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Genetically predicted metabolite mediates the causal relationship between immune cells and autoimmune diseases

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Abstract

Background This study investigates the causal role of metabolites mediating immune cells in rheumatoid arthritis (RA) and ankylosing spondylitis (AS) through a Mendelian randomization (MR) study.

Methods The two-sample and two-step MR methods were used for the current analysis: (1) causal effects of immune cells on RA and AS; (2) mediation effects of metabolites. Inverse variance weighted (IVW) is the main method to analyze causality, and MR results are verified by several sensitive analyses.

Results This study first identified the immune cells and metabolites that are causally associated with RA and AS, respectively. Subsequent mediation analyses revealed that of the 61 metabolic factors that were causally associated with RA, 6 were identified as mediators of the relationship between immune cells and RA, including 4-cholesten-3-one levels (mediation ratio: 8.91%), N-lactoyl isoleucine levels (13%), 3- phosphoglycerate to glycerate ratio (12.9%, 2.31%, respectively), Gamma-glutamyl histidine levels (9.54%), and Citrulline to phosphate ratio (15.6%). Among the 52 metabolic factors that were causally associated with AS, 2 were identified as mediators of the relationship between immune cells and AS, including salicylate levels (10.4%) and Glucose to N-palmitoyl-sphingosine (d18:1 to 16:0) ratio (8.72%). These results performed well in sensitivity analysis.

Conclusions Genetic predictions show causal relationships between immune cells and autoimmune diseases, and that these causal relationships can be mediated by certain metabolites as mediators.

Keywords Autoimmune diseases, Mendelian randomization, Immune cells, Metabolites

Introduction

A heterogeneous group of diseases, autoimmune diseases (ADs), are characterized by damage and dysfunction of specific or multiple organs and tissues caused by abnormal immune responses at various functional parts of the body, including rheumatoid arthritis (RA), ankylosing spondylitis (AS), system lupus erythematosus (SLE), Graves' disease (GD), etc [1–3]. The diversity of ADs influences the prevalence of the condition, which is also influenced by a number of variables including genetic, environmental, infectious, and epigenetic factors [4, 5]. According to epidemiologic research, 5–10%

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of individuals worldwide may be affected by ADs, and the incidence is increasing gradually [6, 7].

As common ADs, the development of RA and AS is intimately associated with tissue damage and immune system dysfunction [8]. Following an autoimmune response, antibodies combine with self-tissues to become autoantibodies and mediate the autoimmune process along with immune cells and other mediators, which is the quintessential molecular mimicry hypothesis for the initiation of autoimmune disease [9, 10]. As an important component of the immune system, immune cells produce cytokines or interact with lymphocytes under the influence of factors of pathogenicity to regulate the dynamic balance and participate in tissue repair [11]. In general, functional and phenotypic defects in certain immune cells mediate the development of ADs, as well as large accumulations of adaptive cells (e.g., T-cell subpopulations, B-cells, plasma cells, and plasmacytoid cells) and innate immune cells (e.g., monocytes, dendritic cells, mast cells, and innate lymphocytes) that contribute to the development of ADs through the activation of cytokines that create positive feedback [12, 13]. For example, a study revealed that initial CD4 T cells from RA patients accelerate senescence and reprogram the metabolic machinery [14]. Upon stimulation, these initial T cells in RA differentiate into hyperproliferative and tissue-invasive effector cells that initiate and maintain aggressive tissue inflammation in the synovium [14]. It has been shown that the percentage of CD8+CD122+T cells in the peripheral blood of AS is higher than that of healthy controls, and that the number of CD27+B cells is decreased, while the number of CD86+and CD27+CD95+B cells is increased [15]. In addition, immunometabolism suggests that associated core metabolic pathways including glycolysis, lipid metabolism, and mitochondrial function can also affect the development of ADs [16].

Human plasma metabolites are the end products of a group of cellular regulatory processes that can be utilized to identify pathophysiological components of complex diseases and to illuminate the complex relationships between genotype and phenotype [17]. Understanding the causal role of metabolites in disease etiology can offer actionable intervention points for treatment. Several epidemiological studies have elucidated the underlying biological mechanisms of the association between specific metabolic profiles and various diseases by investigating the identification of specific metabolic characteristics [18]. The interactions between the metabolic system and the immune system, which are regulated by genetics, nutritional status, and the gut microbiome, have recently attracted considerable attention [19]. Metabolic epigenetics suggests that metabolites can induce permanent changes in cellular organization and genetic structure by modulating the epigenome [20]. A plausible hypothesis is that an aberrant metabolic process within the body may disrupt immune tolerance, with the subsequent aberrations of metabolites in immune cells leading to autoimmune responses [21]. Metabolites are not only involved in the metabolic processes of immune cells, but also transmit information between immune cells through mechanisms such as receptors, transporter proteins, and post-translational modifications [22]. Modified immune-metabolic interactions can lead to the development of ADs [23]. Although observational studies have attempted to elucidate the causal relationship between immune cells, metabolites, and ADs, it is difficult to establish a clear causal relationship because the results may be susceptible to bias due to unanticipated confounding factors or reverse causation [24].

To address confounders and determine causal relationships in observational studies, Mendelian randomization (MR) studies were developed based on Mendelian independent classification rules [25]. In MR studies, genetic variation follows the principle of random assignment of alleles to offspring, similar to randomized controlled experiments. According to the law of segregation assortment and the law of independent assortment, the assignment of genotypes from parent to offspring is random, and therefore the association between genetic variation and outcome is unlikely to be influenced by the environment [26]. Therefore, methods that utilize genetic variation as instrumental variables (IVs) to assess potential causal associations between exposures and outcomes can be effective in addressing confounders and reverse causation in observational epidemiology [27]. The objective of this study was to use a MR study aimed at revealing the potential causal relationship between immune cells and Ads. And importantly, we assessed the mediating role of metabolites on exposure-outcome through mediation analysis. This research approach enhances the comprehension of the pathogenesis and metabolic pathways of RA and AS, while also offering dependable evidence for the development of feasible screening and prevention strategies for ADs.

Materials and methods

Study design

Based on a two-sample MR study, we first assessed causality between exposure (731 immune cell characteristics) and outcome (RA and AS). In addition, the mediated (two-step approach) MR study was employed to delve deeper into the mediating effects between exposure and outcome mediated by metabolites as mediators (Fig. 1). Risk factors are represented by genetic variation also called IVs in MR study. Three key assumptions must be met by IVs in causal inference: (1) IVs must be strongly associated with exposure; (2) IVs are independent of possible confounders between exposure and outcome; and

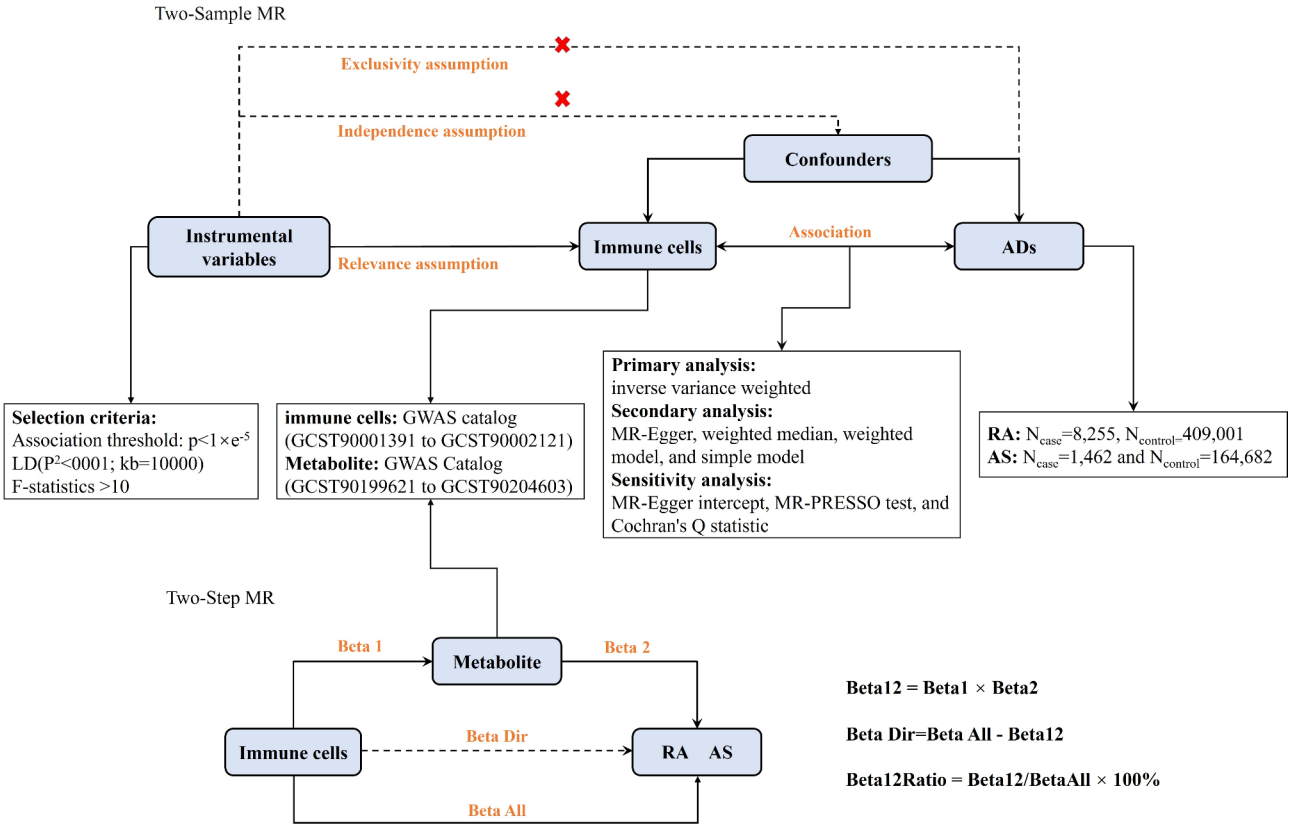

Fig. 1 Flowchart of the analysis performed. ADs, autoimmune diseases; RA, rheumatoid arthritis; AS, ankylosing spondylitis

(3) Genetic variation does not affect outcome through pathways other than exposure (no horizontal pleiotropy). (Fig. 1)

Data sources

The Genome-Wide Association Study (GWAS) catalog (GCST90199621 to GCST90201020) contains summary data for 731 immunophenotypes included in the study [32]. The data from 3757 Sardinians was used to calculate around 22 million genetic variations and validate relationships with autoimmune illnesses in the GWAS data on immunological characteristics. This data identifies molecules and mechanisms involved in the regulation of 459 cellular features by detecting 122 significant independently associated signals at 70 loci, including 118 absolute cell counts (AC), 192 relative cell counts (RC) representing the ratio of cellular levels, 389 median fluorescence intensity (MFI) representing surface antigen levels, and 32 morphological characteristics (MP). The GWAS data for plasma metabolites were retrieved from the GWAS Catalog (GCST0001391 to GCST0002121). The plasma metabolite data comprised 1,091 blood metabolites and 309 metabolite ratios [28]. Summary data can be obtained by visiting: https://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/.

Summary data for both AS and RA were obtained from the MRC-IEU consortium [IEU OpenGWAS project (https://gwas.mrcieu.ac.uk)]. RA included 417,256 participants of European ancestry (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018910/). AS included 166,144 participants of European ancestry (https://gwas.mrcieu.ac.uk/datasets/finn-b-M13_ANKYLOSPON/) [29].

Instrument variables selection

For IVs, we utilized a stringent quality control procedure to ensure data reliability and that sufficient SNPs were available for MR analysis. Therefore, based on previous studies, we set the genome-wide threshold for SNPs associated with two exposures (immune cells and metabolites) to 1×10^{-5} [30, 31]. In addition, we excluded SNPs that are not required using strand linkage disequilibrium (LD, $R^2<0.001$ and clumping distance = 10,000 kb) to ensure that IVs were independent of each other [32]. We selected SNPs with a minor allele frequency (MAF) > 0.01 and excluded palindromic SNPs [33]. Finally, we calculated and excluded F-statistics ($F=R^2(n\text{-}k\text{-}1)/k(1\text{-}R^2)$; R2, exposed variance explained by the selected instrumental variable, we obtained the value of R^2 in the MR Steiger directionality test; n, sample size; k, number of IVs) < 10 SNPs to avoid biasing the results by weak instrumental variables [34].

Statistical analysis

Mendelian randomization statistical analysis

A variety of methods were used to determine whether a causal relationship exists between immune cells with RA and AS, including inverse variance weighted (IVW) [35], MR-Egger [33], weighted median [36], weighted model [37], and simple model [38] methods. Of these, IVW employs meta-analysis to integrate the Wald estimates for each SNP to provide an overall estimate of the effect of exposure on outcome. When horizontal pleiotropy is absent, unbiased causal estimates can be developed by IVW linear regression. Thus, the IVW method is the most direct and important method for evaluating the presence of causality, with the remaining four modalities as auxiliary analysis methods. $P<0.05$ indicated that there was a significant causal relationship between exposure and outcome. In this study, we ensured that the results of all methods were in the same direction as IVW (i.e., the beta and odds ratio (OR) values were in the same direction) [39].

Mediation statistical analysis

In the present study, positive MR analysis was conducted to identify immune cells that were identified as having a potential relationship with RA and AS, respectively (causal effect value of BetaAll). In the subsequent mediation analysis, metabolites with a potential relationship with RA and AS were first identified (causal effect value of Beta2), followed by MR analysis of the above obtained immune cells and metabolites (causal effect value of Beta1). The mediating effect was calculated as Beta12=Beta1 × Beta2; the ratio of the mediating effect to the total effect: Beta12Ratio=Beta12/Beta All×100%; and the direct effect: BetaDirect=BetaAll - Beta12 (Fig. 1) [40]. We also calculated confidence (CI) intervals and *P*-values for the mediating ratio.

Sensitivity statistical analysis

By employing the MR-Egger regression [41] intercept analysis and MR-PRESSO test [33], we tested the results for horizontal pleiotropy. The intercept in MR-Egger regression can be used to assess horizontal pleiotropy, which if significant indicates the presence of horizontal pleiotropy, and vice versa, indicates the absence of horizontal pleiotropy. There are three components of MR-PRESSO, which include: horizontal pleiotropy test (MR-PRESSO global test), correction of horizontal pleiotropy by removing outliers (MR-PRESSO outlier test), and a test for significant differences in causal estimates before and after correcting for outliers (MRPRESSO distortion test).The MR-PRESSO outlier test requires that at least 50% of the genetic variance be valid IVs and relies on the instrumental strength to be independent of the direct effect (InSIDE) condition [33]. After excluding results with horizontal pleiotropy, we conducted subsequent analyses. Heterogeneity of SNP was tested using Cochran's Q statistic, with *P*-values<0.05 indicating significant heterogeneity [42]. In addition, to avoid horizontal pleiotropy caused by a single SNP, we performed "leave-one-out" sensitivity analysis to ensure the reliability of the results [43].

All MR analyses were conducted in R (version 4.3.2; R Foundation for Statistical Computing, https://www.r-project.org/foundation/) using the "TwoSampleMR", "VariantAnnotation", "gwasglue" and "data.table" packages [44].

Result

Summary data on immune cells and metabolites were screened for SNPs and subsequently analyzed by MR using threshold (1×10^{-5}), LD standards and F-statistics. Detailed IVs data are shown in Supplementary Tables 1 and 2. The results of the causal relationship between immune cells with RA and AS are shown in Supplementary Tables 3 and 4, Visualization of scatterplots in Supplementary Figs. 1 and 2.

Two-sample MR analysis

Causal effect of immunophenotypes on RA and AS risk

By MR analysis, we obtained a total of 32 immunophenotypes and 16 immunophenotypes causally associated with RA and AS, respectively (Fig. 2, Supplementary Table 5). The causal relationship between immune cells and RA showed that 19 immunophenotypes were genetically predicted to be associated with a reduced risk of RA and 13 immunophenotypes were associated with an increased risk of RA. The causal relationship between immune cells and AS showed that the genetic prediction of 4 immunophenotypes was associated with decreased risk of AS and 12 immunophenotypes were associated with increased risk of AS. Among them, CD11b on basophil was a common risk factor for RA (OR 1.050, 95% CI, 1.016–1.086; $P=0.004$) and AS (OR 1.111, 95% CI, 1.010–1.222; $P=0.03$).

Two-step MR analysis

Causal effect of metabolites on RA and AS

In the two-step MR analysis, we first performed MR analysis of metabolites with RA and AS to look for metabolites that had a causal relationship with outcome. The causal relationship between metabolites and RA showed that 18 metabolites were genetically predicted to be associated with a reduced risk of RA and 43 were associated with an increased risk of RA. These included 30 currently known metabolites, 11 metabolites not yet studied, and 20 metabolite ratios (Supplementary Table 6). The causal relationship between metabolites and AS showed that 24 metabolites were genetically predicted to be associated with a reduced risk of AS and 28 were associated with an

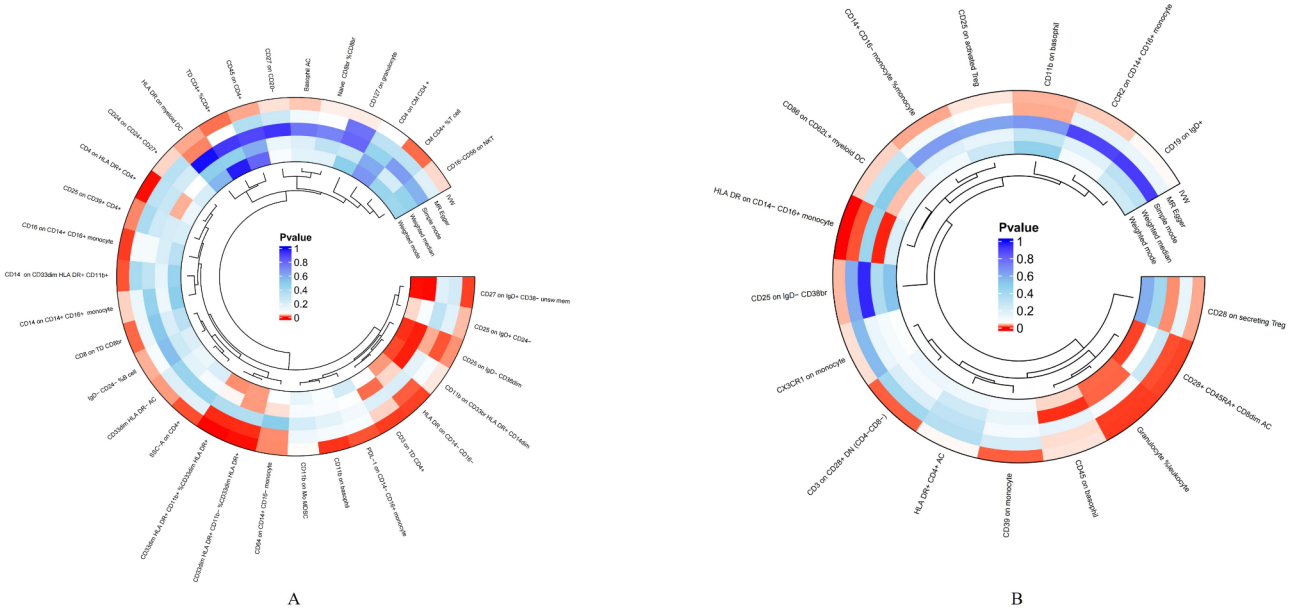

Fig. 2 Visualization of the causal relationship between immune cells and ADs. **(A)**, rheumatoid arthritis; **(B)**, ankylosing spondylitis

increased risk of AS. These included 27 currently known metabolites, 8 metabolites that have not been studied and 17 metabolite ratios (Supplementary Table 7). Among them, Sulfate of piperine metabolite C18H21NO3 (1) levels was a common risk factor for RA (OR 1.105, 95% CI, 1.018-1.200; $P=0.017$) and AS (OR 1.306, 95% CI, 1.015–1.682; $P=0.017$). In addition, Adenosine 5'-monophosphate (AMP) to serine ratio was positively correlated with RA (OR 1.113, 95% CI, 1.016–1.219; $P=0.021$) but negatively correlated with AS (OR 0.734, 95% CI, 0.582–0.926; $P=0.009$). Caffeine to linoleate (18:2n6) ratio was negatively correlated with RA (OR 0.891, 95% CI, 0.796–0.999; $P=0.048$) and positively correlated with AS (OR 1.246, 95% CI, 1.004–1.545; $P=0.046$). It should be mentioned that we excluded metabolites that had not been studied for subsequent MR analysis.

Causal effect of immune cells on metabolites

Specific immunophenotypes (IVW, $P<0.01$) were used for subsequent MR analysis with metabolites (Fig. 3). The immunophenotypes and outcome-related metabolites described above were subjected to MR analysis to determine mediating effects. In subsequent mediation analysis, we found that of the 11 immunophenotypes causally associated with RA, there were 9 immunophenotypes causally associated with a total of 15 metabolites. Three immunophenotypes causally associated with AS were causally associated with a total of 7 metabolites. (Supplementary Table 8)

Mediation analysis of metabolites

Finally, we performed mediation analysis and excluded metabolites with a P value of >0.05 for mediation effects (Table 1). The results showed that CD28+CD45RA+CD8dim AC mediated its effect on AS through Salicylate levels and Glucose to N-palmitoylsphingosine (d18:1 to 16:0) ratio with proportions of 10.4% ($P=0.013$) and 8.72% ($P=0.034$), respectively. CD3 on TD CD4+mediated its effect on RA through 4-cholesten-3-one levels with a proportion of 8.91% ($P=0.040$); PDL-1 on CD14- CD16+monocyte mediated its effect on RA through N-lactoyl isoleucine levels with a proportion of 8.91% ($P=0.040$); CD16 on CD14+CD16+monocyte and HLA DR on CD14- CD16- mediated their effects on RA through the common metabolite 3-phosphoglycerate to glycerate ratio at 12.9% ($P=0.021$) and 2.31% ($P=0.015$); CD14 on CD33dim HLA DR+CD11b+mediated its effect on RA through Gamma-glutamylhistidine levels and Citrulline to phosphate ratio, which were 9.54% ($P=0.018$) and 15.6% ($P=0.008$). (Fig. 4) These ratios emphasize the complex dynamic relationship between specific immune cells, metabolites with RA and AS.

Sensitivity analysis

The analysis performed using the MR-Egger regression intercept method and MR-PRESSO did not reveal any outliers, indicating that there was no cross-sectional pleiotropy ($P>0.05$) (Supplementary Tables 9 and 10). The possibility of horizontal pleiotropy was ruled out by MR-egger intercept (Supplementary Tables 11 and 12). In addition, Cochran's Q statistic, "leave-one-out" sensitivity analysis and funnel plots demonstrated the stability of the results (Supplementary Table 13, Supplementary Figs. 3, 4, 5 and 6).

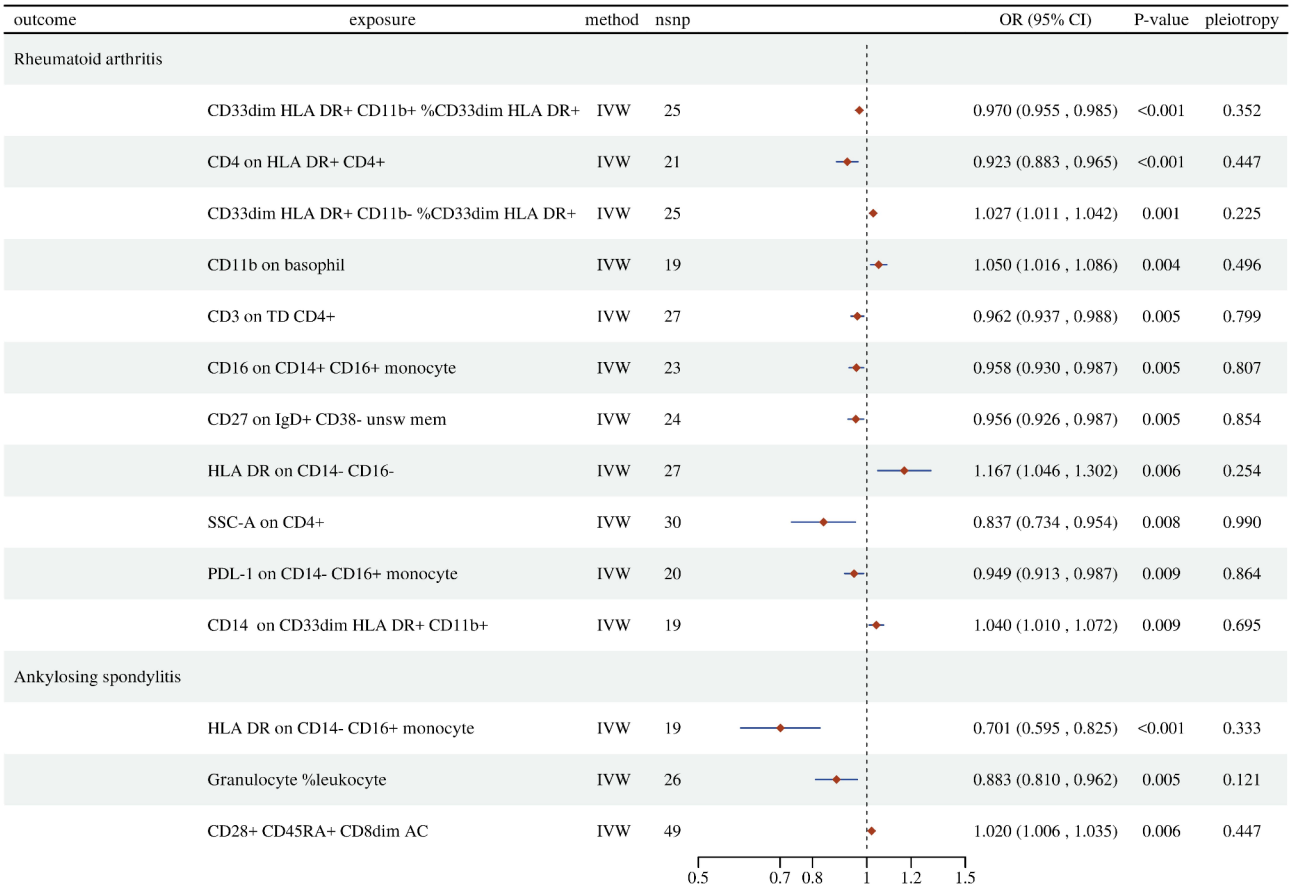

Fig. 3 Forest plot of the causal relationship between immune cells and ADs. (Inverse variance weighted, IVW, $P < 0.01$)

Table 1 The mediation effect of immune cells on RA and AS via metabolites

Exposure	Mediator	Outcome	Total effect βAll (95% CI)	Mediation effect β12 (95% CI)	*P*	Mediated proportion (%) (95% CI)
CD3 on TD CD4 + T cell	4-cholesten-3-one levels	RA	0.04(0.01, 0.07)	0.006(0.002, 0.010)	0.040	8.91(0.406, 17.4)
PDL-1 on CD14- CD16 + monocyte	N-lactoyl isoleucine levels	RA	0.04(0.01, 0.07)	0.004(0.001, 0.007)	0.040	13(0.621, 25.3)
CD16 on CD14 + CD16 + monocyte	3-phosphoglycerate to glycerate ratio	RA	0.15(0.04, 0.26)	0.004(0.000, 0.006)	0.021	12.9(1.92, 23.8)
HLA DR on CD14- CD16- monocyte		RA	-0.04(-0.07, -0.01)	-0.005(-0.010, -0.001)	0.015	2.31(0.441, 4.17)
CD14 on CD33dim HLA DR + CD11b + Myeloid cell	Gamma-glutamylhistidine levels	RA	-0.05(-0.09, -0.01)	-0.001(-0.013, -0.000)	0.018	9.54(1.66, 17.4)
	Citrulline to phosphate ratio	RA	-0.04(-0.06, -0.01)	-0.003(-0.006, -0.000)	0.008	15.6(4.17, 27.1)
CD28 + CD45RA + CD8dim AC Treg cell	Salicylate levels	AS	0.02(0.01, 0.03)	0.002(0.000, 0.004)	0.013	10.4(2.19, 18.6)
	Glucose to N-palmitoyl-sphingosine (d18:1 to 16:0) ratio	AS	0.02(0.01, 0.03)	0.002(0.001, 0.003)	0.034	8.72(0.646, 16.8)

Discussion

The complex relationship between immune cells, metabolites, and immune-mediated diseases has been a topic of interest in recent years. Immune cells and metabolites have a complex relationship that affects health and disease, and disruption of the dynamic balance can lead to the development of autoimmune diseases [10]. We have taken the summary data obtained from the GWAS database and used MR methods to delve deeper into the relationship between specific immune cells with RA and AS, and explored the mediating effects of metabolites in this context, shedding light on the complex relationship between the three in terms of gene prediction, providing compelling insights into this complex interaction, and confirming that some immunophenotypes can have an effect on RA and AS mediated by metabolites. This is the first time that we have used genetic prediction to investigate the potential causal relationships and

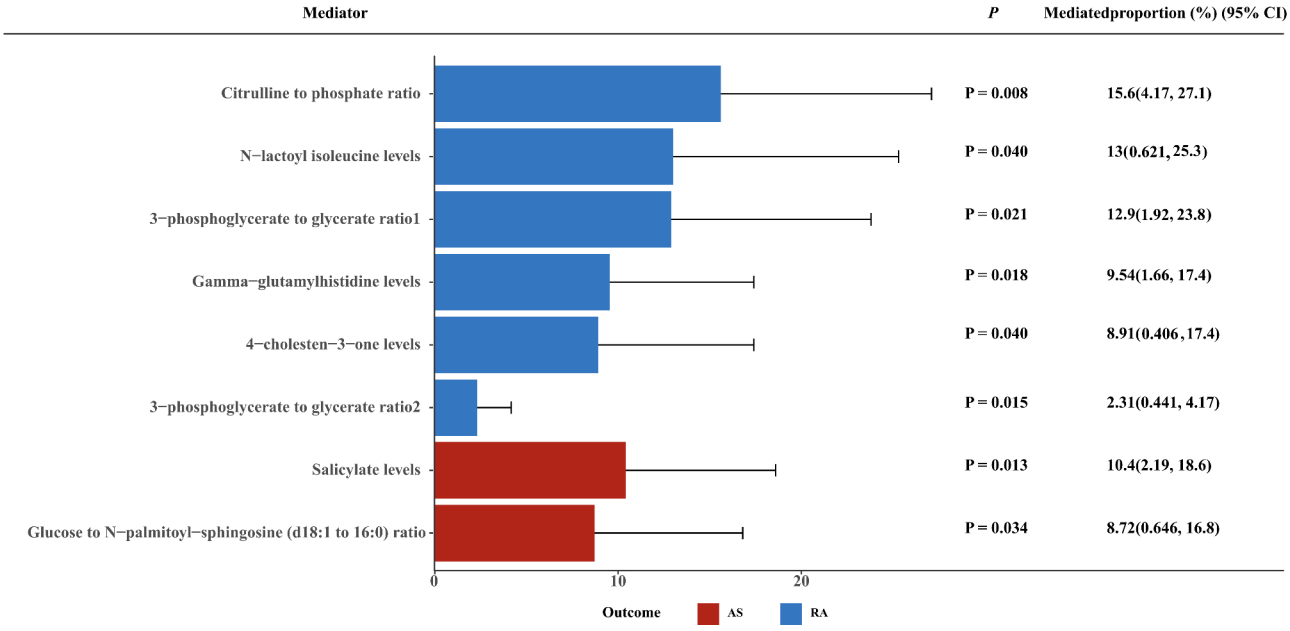

Fig. 4 Mediating proportions of each mediator in the causal relationship between immune cells and ADs

mediating effects among the above three, and our findings can better guide our understanding of RA and AS, as well as shed light on the prevention and treatment of the diseases.

The immunopathogenesis of RA, characterized by immune cell infiltration in the joints, has spanned decades [45, 46]. According to research, several immune cell types, including T cells, B cells, monocytes, and myeloid cells, have been related to the pathogenesis of RA [14]. In this work, we identified 32 immunophenotypes that are causally linked to RA genetically. More significantly, for the first time, we screened 5 immunophenotypes (CD3 on TD CD4+T cell, PDL-1 on CD14- CD16+monocyte, CD16 on CD14+CD16+monocyte, HLA DR on CD14- CD16- monocyte, CD14 on CD33dim HLA DR+CD11b+Myeloid cell) from this group, which demonstrated the potential to influence the progression of RA by modulating related metabolites. Innate blood cells, referred to as monocytes, are responsible for preserving vascular homeostasis and acting as early responders to pathogens in acute infections. Moreover, classical monocytes contain the ability to develop into macrophages in tissues, which can result in long-term illnesses [47]. Subgroups of peripheral blood monocytes can be distinguished by their CD14/CD16 surface characteristics. Our research indicates that the expression of PDL-1, CD16, and HLA DR in different monocyte subtypes influences (CD14- CD16+monocyte, CD14+CD16+monocyte, CD14- CD16- monocyte) the development of RA through specific metabolites. Previous studies have shown that chronic otitis media typically coexists with increased PDL-1, a checkpoint molecule in circulating monocytes [48]. However, in RA, more studies have focused on the role of PDL-1 in regulating the activity of peripheral T cells [49, 50]. Currently, there are just a few studies on N-lactoyl isoleucine, a critical metabolite in PDL-1 on CD14- CD16+monocyte-mediated RA illness. Only a single population-based longitudinal investigation demonstrated that N-lactoyl isoleucine is substantially linked to the development of diabetic retinopathy in individuals with type 2 diabetes mellitus [51]. Generally speaking, most human peripheral blood monocytes strongly express surface CD14, but not CD16 (CD14+ +/CD 16-) [52]. When evaluating the frequency of CD16+cells in all blood mononuclear cells between RA patients and healthy controls, Andrew et al. observed a statistically significant increase [52]. The involvement of HLA-DR in RA was brought to the attention of researchers long before 1989, whose study revealed that, compared to normal controls, patients with active RA presented lower densities of monocytes expressing HLA-DR [53]. This provides additional evidence that HLA-DR in peripheral monocytes can be employed as a therapeutic target to influence disease progression in RA. Surprisingly, our study also revealed that the 3-phosphoglycerate to glycerate ratio is the metabolite status that mediates the effects of CD16 on CD14+CD16+monocyte and HLA DR on CD14- CD16- monocyte on RA progression. Many studies have demonstrated the critical role that glucose metabolism plays in RA etiology. 3-phosphoglycerate is a glycolysis intermediate metabolite. Increased expression of phosphoglycerate kinase 1 (PGK1), a glycolytic enzyme related to 3-phosphoglycerate, in RA synovial tissue and blood may be associated

with the pro-inflammatory and synovial proliferation of the disease [54]. In addition, the research by Samantha et al. suggests the possibility that glycerate dysregulation is a key mechanism leading to early RA [55]. Undoubtedly, our study provides new insights into the matter.

According to this study, CD3 on TD CD4+T cells also mediates 4-cholesten-3-one levels affecting RA progression. Growing evidence reveals that RA is a T-cell-mediated autoimmune disease. Abnormalities in the quantity and function of CD4+T lymphocytes in the peripheral blood are strongly associated with the development and progression of RA [56]. Several studies have also indicated that CD4+T-cells and their cytokine products represent potential therapeutic and diagnostic targets in RA [57]. Xiong et al. examined the expression of CD3 in synovial tissues of RA and osteoarthritis (OA) patients, respectively, and revealed that the synovial tissues of the RA showed increased expression of CD3, suggesting that high levels of CD3 may promote the progression of RA [58]. Several of the most comprehensive studies concerning 4-cholesten-3-one focus on the metabolism of bile acids, which are not currently recognized to perform a role in RA [59]. Furthermore, CD14 on CD33dim HLA DR+CD11b+Myeloid cell can influence RA by modulating Gamma-glutamyl histidine levels and Citrulline to phosphate ratio. The first investigation into the function of Gamma-glutamyl histidine in RA was conducted in 1984 by Dixon et al., when researchers examined plasma from patients with RA and Behçet's syndrome and revealed biochemical variations in A between the two conditions [60]. Similar to 3-phosphoglycerate, Citrulline to phosphate ratio typically contributes significantly to the regulation of associated disorders through energy metabolism [61, 62].

Furthermore, this study discovered that AS is related to CD28+CD45RA+CD8dim AC Treg cell by modulating Salicylate levels and Glucose to N-palmitoyl-sphingosine (d18:1 to 16:0) ratio. AS is an autoimmune disease characterized by clonal proliferation of T-cells. Previous research has demonstrated that patients with AS exhibit different CD8+Treg cell differentiation patterns from normal participants, which may contribute to the pathogenesis of AS [63]. Compared to male controls, male patients with AS exhibited a 1.5- to 2-fold increase in Th17 (P=0.013) among CD45+lymphocytes [64]. A copper-salicylate preparation, permalon, earlier employed as an effective drug in the treatment of rheumatoid and other degenerative diseases [65]. It has been demonstrated that the metabolism of salicylic acid regulates immunity to promote both growth and defense in plants, which implies that it could have an impact on AS progression via the same pathways [66]. A sphingolipid involved in cell signaling, ceramide, frequently accumulates in tissues and plasma during metabolic dysfunction, dyslipidemia and inflammation. N-palmitoyl-sphingosine (d18:1 to 16:0) is a special type of ceramide. Increased plasma N-palmitoyl-sphingosine (d18:1 to 16:0) may be substantially correlated with inflammatory bowel disease, according to a study of mucosal and plasma metabolomes in new-onset pediatric inflammatory bowel disease [67]. However, the above metabolite profiles have not been investigated in AS patients. Undoubtedly, our findings will enrich the theory of metabolite pathogenesis in AS patients.

Overall, this study has several strengths. Firstly, a total of 32 immunophenotypes and 16 immunophenotypes may be causally associated with RA and AS employing MR analysis. Secondly, our study also identified 61 and 52 metabolites at the genetic level that may contribute to the progression of RA and AS, respectively. Importantly, we also found that specific immunophenotypes may influence disease progression by regulating specific metabolites, which were not mentioned in previous studies. Without a doubt, this research offers fresh perspectives on the etiology of particular autoimmune diseases, such as RA and AS. Our study is not without limits, though. First, given that the majority of those participating in the data aggregated in this study were of European descent, the ability to extrapolate findings to other ethnic groups may be limited. Second, the current research samples were not evaluated by additional subgroup stratification analysis since individual patient information is not currently included in the GWAS data. Third, while immunophenotypes and metabolites that might be associated with the development of RA and AS were identified by MR analysis, biological mechanisms should also be taken into account when interpreting the data. Biological mechanisms are not disclosed by the statistical effect values alone. This means, of course, that stronger biological evidence is needed to support this study.

Conclusions

In conclusion, we employed MR analysis to further clarify the patterns of RA and AS interactions with the immune system as well as provide insight into the associations between immunological phenotypes, metabolites, and diseases. In addition to offering believable genetic evidence, this finding also provides valuable guidelines for future mechanistic and clinical research on ADs.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-024-03445-z.

Supplementary Material 1: Table 1 IVs of immune cells after removal of linkage disequilibrium and weak instrumental variables; Table 2 IVs of Metabolites after removal of linkage disequilibrium and weak instrumental variables; Table 3 Causal relationship between immune cells and RA; Supplementary Table 4 Causal relationship between immune cells and AS; Supplementary Table 5 Immune cells with IVW less than 0.05 in the

causal outcome of immune cells and RA and AS; Table 6 Metabolite with IVW less than 0.05 in the causal outcome of immune cells and RA; Table 7 Metabolite with IVW less than 0.05 in the causal outcome of immune cells and AS; Table 8 Causal effect of immunophenotypes on metabolites; Table 9 MR-PRESSO test of immune cells and rheumatoid arthritis; Table 10 MR-PRESSO test of immune cells and ankylosing spondylitis; Table 11 MR analytical horizontal pleiotropy test of immune cells and ADs; Table 12 MR analytical horizontal pleiotropy test of metabolite and ADs; Table 13 MR analytical Cochran's Q statistic of immune cells and ADs.

Supplementary Material 2: Fig. 1 Scatter plots for the causal association between immune cells and AS (Supplementary figures were numbered using the last four digits of the GWAS ID of the immune cells, and this applies to the rest of the supplementary file);

Supplementary Material 3: Fig. 2 Scatter plots for the causal association between immune cells and RA;

Supplementary Material 4: Fig. 3 Funnel plots for the causal association between immune cells and AS;

Supplementary Material 5: Fig. 4 Funnel plots for the causal association between immune cells and RA;

Supplementary Material 6: Fig. 5 Leave-one-out plots for the causal association between immune cells and AS;

Supplementary Material 7: Fig. 6 Leave-one-out plots for the causal association between immune cells and RA.

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Author contributions
WJP and LJ designed the study and performed the statistical analysis; WJP, LJ, LTY, and XT participated in data interpretation; WJP, LTY, and ZYC wrote the first draft, and LJ, YSH, WH and HHH revised the article. WH and HHH had direct access and responsibility for verifying all data reported in the manuscript. All authors read and approved the submitted version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All summarized statistics utilized in the MR analyses were generated by previous studies, for which ethical approval and individual consent were obtained for all original studies.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Data sharing statement
Summary statistics for GWAS are publicly available for download. All scripts for the analysis are available from the authors upon request.

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