Two-sample MR analyses were employed to explore the causal relationship between circu-

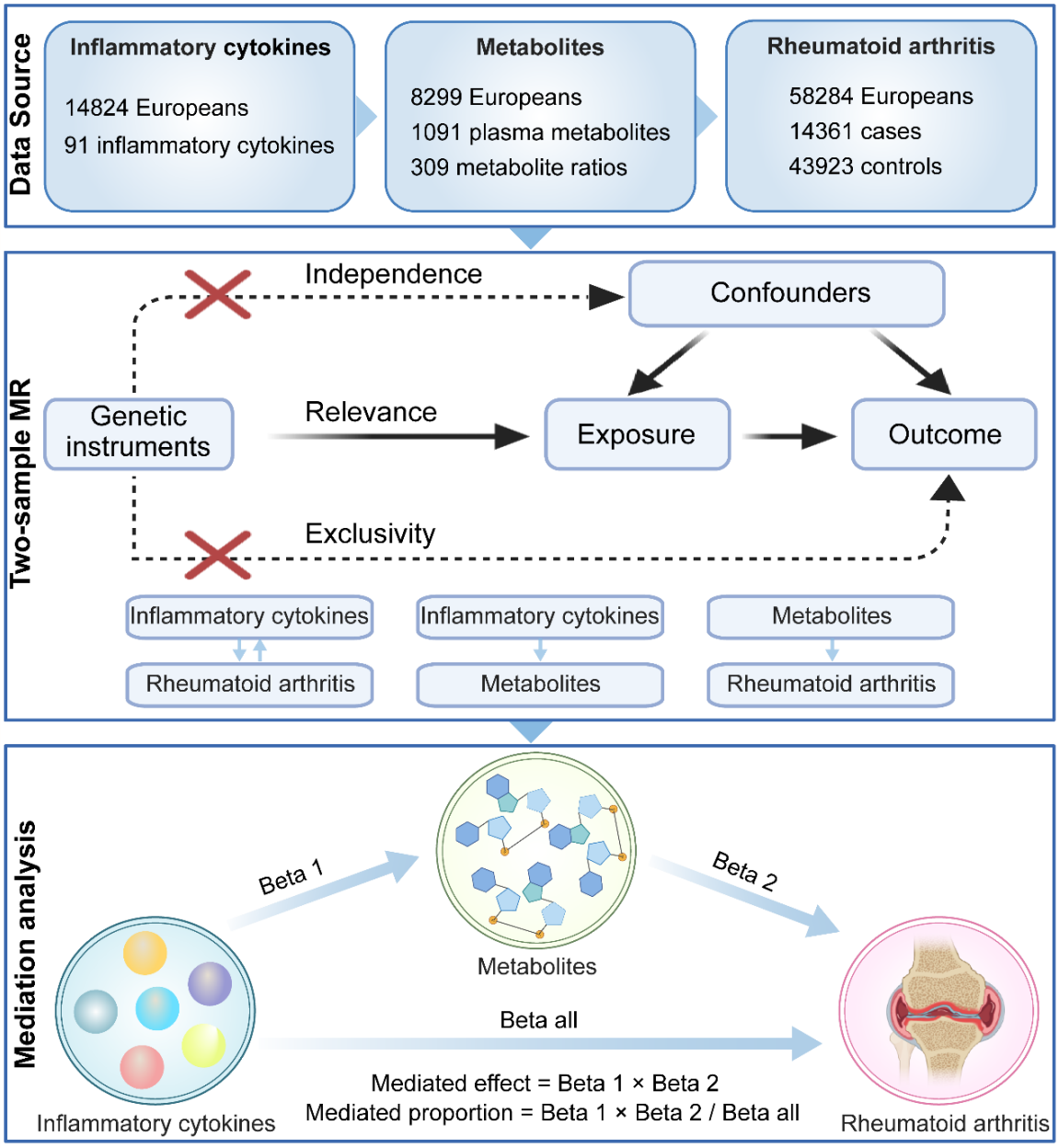

Figure 1. Flow chart of the study.

Table 1. Summary of GWAS datasets used in this study

Traits	Sample size	Ancestry	Accession codes
91 inflammatory cytokines	14,824 participants	European	GCST90274758-GCST90274848
1091 plasma metabolites and 309 plasma metabolite ratios	8,299 participants	European	GCST90199621-GCST90201020
Rheumatoid arthritis	58,284 participants (14,361 cases; 43,923 controls)	European	ebi-a-GCST90013534

lating inflammatory cytokines and RA. In our MR analysis, inflammatory cytokines served as the exposure, RA as the outcome, and SNPs worked as IVs. Inverse variance weighted (IVW)

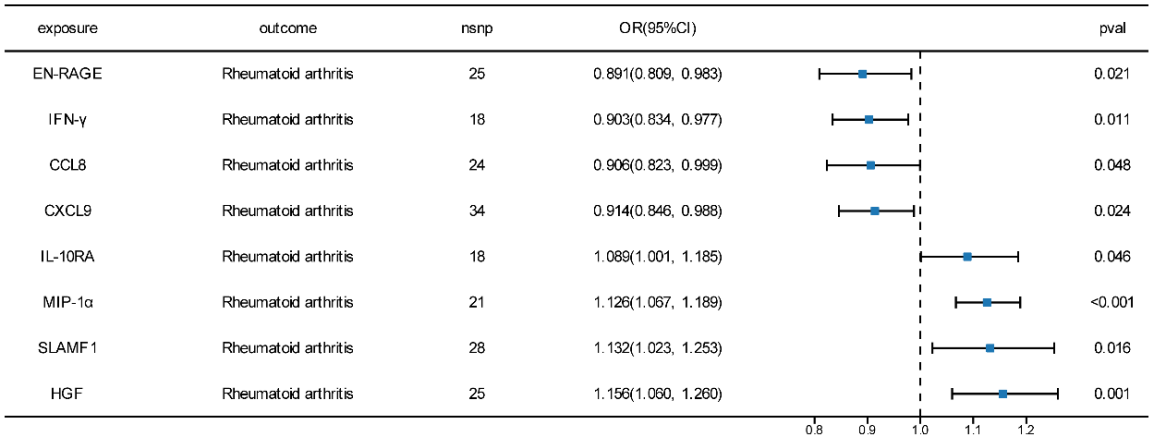

Figure 2. Forest plot of positive causal associations between circulating inflammatory cytokines and RA.

method was employed as the primary method, and $P < 0.05$ indicated a statistically significant causal relationship between the exposure and outcome. In addition, we utilized the Weighted Median, Simple Mode, Weighted Mode and MR Egger regression methods as supplementary analyses. Subsequently, reverse MR analyses were performed with positive inflammatory cytokines as the outcome and RA as the exposure to eliminate reverse causality bias. In a similar way, causality between 1400 metabolites and RA was assessed to obtain positive metabolites. Then, mediation analysis was performed using two-step MR. The total effect of the screened positive inflammatory cytokines on RA (beta All) was divided into the direct effect of the inflammatory cytokines on RA, and the indirect effect mediated through plasma metabolites. The effect of positive inflammatory cytokines on positive metabolites (beta 1) was obtained firstly, followed by revealing the effect of positive metabolites on RA (beta 2). The indirect (mediated) effect was then calculated as the product of coefficients (beta 1* beta 2). The proportion of the total effect mediated by the metabolite was calculated as beta 1* beta 2/beta All.

We conducted a series of sensitivity analyses to determine the validity of our findings.

The leave-one-out method was used to determine if results were driven by any single influential SNP. the MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) global test, where $P < 0.05$ suggested there was no major pleiotropy, were used to determine horizontal pleiotropy. Cochran's Q statistic was used to assess diversity, with $P > 0.05$ indicating presence.

Statistical analysis

All analyses were run in R (version 4.3.1). A *P*-value below 0.05 was considered statistically significant. We relied on several core R packages for the Mendelian randomization workflow: the 'Mendelian Randomization' package implemented the main IVW, MR-Egger, and related methods for causal estimation. Data handling and basic visualization for the two-sample MR framework were managed using the 'Two Sample MR' package. To identify and correct for potential horizontal pleiotropy, we applied the 'MRPRESSO' package. Figures, including scatter, forest, and funnel plots, were created with 'ggplot2'.

Results

Genetic evidence for inflammatory drivers and protectors in RA

MR analysis of 91 inflammatory cytokines identified eight with significant associations with RA (**Figure 2** and Supplementary Figure 1). Genetically predicted higher levels of Protein S100-A12 levels (S100A12/EN-RAGE) (OR = 0.891, 95% CI: 0.809-0.983, $P = 0.021$), Interferon gamma levels (IFN-γ) (OR = 0.903, 95% CI: 0.834-0.977, $P = 0.011$), Monocyte chemoattractant protein-1 levels (MCP-1/CCL8) (OR = 0.906, 95% CI: 0.823-0.999, $P = 0.048$), and C-X-C motif chemokine 9 levels (CXCL9) (OR = 0.914, 95% CI: 0.846-0.988, $P =$

Table 2. The heterogeneity and pleiotropy in MR analysis

Exposure	Outcome	Heterogeneity test				Pleiotropy test				
		MR Egger		IVW		MR Egger regression			MR PRESSO	
		Cochran Q	pval	Cochran Q	pval	Intercept	se	pval	RSSobs	pval
CXCL9	Rheumatoid arthritis	33.464	0.396	33.947	0.422	-0.004	0.007	0.501	36.304	0.414
EN-RAGE	Rheumatoid arthritis	28.185	0.209	31.046	0.152	0.016	0.010	0.140	36.378	0.132
HGF	Rheumatoid arthritis	16.891	0.815	17.627	0.821	-0.008	0.009	0.400	19.664	0.830
IFN-γ	Rheumatoid arthritis	11.192	0.798	12.540	0.766	-0.010	0.009	0.263	13.733	0.810
IL-10RA	Rheumatoid arthritis	16.910	0.391	17.119	0.446	0.004	0.008	0.663	20.533	0.464
CCL8	Rheumatoid arthritis	44.888	0.003	46.306	0.003	0.008	0.010	0.413	48.889	0.008
MIP-1α	Rheumatoid arthritis	15.047	0.720	16.341	0.695	0.007	0.006	0.270	18.016	0.746
SLAMF1	Rheumatoid arthritis	47.892	0.006	48.862	0.006	0.008	0.010	0.474	53.278	0.006
CXCL9	Eicosapentaenoate (EPA; 20:5n3) levels	28.149	0.613	29.286	0.605	0.009	0.008	0.295	31.175	0.618
EN-RAGE	Salicyluric glucuronide levels	27.602	0.277	28.032	0.306	-0.008	0.014	0.547	29.806	0.325
IFN-γ	1-arachidonoyl-gpc (20:4n6) levels	14.369	0.705	14.813	0.734	-0.007	0.010	0.513	16.240	0.739
IFN-γ	Glycine to pyridoxal ratio	15.362	0.637	16.159	0.647	-0.009	0.010	0.384	17.960	0.665
IL-10RA	3,7-dimethylurate levels	19.028	0.390	19.070	0.452	0.003	0.013	0.844	21.040	0.517
IL-10RA	N,N-dimethylalanine levels	21.396	0.260	21.428	0.314	-0.002	0.012	0.871	23.852	0.308
CCL8	Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	29.723	0.327	29.829	0.371	0.002	0.008	0.759	31.321	0.421
Eicosapentaenoate (EPA; 20:5n3) levels	Rheumatoid arthritis	19.298	0.627	22.020	0.519	-0.016	0.009	0.113	24.034	0.527
Salicyluric glucuronide levels	Rheumatoid arthritis	5.561	0.901	6.010	0.916	-0.010	0.015	0.517	7.197	0.903
1-arachidonoyl-gpc (20:4n6) levels	Rheumatoid arthritis	22.347	0.439	23.143	0.452	0.005	0.005	0.386	24.231	0.586
Glycine to pyridoxal ratio	Rheumatoid arthritis	11.966	0.287	11.966	0.366	0.00004	0.013	0.998	18.643	0.334
3,7-dimethylurate levels	Rheumatoid arthritis	27.869	0.525	27.896	0.576	0.002	0.009	0.872	29.993	0.597
N,N-dimethylalanine levels	Rheumatoid arthritis	25.454	0.327	25.560	0.375	-0.003	0.009	0.760	26.515	0.484
Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	Rheumatoid arthritis	29.217	0.109	29.259	0.138	-0.002	0.012	0.864	32.515	0.151

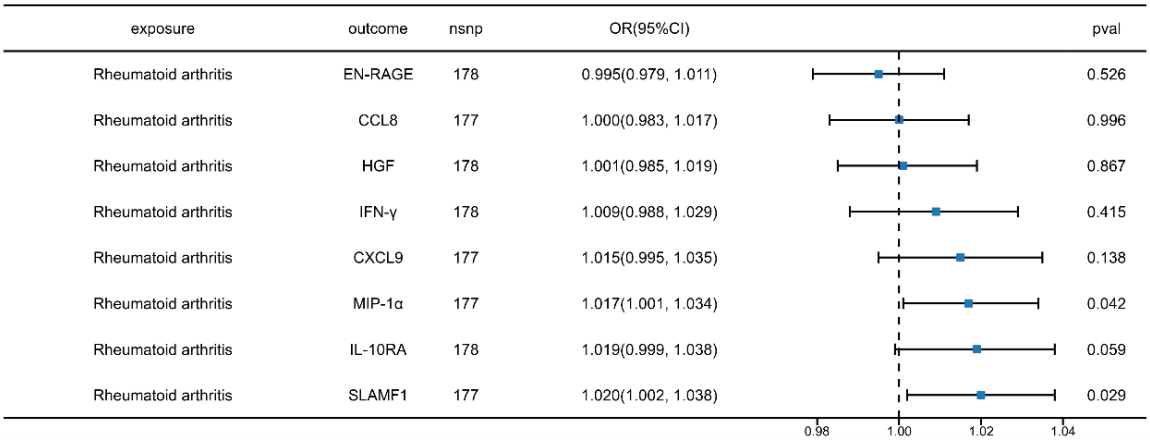

Figure 3. Forest plot of reverse causal association between RA and circulating inflammatory cytokines.

0.024) were significantly associated with a reduced risk of RA. Conversely, elevated levels of Interleukin-10 receptor subunit alpha levels (IL-10RA) (OR = 1.089, 95% CI: 1.001-1.185, P = 0.046), Macrophage inflammatory protein 1a levels (MIP-1α) (OR = 1.126, 95% CI: 1.067-1.189, P < 0.001), Signaling lymphocytic activation molecule levels (SLAMF1) (OR = 1.132, 95% CI: 1.023-1.253, P = 0.016), and Hepatocyte growth factor levels (HGF) (OR = 1.156, 95% CI: 1.060-1.260, P = 0.001) were associated with increased RA risk.

Sensitivity analyses revealed no evidence of horizontal pleiotropy or significant heterogeneity for most inflammatory cytokines, as confirmed by MR-Egger intercept, Cochran's Q tests and MR-PRESSO tests (P > 0.05) (**Table 2**). However, CCL8 and SLAMF1 exhibited potential pleiotropy and heterogeneity (P < 0.05), suggesting that their associations with RA may be influenced by pleiotropic effects, and these findings necessitate cautious interpretation. The robustness of the findings was further supported by leave-one-out analyses and funnel plot symmetry (Supplementary Figure 2).

Reverse MR analysis

Reverse MR analysis was conducted to assess the potential for reverse causality, that is, whether RA itself might influence the levels of inflammatory cytokines previously identified as having causal effects on RA. Among the eight cytokines initially found to be causally associated with RA, two were identified as potentially affected by RA, and were therefore excluded (P < 0.05). For the remaining six cytokines, no evidence of reverse causation was observed based on IVW analysis (P > 0.05): CXCL9 (OR = 1.015, 95% CI: 0.995-1.035, P = 0.138), EN-RAGE (OR = 0.995, 95% CI: 0.979-1.011, P = 0.526), HGF (OR = 1.001, 95% CI: 0.985-1.019, P = 0.867); IFN-γ (OR = 1.009, 95% CI: 0.988-1.029, P = 0.415); IL-10RA (OR = 1.019, 95% CI: 0.999-1.038, P = 0.059); CCL8 (OR = 1.000, 95% CI: 0.983-1.017, P = 0.996) (**Figure 3**).

MR analysis identifies plasma metabolites potentially causal for RA risk

We systematically assessed the potential causal association between 1,400 plasma metabolites and RA using the IVW approach. A total of 69 metabolites demonstrated statistically significant associations with RA risk (**Figure 4**). Among them, 28 metabolites were identified as potential risk factors (OR > 1), while 41 metabolites appeared to be protective (OR < 1).

Notable protective metabolites included X-07765 (OR = 0.881, 95% CI: 0.819-0.949, P = 0.001), adenosine 5'-monophosphate (AMP) to EDTA ratio (OR = 0.882, 95% CI: 0.792-0.983, P = 0.023), and 1,3-dimethylurate (OR = 0.882, 95% CI: 0.814-0.955, P = 0.002). Amino acid metabolism-related ratios, such as glycine to pyridoxal and glutamate to glutamine, also showed inverse associations with RA, suggesting their potential protective roles in disease modulation. Conversely, elevated levels of certain metabolites were associated with increased RA risk, including methylsuccinate

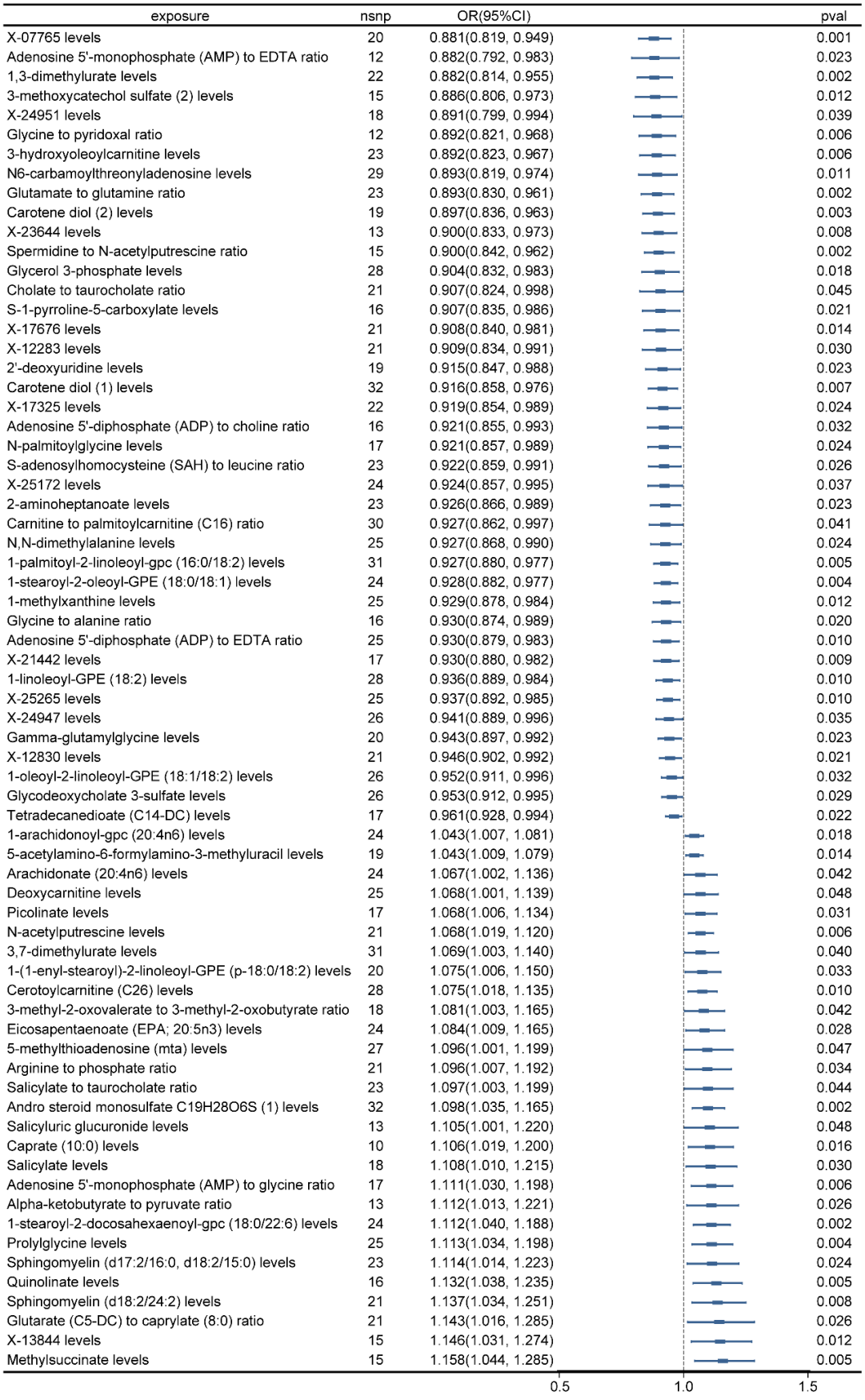

Figure 4. Forest plot of causal associations between plasma metabolites and RA.

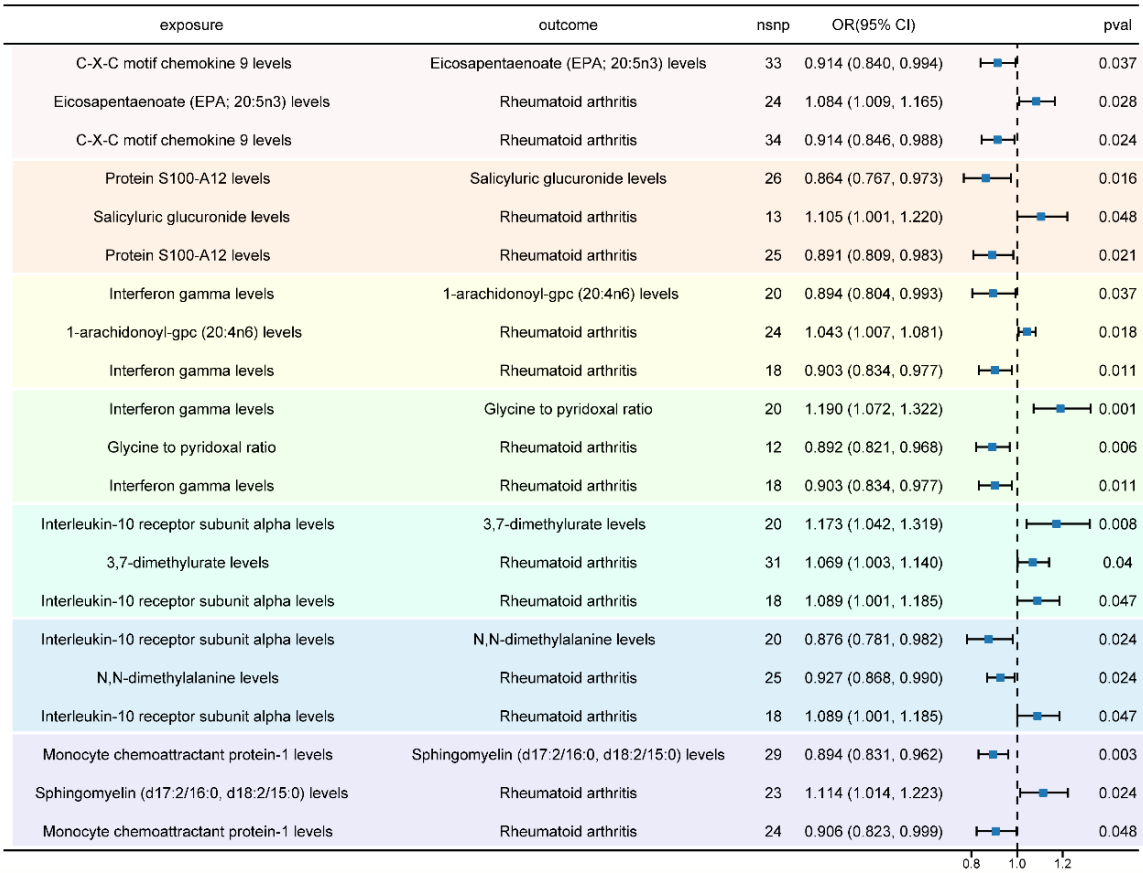

Figure 5. Forest plot of mediation effect analysis of circulating inflammatory cytokines, plasma metabolites, and RA.

(OR = 1.158, 95% CI: 1.044-1.285, *P* = 0.005), X-13844 (OR = 1.146, 95% CI: 1.031-1.274, *P* = 0.012), and sphingomyelin (d18:2/24:2) (OR = 1.137, 95% CI: 1.034-1.251, *P* = 0.008). Interestingly, metabolites associated with RA risk were significantly enriched in lipid categories, particularly sphingolipids and long-chain polyunsaturated fatty acids.

Mediation analysis reveals metabolite-driven causal pathways linking inflammatory cytokines to RA

We performed a two-step MR-based mediation analysis to reveal the metabolic pathways in which inflammatory cytokines influence RA. Five previously identified inflammatory cytokines and 69 plasma metabolites were included in the model. Firstly, the causal effects of these cytokines on metabolite levels were assessed using two-sample MR. Subsequently, those metabolites that showed significant genetic associations with the cytokines were used as exposure factors to further examine their downstream effects on RA. Only associations that demonstrated consistent directionality across five MR methods and statistical significance based on the IVW approach were considered robust.

Figure 5 summarizes the bidirectional MR findings supporting metabolite-mediated causal pathways between inflammatory cytokines and RA. Specifically, seven plasma metabolites were identified as mediators of the causal effects exerted by the five inflammatory cytokines on RA (**Figure 5** and Supplementary Figures 3, 4; **Table 3**). For instance, the risk of RA was reduced by CXCL9 through a decrease in eicosapentaenoate (EPA; 20:5n3) levels (mediated effect = -0.007; 95% CI: -0.017-0.003; mediated proportion = 8.10%). ENRAGE was found to reduce RA risk by lowering salicyluric glucuronide levels (mediated effect = -0.015; 95% CI: -0.036-0.006; mediated proportion = 12.70%). IFN-γ was observed

Table 3. The mediation effect of circulating inflammatory cytokines on RA via metabolites

Inflammatory factor	Metabolite	Outcome	betaAll	Mediated effect	Mediated proportion
CXCL9	Eicosapentaenoate (EPA; 20:5n3) levels	Rheumatoid arthritis	-0.090	-0.007 (-0.017, 0.003)	8.10%
EN-RAGE	Salicyluric glucuronide levels	Rheumatoid arthritis	-0.115	-0.015 (-0.036, 0.006)	12.70%
IFN-γ	1-arachidonoyl-gpc (20:4n6) levels	Rheumatoid arthritis	-0.102	-0.005 (-0.017, 0.008)	4.67%
IFN-γ	Glycine to pyridoxal ratio	Rheumatoid arthritis	-0.102	-0.020 (-0.042, 0.002)	19.60%
IL-10RA	3,7-dimethylurate levels	Rheumatoid arthritis	0.086	0.011 (-0.100, 0.031)	12.50%
IL-10RA	N,N-dimethylalanine levels	Rheumatoid arthritis	0.086	0.010 (-0.007, 0.027)	11.70%
CCL8	Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	Rheumatoid arthritis	-0.098	-0.012 (-0.023, -0.001)	12.30%

to reduce RA risk either by decreasing 1-arachidonoyl-gpc (20:4n6) levels (mediated effect = -0.005; 95% CI: -0.017-0.008; mediated proportion = 4.67%) or by increasing the glycine-to-pyridoxal ratio (mediated effect = -0.020; 95% CI: -0.042-0.002; mediated proportion = 19.60%). In contrast, IL-10RA was associated with increased RA risk via elevation of 3,7-dimethylurate levels (mediated effect = 0.011; 95% CI: -0.100-0.031; mediated proportion = 12.50%) or suppression of N, N-dimethylalanine levels (mediated effect = 0.010; 95% CI: -0.007-0.027; mediated proportion = 11.70%). Additionally, monocyte chemoattractant protein-1 (CCL8) was found to reduce RA risk by decreasing sphingomyelin (d17:2/16:0, d18:2/15:0) levels (mediated effect = -0.012; 95% CI: -0.023 - -0.001; mediated proportion = 12.30%). Furthermore, the robustness of the results was further supported by leave-one-out analyses and funnel plot symmetry (Supplementary Figures 5, 6).

Discussion

The occurrence and development of rheumatoid arthritis are influenced by a combination of factors. Among them, immune response dysregulation is a key link in promoting the continuous worsening of chronic synovial inflammation and joint injury, which is mainly driven by cytokines, chemokines and soluble receptors [15, 16]. Using Mendelian randomization, we explored potential immunometabolic pathways connecting systemic inflammation to RA. Our analysis identified a causal role for six inflammatory mediators: CXCL9, EN-RAGE, HGF, IFN-γ, IL-10RA, and CCL8. Among these, HGF and IL-10RA were linked to increased RA risk, while the others appeared protective. Reverse MR analysis was performed to exclude most reverse causal relationships from RA to these inflammatory exposures. We also evaluated around 1400 plasma metabolites and found that 69 were significantly associated with RA risk, of which 41 were protective and 28 increased risks. A higher glycine-to-pyridoxal ratio and elevated 1,3-dimethylurate emerged as protective factors, whereas several sphingomyelins and long-chain polyunsaturated fatty acids were associated with greater risk, suggesting that disturbances in lipid metabolism may be involved in the disease process of RA by affecting signal transduction or cell membrane function. Mediation MR analyses suggest that specific metabolites explain the relationship between cytokines and RA to some extent. The current study provides mechanistic insights into potential immunometabolic pathways that properly explain RA pathogenesis by identifying metabolites that mediate inflammatory signal transduction.

To elucidate the biological logic behind the above causal associations, we analyzed some of the key inflammatory mediators. HGF is known to be a risk factor for an increased risk of RA due to its pro-inflammatory effects. In RA synovium, HGF is overexpressed and promotes angiogenesis, synoviocyte growth, and tissue remodeling, which contributes to joint damage [17-20]. Preclinical studies showing that HGF inhibition reduces synovitis and bone erosion lend further support to a causal role [21]. Related to IL-10RA's ability to regulate anti-inflammatory IL-10 signals, genetic variants such as rs9610 have nevertheless been tied to higher RA risk [22, 23]. This may be a result of compensation overexpression resulting from impaired IL-10 indicating, which reveals how destructive hormone pathways may turn into harmful. In comparison, our MR analysis revealed that the pro-inflammatory cytokines CXCL9, IFN-γ, EN-RAGE, and CCL8 were protec-

tive [24-26]. This seemingly paradoxical finding highlights the context-dependent actions of immune mediators. It also demonstrates how MR captures the net lifelong effects of genetic predisposition. These effects can differ from observations made after disease onset. Several explanations are plausible. First, these cytokines may participate in long-term feedback or compensatory circuits that suppress inflammation over time. For example, IFN-γ is a powerful inhibitor of osteoclast formation, which helps limit bone erosion, a key factor in RA disability [27]. EN-RAGE, though often elevated in active RA, might engage counter-regulatory mechanisms once inflammation is established. Second, CXCL9 could help recruit regulatory T cells or otherwise modulate local immune responses [28]. Finally, the genetic variants used in MR analysis reflect the moderately altered levels of cytokine exposure that individuals face throughout their lives. This long-term pattern of exposure may help the immune system mature or "tune" in a protective way, an effect that is difficult to capture only in observational studies measuring cytokine levels after the onset of disease. Thus, our findings do not contradict the documented pro-inflammatory roles of these molecules, instead, they suggest that a long-term, systemic genetic predisposition influences RA risk through a distinct, potentially regulatory, net pathway.

The most important advancement in this study is the demonstration that plasma metabolites partially mediate the effect of cytokines on RA risk. Mediation MR demonstrated that inflammatory signals propagate through specific metabolic routes, reinforcing the emerging concept of immunometabolic crosstalk in autoimmunity. For instance, the protective cytokine CXCL9 was linked to lower levels of eicosapentaenoic acid (EPA), an omega-3 fatty acid with known anti-inflammatory properties [29]. EN-RAGE also exerted protective effects via decreased salicyluric glucuronide, a metabolite of aspirin, suggesting overlap between endogenous anti-inflammatory responses and pharmacologic mechanisms. IFN-γ influenced RA risk via two metabolic changes: lower levels of the pro-inflammatory lipid 1-arachidonoyl-GPC (20:4n6) and a higher glycine-to-pyridoxal ratio, implicating amino acid and vitamin B6 metabolism in immune regulation. Glycine can inhibit NF-κB signaling, while pyridoxal supports methylation and one-carbon metabolism [30, 31]. In contrast, IL-10RA increases the risk of RA by increasing 3,7-dimethylurate, a purine breakdown product, and decreasing levels of N, N-dimethyl alanine, suggesting that purine metabolism may be disturbed with methylation processes. In addition, the role of the protective cytokine CCL8 is related to the reduction of sphingomyelin levels, which confirms the view that "membrane lipid composition can regulate immune cell signaling" [32]. These findings underscore a bidirectional crosstalk between inflammation and metabolism, where cytokine-driven metabolic changes influence immune responses and vice versa. This dynamic interface offers novel targets for therapeutic modulation of immune activity through metabolic pathways.

Causal inference was strengthened by the integration of two-sample MR with GWAS datasets, which minimized the potential for confounding and reverse causation [33]. We further validated the sensitivity with multiple MR methods and sensitivity validation, including IVW, MR-Egger regression, and leave-one-out tests, and the consistency of the results supported the robustness of the findings. The mediation framework allowed us to uncover previously unrecognized sequential links between cytokines, metabolites, and RA, extending earlier work that often examined these factors in isolation. We recognize several limitations. First, since our data came exclusively from European-ancestry populations, the results may not generalize to other groups. Second, as GWAS resources for RA and metabolomic traits grow, future updates may refine the estimates we observed. Third, although MR excels at estimating lifelong genetic effects, it may not fully capture time-varying or acute influences that could be relevant in a heterogeneous disease like RA. Fourth, despite MR's ability to limit confounding, residual or unmeasured confounding in the underlying GWAS data could still affect our estimates. Finally, there are differences between the results of this study and previous observational reports, such as the well-proven pro-inflammatory effects of EN-RAGE and IFN-γ, which may be more due to differences in study design, specific biological context, or residual confounders, rather than the flaws of the Mendelian randomization method itself.

Conclusions

This study provides strong causal evidence for circulating inflammatory cytokines to influence RA risk and identifies the key mediating roles of plasma metabolites in these impact pathways. The specific immune-metabolic axes identified, such as CXCL9-EPA, IFN-γ-glycine/pyridoxal, and IL-10RA-purine metabolism axes, not only provide new mechanisms for elucidating RA disease susceptibility, but also suggest potential therapeutic targets. These findings have improved our understanding of the pathogenesis of RA and provided a theoretical basis for the subsequent development of RA prevention and treatment strategies for immunometabolic links.

Acknowledgements

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Disclosure of conflict of interest

None.

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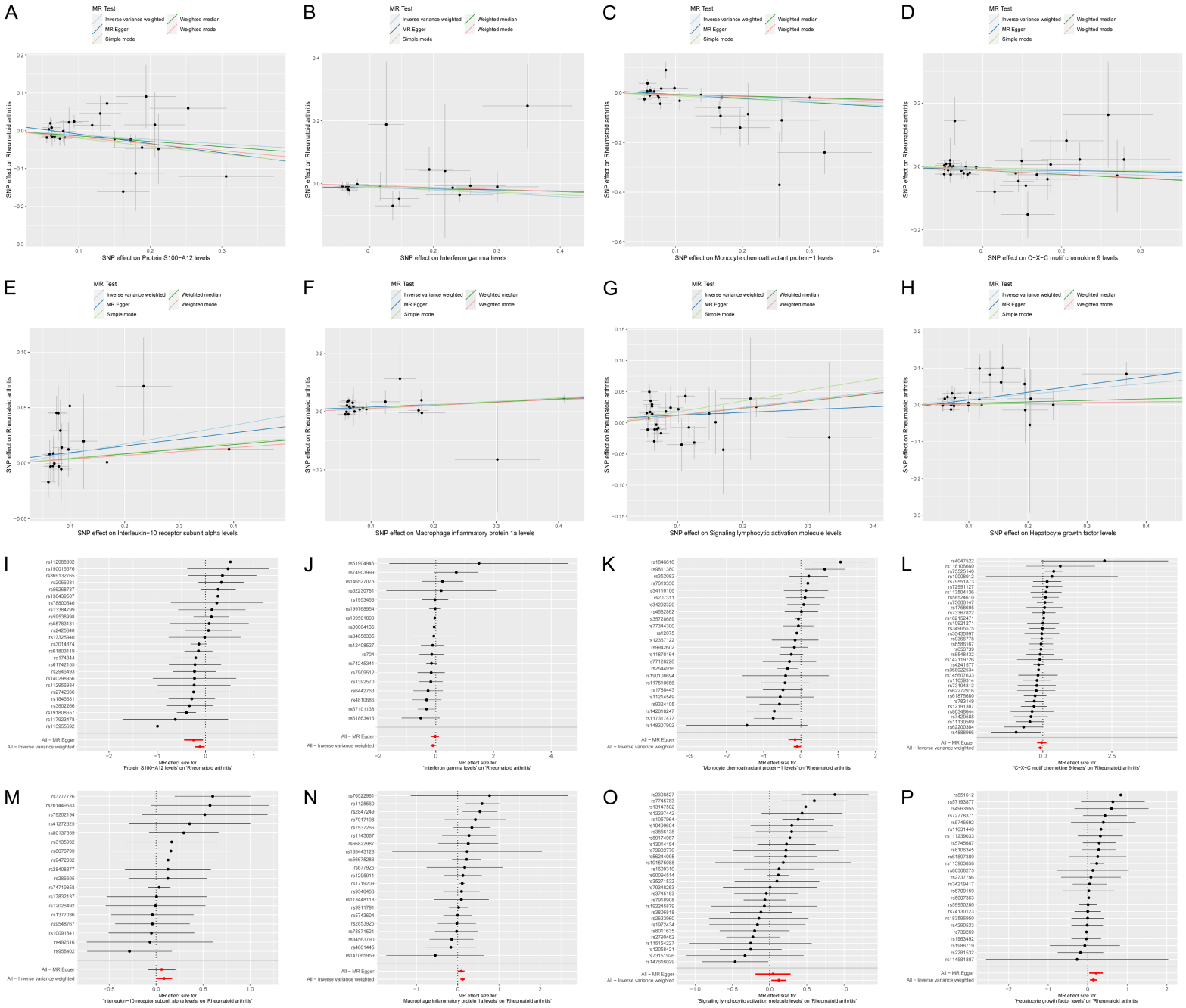
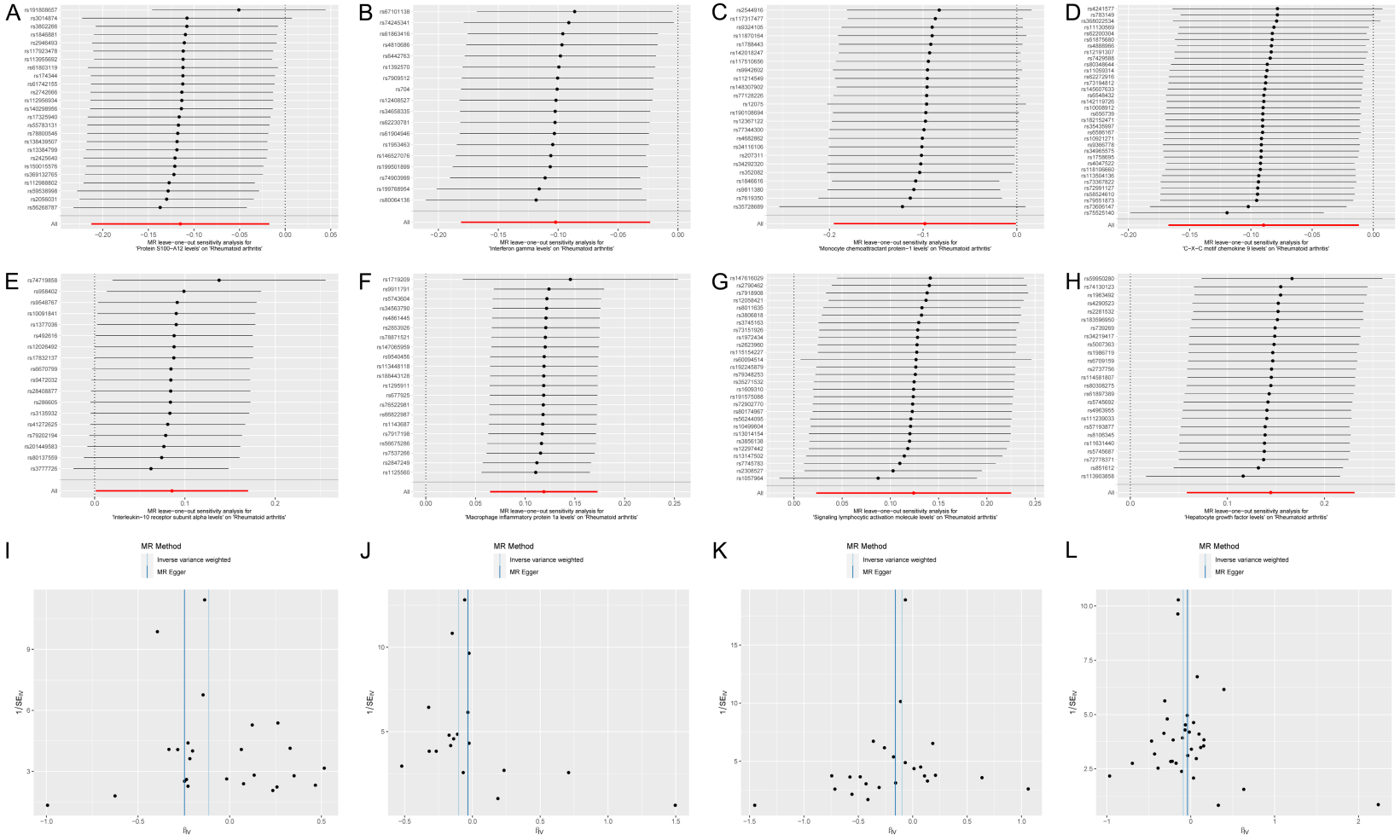
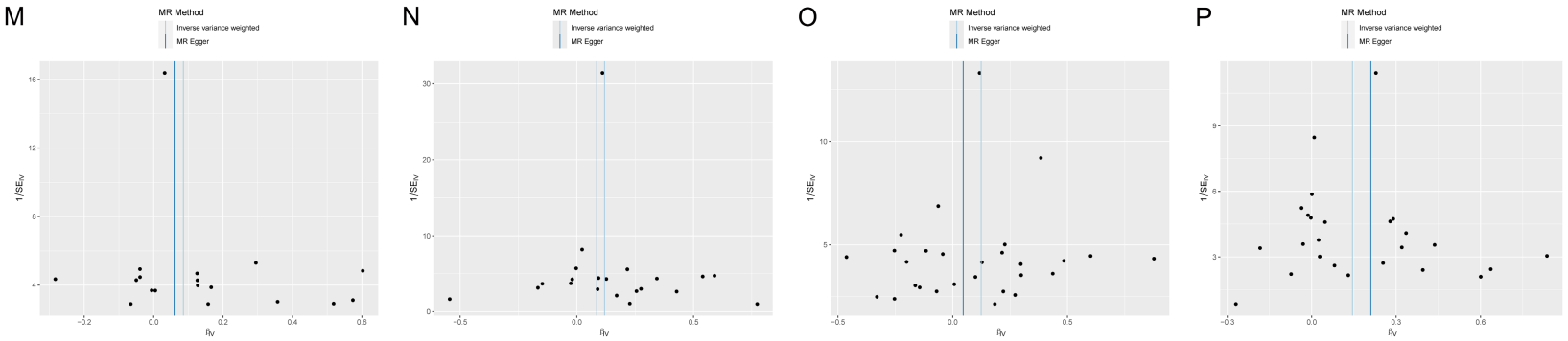
Immune-metabolic crosstalk in rheumatoid arthritis

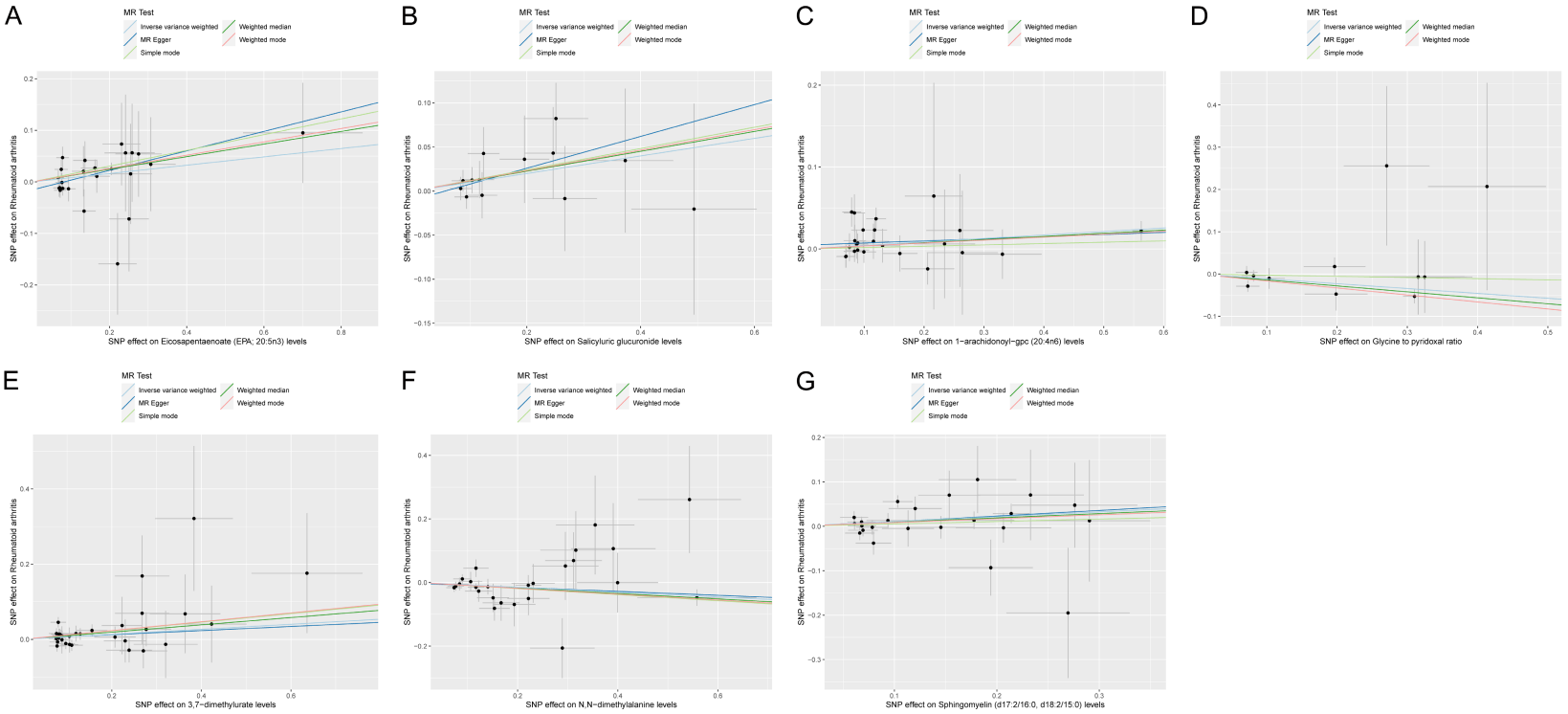


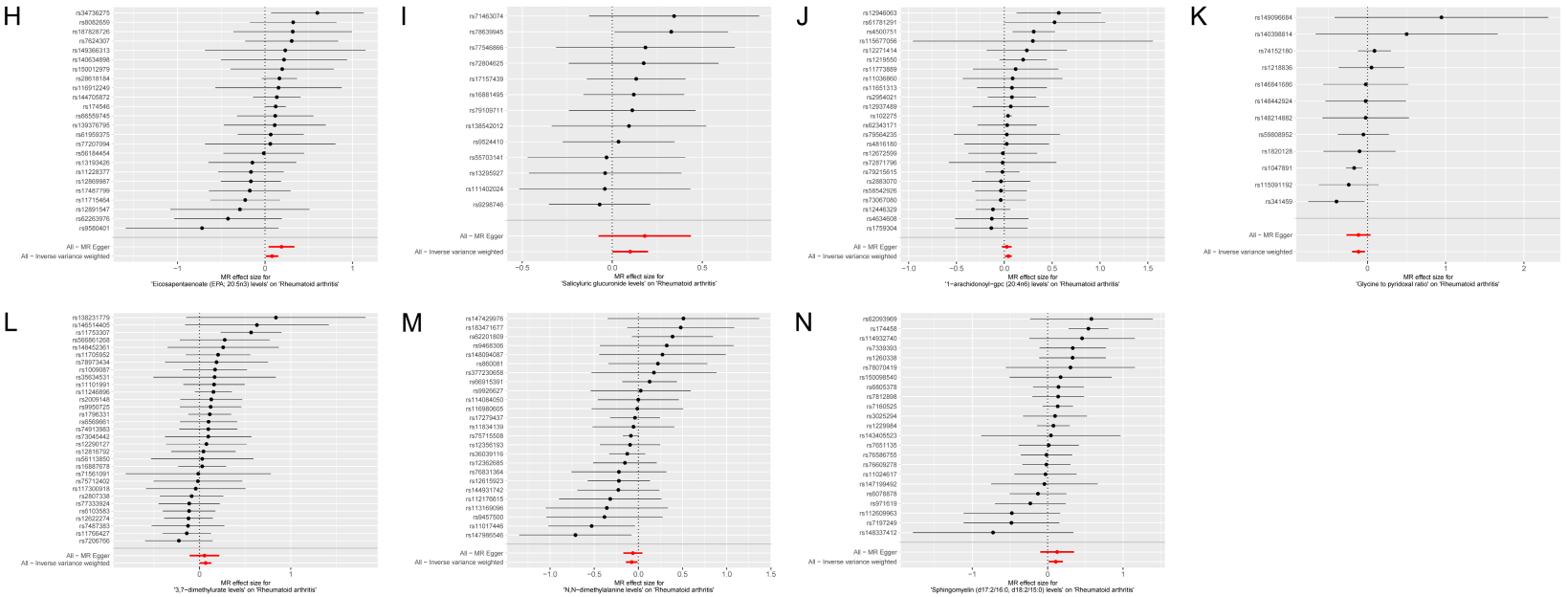
Supplementary Figure 1. MR results of inflammatory factors to RA. Scatter plot of MR for inflammatory factors. (A) EN-RAGE, (B) IFN-γ, (C) CCL8, (D) CXCL9, (E) IL-10RA, (F) MIP-1α, (G) SLAMF1, (H) HGF with RA. Forest plot of MR for inflammatory factors (I) EN-RAGE, (J) IFN-γ, (K) CCL8, (L) CXCL9, (M) IL-10RA, (N) MIP-1α, (O) SLAMF1, (P) HGF with RA.



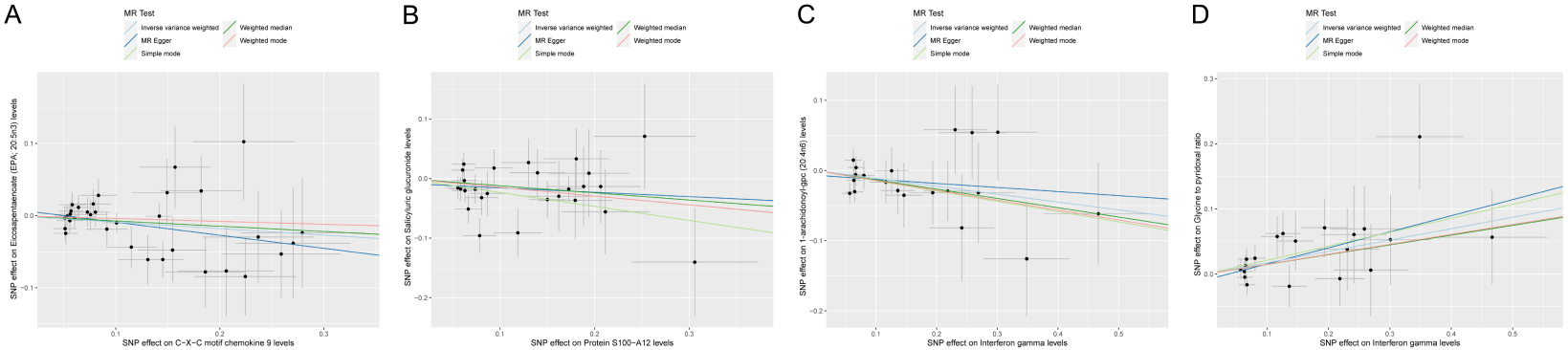


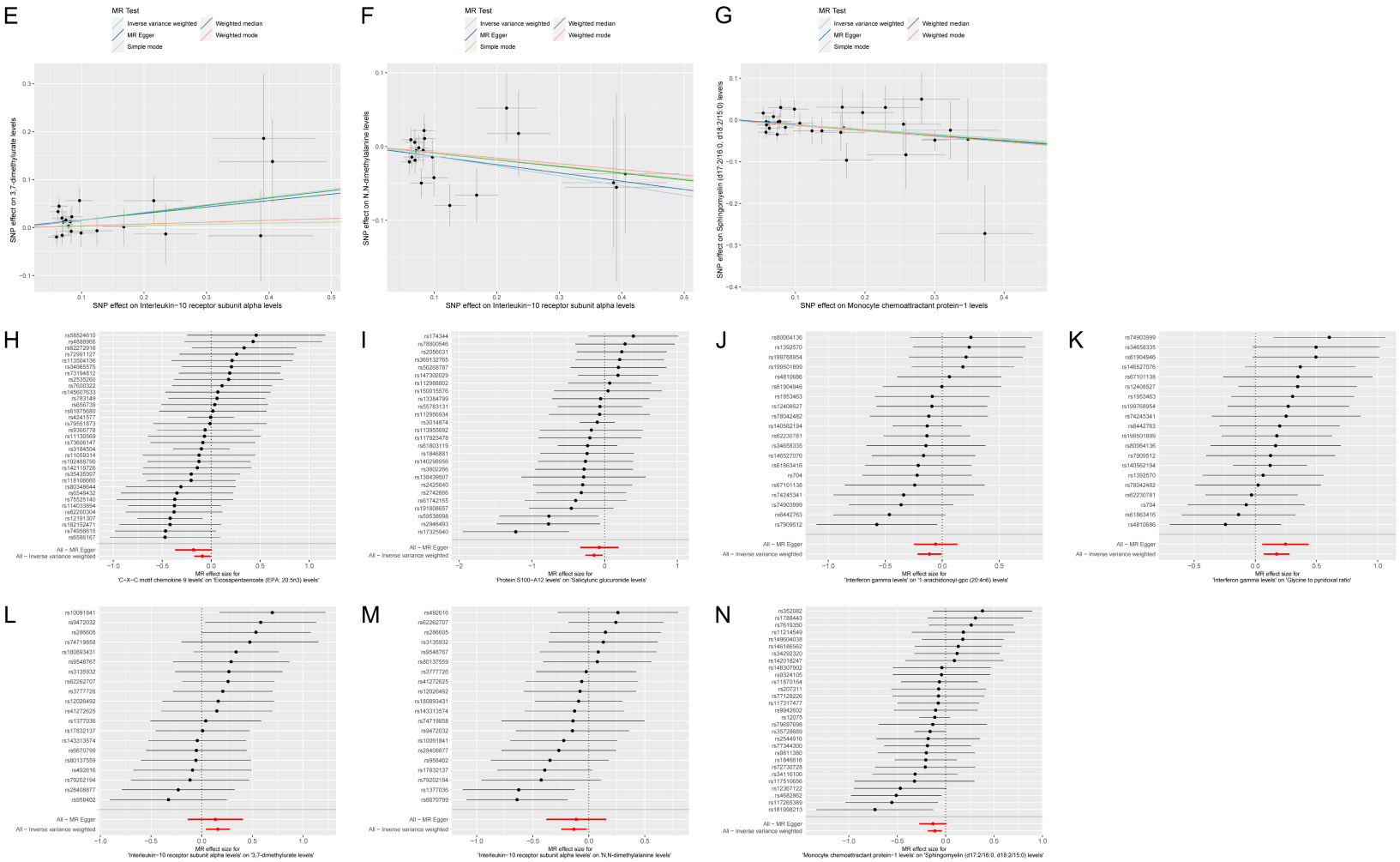
Supplementary Figure 2. MR results of inflammatory factors to RA. Leave-one-out analysis of MR for (A) EN-RAGE, (B) IFN-γ, (C) CCL8, (D) CXCL9, (E) IL-10RA, (F) MIP-1α, (G) SLAMF1, (H) HGF with RA. Funnel plot of MR for (I) EN-RAGE, (J) IFN-γ, (K) CCL8, (L) CXCL9, (M) IL-10RA, (N) MIP-1α, (O) SLAMF1, (P) HGF with RA.



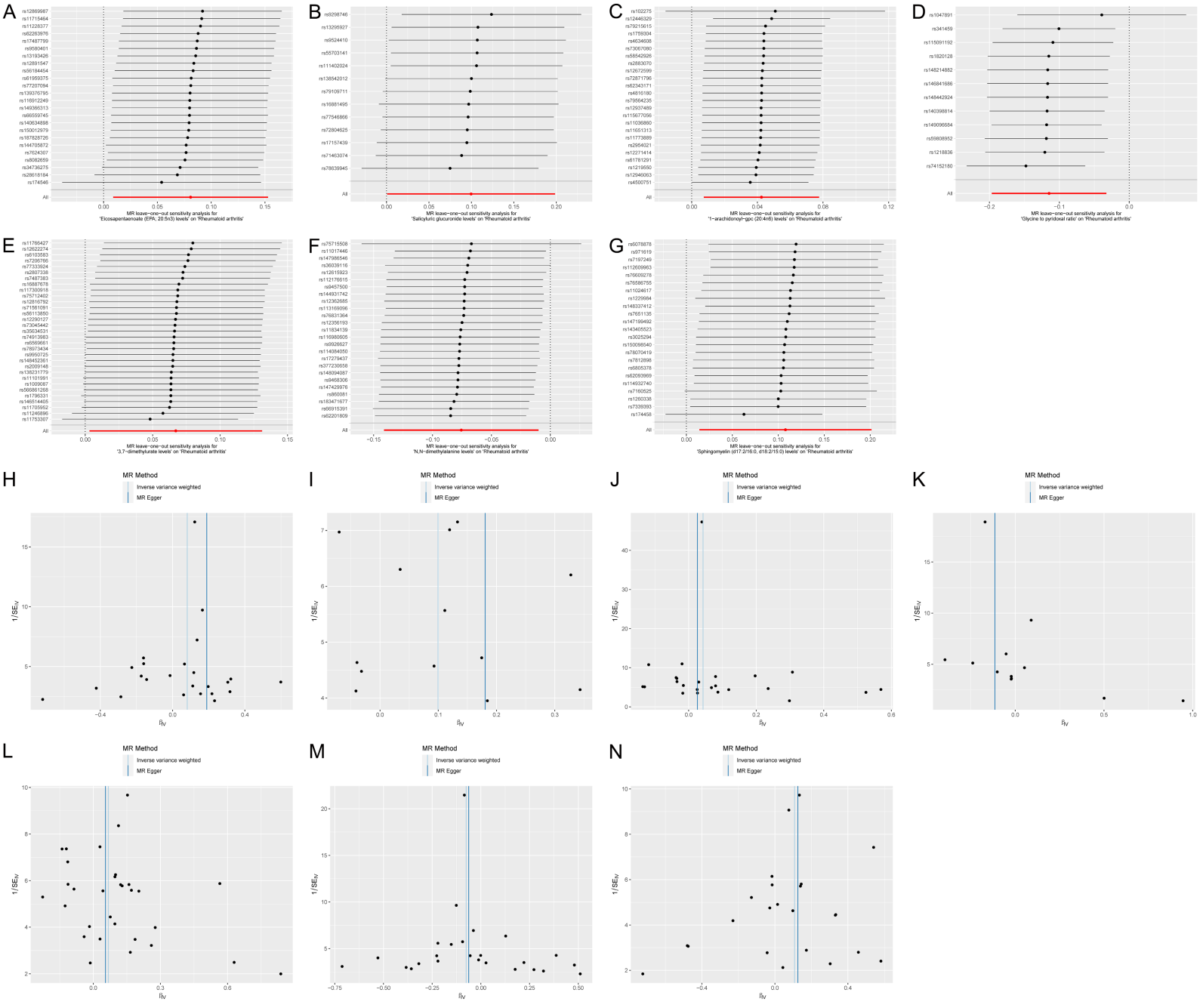


Supplementary Figure 3. MR results of plasma metabolites to RA. Scatter plot of MR for plasma metabolites (A) Eicosapentaenoate (EPA; 20:5n3) levels (B) Salicyluric glucuronide levels (C) 1-arachidonoyl-gpc (20:4n6) levels, (D) Glycine to pyridoxal ratio, (E) 3,7-dimethylurate levels, (F) N, N-dimethylalanine levels, (G) Sphingomyelin (d17:2/16:0, d18:2/15:0) levels with RA. Forest plot of MR for plasma metabolites (H) Eicosapentaenoate (EPA; 20:5n3) levels, (I) Salicyluric glucuronide levels (J) 1-arachidonoyl-gpc (20:4n6) levels, (K) Glycine to pyridoxal ratio, (L) 3,7-dimethylurate levels, (M) N, N-dimethylalanine levels, (N) Sphingomyelin (d17:2/16:0, d18:2/15:0) levels with RA.

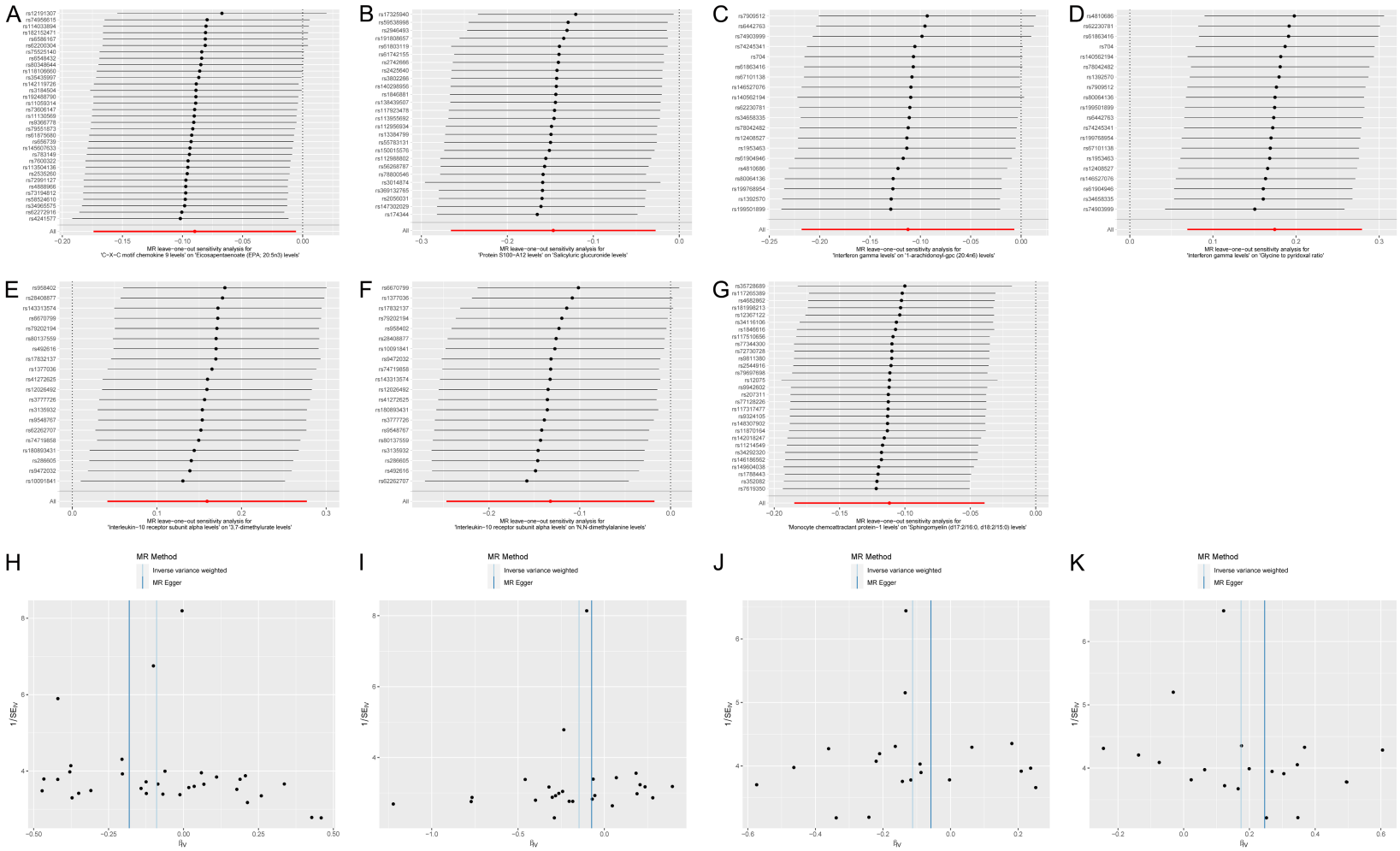


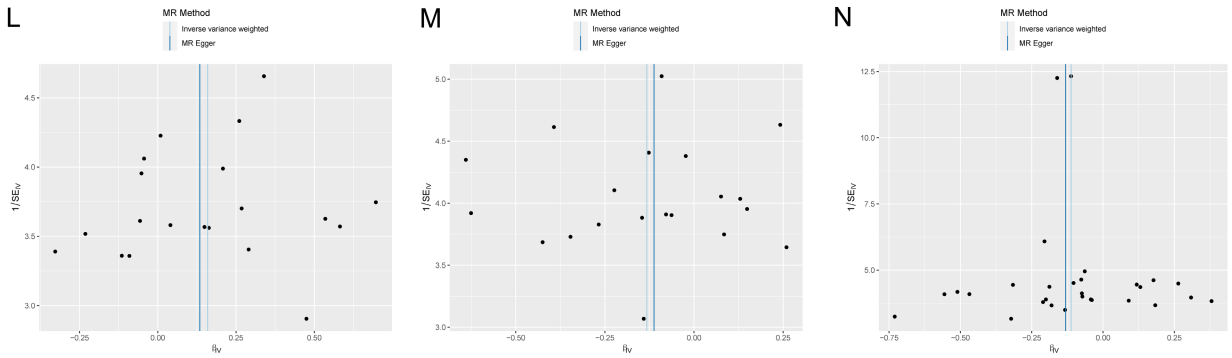


Supplementary Figure 4. MR results of inflammatory factors and plasma metabolites. Scatter plot of MR for (A) CXCL9 and Eicosapentaenoate (EPA; 20:5n3) levels, (B) EN-RAGE and Salicyluric glucuronide levels (C) IFN-γ and 1-arachidonoyl-gpc (20:4n6) levels, (D) IFN-γ and Glycine to pyridoxal ratio, (E) IL-10RA and 3,7-dimethylurate levels, (F) IL-10RA and N, N-dimethylalanine levels, (G) CCL8 and Sphingomyelin (d17:2/16:0, d18:2/15:0) levels. Forest plot of MR for (H) CXCL9 and Eicosapentaenoate (EPA; 20:5n3) levels, (I) EN-RAGE and Salicyluric glucuronide levels (J) IFN-γ and 1-arachidonoyl-gpc (20:4n6) levels, (K) IFN-γ and Glycine to pyridoxal ratio, (L) IL-10RA and 3,7-dimethylurate levels, (M) IL-10RA and N, N-dimethylalanine levels, (N) CCL8 and Sphingomyelin (d17:2/16:0, d18:2/15:0) levels.


A
MR leave−one−out sensitivity analysis for
'Eicosapentaenoate (EPA, 20:5n3) levels' on 'Rheumatoid arthritis'
B
MR leave−one−out sensitivity analysis for
'Salicyluric glucuronide levels' on 'Rheumatoid arthritis'
C
MR leave−one−out sensitivity analysis for
'1−arachidonoyl−gpc (20:4n6) levels' on 'Rheumatoid arthritis'
D
MR leave−one−out sensitivity analysis for
'Glycine to pyridoxal ratio' on 'Rheumatoid arthritis'
E
MR leave−one−out sensitivity analysis for
'3,7−dimethylurate levels' on 'Rheumatoid arthritis'
F
MR leave−one−out sensitivity analysis for
'N,N−dimethylalanine levels' on 'Rheumatoid arthritis'
G
MR leave−one−out sensitivity analysis for
'Sphingomyelin (d17:2/16:0, d18:2/15:0) levels' on 'Rheumatoid arthritis'
H
I
J
K
L
M
N
MR Method
Inverse variance weighted
MR Egger
1/SE_IV
β_IV

Supplementary Figure 5. MR results of plasma metabolites to RA. Leave-one-out analysis of MR for plasma metabolites (A) Eicosapentaenoate (EPA; 20:5n3) levels (B) Salicyluric glucuronide levels (C) 1-arachidonoyl-gpc (20:4n6) levels, (D) Glycine to pyridoxal ratio, (E) 3,7-dimethylurate levels, (F) N, N-dimethylalanine levels, (G) Sphingomyelin (d17:2/16:0, d18:2/15:0) levels with RA. Funnel plot of MR for plasma metabolites (H) Eicosapentaenoate (EPA; 20:5n3) levels, (I) Salicyluric glucuronide levels (J) 1-arachidonoyl-gpc (20:4n6) levels, (K) Glycine to pyridoxal ratio, (L) 3,7-dimethylurate levels, (M) N, N-dimethylalanine levels, (N) Sphingomyelin (d17:2/16:0, d18:2/15:0) levels with RA.





Supplementary Figure 6. MR results of inflammatory factors and plasma metabolites. Leave-one-out analysis of MR for (A) CXCL9 and Eicosapentaenoate (EPA; 20:5n3) levels, (B) EN-RAGE and Salicyluric glucuronide levels (C) IFN-γ and 1-arachidonoyl-gpc (20:4n6) levels, (D) IFN-γ and Glycine to pyridoxal ratio, (E) IL-10RA and 3,7-dimethylurate levels, (F) IL-10RA and N, N-dimethylalanine levels, (G) CCL8 and Sphingomyelin (d17:2/16:0, d18:2/15:0) levels. Funnel plot of MR for (H) CXCL9 and Eicosapentaenoate (EPA; 20:5n3) levels, (I) EN-RAGE and Salicyluric glucuronide levels (J) IFN-γ and 1-arachidonoyl-gpc (20:4n6) levels, (K) IFN-γ and Glycine to pyridoxal ratio, (L) IL-10RA and 3,7-dimethylurate levels, (M) IL-10RA and N, N-dimethylalanine levels, (N) CCL8 and Sphingomyelin (d17:2/16:0, d18:2/15:0) levels.