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Original Article

Bidirectional causal relationship between rheumatoid arthritis and systemic lupus erythematosus: a Mendelian randomization study

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Abstract: Background: Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are systemic, autoimmune inflammatory diseases. Although overlapping clinical and genetic features have been reported, the presence of a direct causal relationship between them remains uncertain. Methods: A bidirectional two-sample Mendelian randomization (MR) analysis was performed using genetic variants as instrumental variables to evaluate potential causal associations between RA and SLE. Sensitivity analyses were performed to assess heterogeneity, horizontal pleiotropy and the robustness of the findings. Results: Genetically predicted RA did not have a significant causal effect on susceptibility to SLE. Conversely, genetically predicted SLE did not demonstrate a significant causal effect on RA risk. Sensitivity analyses supported the stability and reliability of these findings, with no evidence of substantial heterogeneity or directional pleiotropy. Conclusion: We concluded that RA and SLE may share genetic susceptibility and environmental triggers without a unidirectional causal relationship.

Keywords: Systemic lupus erythematosus, rheumatoid arthritis, Mendelian randomization, autoimmune diseases, genome-wide association study

Introduction

Autoimmune diseases are characterized by immune system dysfunction, in which normal tissues and organs are mistakenly targeted and damaged [1, 2]. A central feature of these disorders is the production of autoantibodies, which trigger sustained immune activation [3]. Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are prototypical autoimmune diseases that share overlapping immunopathogenic mechanisms and certain clinical manifestations [4].

The global incidence of SLE has been estimated at 43.7 (15.87-108.92)/100,000 individuals, with approximately 3.41 million affected worldwide [5]. This disease shows significant variation across sex, age, and some ethnic groups [6]. SLE is classically considered a type III hypersensitivity disorder and is characterized by the production of autoantibodies against double-stranded DNA, histones, nucleosomes and other chromatin components [7]. RA is a common systemic autoimmune disease of unclear etiology. It is characterized by the production of autoantibodies in the synovium, leading to chronic synovial inflammation and joint destruction [8]. In addition to synovial hyperplasia and persistent inflammation, RA may result in severe systemic complications [9].

Both SLE and RA are associated with systemic inflammation and multi-organ involvement, including the brain and vascular system [10]. Observational studies reported that some patients with RA may develop a lupus-like syndrome after penicillamine treatment [11, 12]. Although RA and SLE exhibit overlapping clinical and serological features, observational studies cannot determine whether their co-occurrence reflects shared genetic and environmental susceptibility or a direct causal influence of one disease on the other. In this study, this limita-

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tion was addressed by applying Mendelian randomization (MR) to evaluate the potential lifelong causal relationship between RA and SLE.

MR is based on the random allocation of single nucleotide polymorphism (SNP) at conception [13]. Genetic variants are randomly inherited from parents and are generally independent of postnatal environmental influences [14]. This approach relies on three core assumptions: genetic variants are associated with the exposure of interest and are not associated with confounders, and they influence the outcome only through exposure [15, 16]. By leveraging genetic associations, potential causal relationships between exposures and outcomes can be inferred [17]. MR reduces confounding factors and reverse causation compared with traditional observational studies, thereby strengthening causal inference [18]. Additionally, publicly available genome-wide association data can be used for two-sample MR analyses [19]. Accordingly, a bidirectional two-sample MR analysis was performed to investigate the potential causal relationship between SLE and RA.

Methods

Patients

Thirteen patients with SLE, thirteen patients with RA, and thirteen healthy volunteers aged between 18 and 84 years were recruited at the Affiliated Hospital of Nantong University for this investigation. The study received approval from the Ethics Committee of the Affiliated Hospital of Nantong University (2017-K003). Fresh peripheral blood and serum samples, each totaling 5 mL, were collected from individuals diagnosed with SLE or RA, as well as from healthy subjects, for the purpose of conducting quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA).

qRT-PCR assay

Total RNA was isolated utilizing TRIzol Reagent (15596026, Invitrogen). Approximately 500 ng of RNA was obtained from each sample for the reverse transcription process. The PrimeScript™ II 1st Strand cDNA Synthesis Kit (6210A, Takara) was employed for reverse transcription, resulting in the synthesis of the first strand cDNA. TB Green Premix Ex TaqII (RR820, Takara) served as the fluorescent reporter dye, and qPCR analysis was conducted using the LightCycler® 96 Real-Time PCR System (Roche). Primer sequences were as follows: Signal transducer and activator of transcription 4 (STAT4)-F: AAGGGATGGGTAGCCAGGAT; STAT4-R: AGGAGGCTAGGTCAGGAAGG; Interferon regulatory factor 5 (IRF5)-F: TCTTCCTCCTCCTCTCCTGC; IRF5-R: GGACTTCCGCCTCATCTACG; Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-F: GTCAAGGCTGAGAACGGGAA; GAPDH-R: AAATGAGCCCCAGCCTTCTC.

Tumor necrosis factor-α (TNF-α) assay

The serum levels of TNF-α in patients with SLE or RA, along with those in healthy volunteers, were assessed utilizing a commercial ELISA kit (MULTI SCIENCES, EK182) in accordance with the manufacturer's guidelines.

Source of GWAS data for exposure and outcome

Genome-wide association study (GWAS) datasets derived from comparable populations were selected for both exposure and outcome analyses to ensure balanced sex representation and minimize population stratification bias. The GWAS dataset for SLE (ebi-a-GCST003156) was obtained from the IEU OpenGWAS database, which included 14,267 samples and 7,071,163 SNPs (https://GWAS.mrcieu.ac.uk/). The GWAS dataset for RA (ebi-a-GCST005569) was retrieved from the same database, with 47,580 samples and 112,654 SNPs. Detailed information on the selected GWAS datasets is summarized in **Table 1**.

Bidirectional MR analysis

A bidirectional univariate MR analysis was performed to evaluate the causal association between SLE and RA. SNPs strongly associated with the exposure were selected using a genome-wide significance threshold ($P < 5 \times 10^{-8}$). SNPs were pruned using independence criteria ($r^2 < 0.001$, distance = 10,000 kb) to minimize bias due to linkage disequilibrium (LD). Selected SNPs were further screened in PhenoScanner (www.PhenoScanner.medschl.cam.ac.uk) and SNPs associated with potential confounding factors or outcomes were excluded.

Instrument strength was assessed using the F-statistic ($F = R^2(N-1-k)/(1-R^2)k$ or $F = \beta^2/se^2$) to

Table 1. GWAS datasets for RA and SLE

	Systemic Lupus Erythematosus	Rheumatoid Arthritis
Population	European	European
Ncase	5201	13838
Ncontrol	9066	33742
Sample size	14267	47580
SNPs	7071163	112654
GWAS ID	ebi-a-GCST003156	ebi-a-GCST005569

GWAS, genome-wide association studies; Ncase, number of cases; Ncontrol, number of controls; SNPs, single-nucleotide polymorphisms; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.

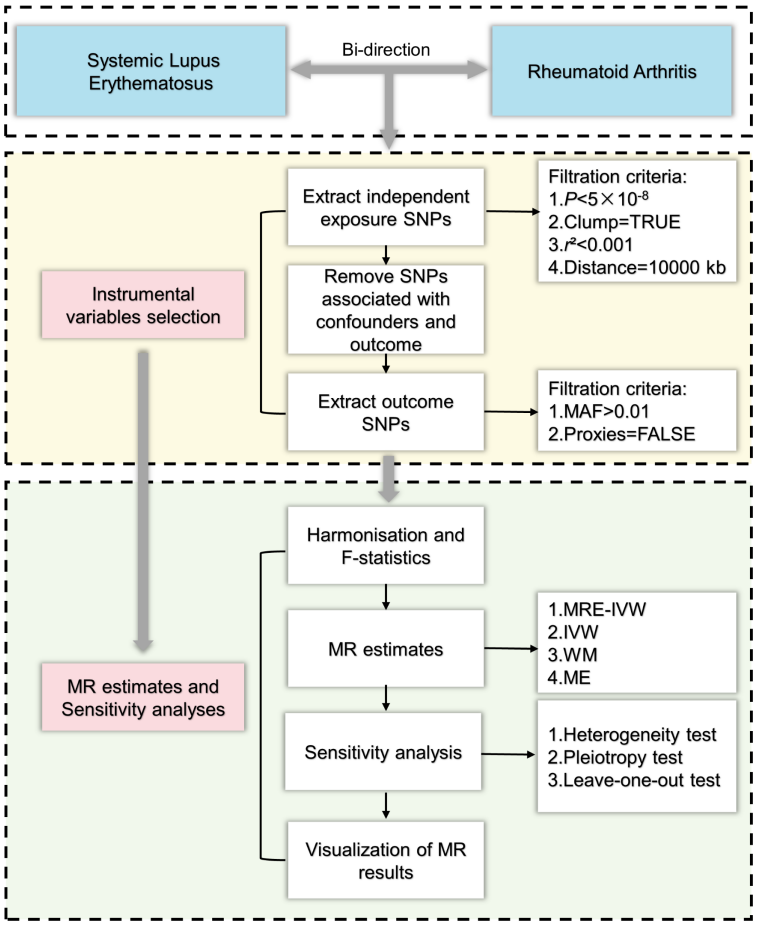

Figure 1. Flow chart of the bidirectional two-sample MR study. GWAS, genome-wide association studies; SNPs, single-nucleotide polymorphisms; MAF, minor allele frequency; MR, Mendelian randomization; IVW, inverse variance weighted; MRE-IVW, multiplicative random effects-inverse variance weighted; WM, weighted median; ME, MR-Egger.

reduce weak instrument bias [20, 21]. SNP-outcome effect estimates were extracted from the corresponding GWAS dataset, with a minor allele frequency (MAF) threshold > 0.01. Causal estimates were calculated using multiple MR methods, including multiplicative random effects-inverse variance weighted (MRE-IVW), inverse variance weighted (IVW), weighted median (WM), and MR Egger (ME) regression. The IVW method was considered the primary analysis under the assumptions that all instrumental variables satisfied the core MR assumptions. The WM method provides reliable estimates when at least 50% of the weight originates from valid instruments [22]. ME allows the detection of directional pleiotropy through its intercept term, providing adjusted estimates when pleiotropy is present. The RE-IVW method accounts for heterogeneity among instruments, yielding more conservative estimates when heterogeneity is present.

Statistical analysis

Heterogeneity among instrumental variables was assessed using Cochran Q statistic and visualized with funnel plots [23, 24]. The MRE-IVW model was applied when significant heterogeneity was detected ($P < 0.05$). A leave-one-out analysis was performed to evaluate the influence of individual SNPs on the overall estimates and to assess result stability [25]. Horizontal pleiotropy was examined using the ME intercept test. A statistically significant intercept ($P < 0.05$) indicated potential directional pleiotropy [26]. A flow chart of the study design is represented in **Figure 1**.

Functional experimental data are depicted as mean + standard error of the mean (SEM). Statistical analysis was carried out using GraphPad Prism software Version 6.01 (GraphPad Inc., La Jolla, CA, USA). One-way ANOVA was utilized for comparisons among multiple groups with Dunnett's comparisons test. Significance levels

Table 2. SNPs used as valid instrumental variables for RA on SLE

SNP	Expose	Outcome	Chr	EA	OA	B (expose)	B (outcome)	SE (expose)	SE (outcome)	EAF	P (expose)	P (outcome)	KEEP	F-statistic
rs10209110	RA	SLE	2	T	C	-0.1058	-0.04085	0.018527	0.029876	NA	1.13E-08	0.171476	TRUE	32.61042
rs17630466	RA	SLE	4	G	A	0.126721	-0.0202	0.020018	0.02481	NA	2.45E-10	0.415467	TRUE	40.07417
rs2228145	RA	SLE	1	C	A	-0.1087	0.04879	0.019124	0.028077	NA	1.32E-08	0.082262	TRUE	32.30745
rs2240339	RA	SLE	1	T	C	-0.1125	0.00995	0.01981	0.028124	NA	1.36E-08	0.723486	TRUE	32.24778
rs2812378	RA	SLE	9	A	G	-0.11038	0.02020	0.019415	0.030501	NA	1.31E-08	0.507745	TRUE	32.32107
rs3129294	RA	SLE	6	C	A	-0.17698	-0.04082	0.01994	0.033437	NA	6.96E-19	0.222144	TRUE	78.77635
rs8026898	RA	SLE	15	A	G	0.124604	-0.01005	0.020834	0.029432	NA	2.22E-09	0.732745	TRUE	35.7686
rs9262218	RA	SLE	6	T	C	-0.44723	0.10436	0.069481	0.086959	NA	1.22E-10	0.230098	TRUE	41.43108

SNPs, single-nucleotide polymorphisms; Chr, chromosome; EA, effect allele; OA, other allele; SE, standard error; EAF, effect allele frequency; β, beta coefficient; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; P, *P*-value; F-statistic, Fisher's F-statistic.

Table 3. SNPs used as valid instrumental variables for SLE on RA

SNP	Expose	Outcome	Chr	EA	OA	B (expose)	B (outcome)	SE (expose)	SE (outcome)	EAF	P (expose)	P (outcome)	KEEP	F-statistic
rs1143679	SLE	RA	16	A	G	0.58221	0.038451	0.039987	0.029505	NA	5.03E-48	0.1925	TRUE	212.0017
rs17849501	SLE	RA	1	T	C	0.81093	0.071483	0.049864	0.062535	NA	1.81E-59	0.253	TRUE	264.4777
rs2431697	SLE	RA	5	C	T	-0.22314	-0.03376	0.029296	0.018771	NA	2.60E-14	0.072059	TRUE	58.01522
rs2459611	SLE	RA	2	T	C	0.26136	0.036249	0.045245	0.030838	NA	7.62E-09	0.2398	TRUE	33.36981
rs35000415	SLE	RA	7	T	C	0.58778	0.11876	0.041539	0.0279	NA	1.86E-45	2.08E-05	TRUE	200.2294
rs353608	SLE	RA	11	G	A	0.18633	-0.02946	0.02802	0.018569	NA	2.93E-11	0.1126	TRUE	44.22171
rs389884	SLE	RA	6	G	A	0.92821	-0.06507	0.043232	0.0291	NA	2.92E-10	0.02534	TRUE	460.9908
rs4916215	SLE	RA	1	T	C	0.22314	0.053612	0.033969	0.02116	NA	5.07E-11	0.01129	TRUE	43.15163
rs58688157	SLE	RA	11	G	A	-0.22314	-0.01177	0.033565	0.020995	NA	2.97E-11	0.5751	TRUE	44.19823
rs6889239	SLE	RA	5	C	T	0.27763	0.070086	0.03174	0.021041	NA	2.19E-18	0.000866	TRUE	76.51123
rs7097397	SLE	RA	10	A	G	-0.18633	0.006876	0.028712	0.019429	NA	8.60E-11	0.723401	TRUE	42.11577
rs73068668	SLE	RA	19	A	G	-0.31471	-0.01765	0.05749	0.035769	NA	4.40E-08	0.6216	TRUE	29.96641
rs7768653	SLE	RA	6	T	C	-0.20701	-0.04421	0.029689	0.018744	NA	3.11E-12	0.01835	TRUE	48.61893

SNPs, single-nucleotide polymorphisms; Chr, chromosome; EA, effect allele; OA, other allele; SE, standard error; EAF, effect allele frequency; β, beta coefficient; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; P, *P*-value; F-statistic, Fisher's F-statistic.

Table 4. Bidirectional two-sample MR analysis between RA and SLE

Exposure	Outcome	Method	nSNP	OR	95% CI	*P* value
RA	SLE	IVW	8	0.931	0.78-1.11	0.419
		ME		0.899	0.52-1.57	0.720
		WM		0.884	0.72-1.09	0.245
		MRE-IVW		0.931	0.78-1.11	0.419
SLE	RA	IVW	13	1.061	0.99-1.14	0.100
		ME		0.992	0.87-1.13	0.910
		WM		1.063	0.99-1.14	0.105
		MRE-IVW		1.061	0.99-1.14	0.100

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNPs, single-nucleotide polymorphisms; IVW, inverse variance weighted; ME, MR-Egger; WM, weighted median; MRE-IVW, multiplicative random effects-inverse variance weighted; OR, odds ratio; CI, confidence interval; P > 0.05.

were denoted as follows: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Results

Extraction of instrumental variables for MR

A bidirectional two-sample MR analysis was performed. Independent SNPs strongly associated with the exposure were identified within a 10,000 kb window using a genome-wide significant threshold of $P < 5 \times 10^{-8}$, prioritizing SNPs showing low correlation with other SNPs in the region (LD, $r^2 < 0.001$). These SNPs were screened in PhenoScanner to remove those associated with potential confounders and outcomes. Common risk factors for SLE included, but were not limited to smoking, alcohol consumption, obesity, endometriosis, silica exposure, air pollution, infections, and vitamin D deficiency [27, 28]. Established risk factors for RA included smoking, unhealthy lifestyle choices, dietary factors, and infections [29].

When RA was treated as the exposure variable, 15 SNPs significantly associated with RA were initially identified. After PhenoScanner screening, rs11758312, rs13426947, and rs6679677 were excluded due to strong associations with the outcome. Additionally, rs3129886, rs660895, rs6920220, and rs71624119 were excluded because they were associated with immune diseases, including Crohn's disease and inflammatory bowel disease. Ultimately, 8 instrumental variables were identified for MR analysis (**Table 2**). In the reverse MR analysis, SLE was considered as the exposure variable, and two SNPs, namely rs58721818 and rs6679677, were removed using the same exclusion criteria previously described (**Table 3**), and 13 instrumental variables were retained for further analysis.

SNP effect estimates were extracted from the corresponding outcome GWAS datasets and filtered using MAF threshold > 0.01. After harmonization of exposure and outcome alleles, as well as exclusion of weak instrument F-statistics < 10 or incongruent SNPs, effective instrumental variables were identified and included in MR analysis using MRE-IVW, IVW, WM and ME regression methods.

Causal effects of RA on SLE

Eight instrumental variables were identified to evaluate the causal association between RA (exposure) and SLE (outcome), and the *P* values from the MR analyses exceeded 0.05 (IVW: OR = 0.931, 95% CI 0.78-1.11, *P* = 0.419; ME: OR = 0.899, 95% CI 0.52-1.57, *P* = 0.720; WM: OR = 0.884, 95% CI 0.72-1.09, *P* = 0.245; MRE-IVW: OR = 0.931, 95% CI 0.78-1.11, *P* = 0.419), indicating a lack of significant correlation (**Table 4** and **Figure 2**).

Causal effects of SLE on RA

A total of 13 SNPs were chosen for evaluation through the reverse MR analysis, with the outcomes (**Table 4** and **Figure 2**) also yielding non-significant results (IVW: OR = 1.061, 95% CI 0.99-1.14, *P* = 0.100; ME: OR = 0.992, 95% CI 0.87-1.13, *P* = 0.910; WM: OR = 1.063, 95% CI 0.99-1.14, *P* = 0.105; MRE-IVW: OR = 1.061, 95% CI 0.99-1.14, *P* = 0.100).

Sensitivity analysis

F-statistics were calculated for each instrumental variable to assess instrument strength, and all selected variants exceeded the threshold of 10 (**Tables 2** and **3**). Heterogeneity among instrumental variables used in the present MR analysis was evaluated using Cochran's Q statistics under both MR-Egger (ME) and inverse

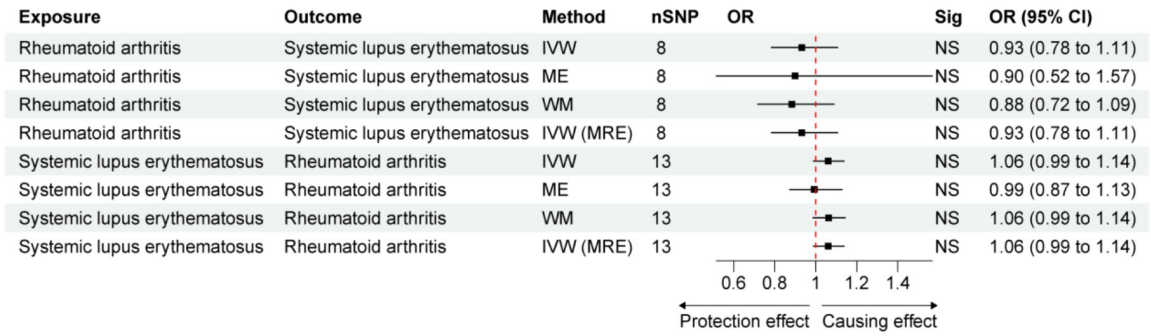

Figure 2. Forest plots of the bidirectional causal relationships between SLE and RA indicating no significant causal effect. Forward MR (RA to SLE) did not identify a significant causal effect of RA on SLE, and reverse MR (SLE to RA) similarly showed no evidence of a direct genetic causal association. RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism; IVW, inverse variance weighted; ME, MR Egger; WM, Weighted median; IVW (MRE), multiplicative random effects inverse variance weighted; nSNP, number of single-nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; NS, not significant.

Table 5. Heterogeneity tests in univariable MR analysis

Exposure	Outcome	Method	Cochran's Q	Q-df	Q-*P*-value
RA	SLE	ME	8.36	6	0.21
		IVW	8.38	7	0.30
SLE	RA	ME	41.12	11	2.30×10^{-5}
		IVW	46.54	12	5.59×10^{-6}

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; ME, MR-Egger; IVW, Inverse variance weighted; Q-df, Q-degrees of freedom; Q-*P* value, *P* value of Cochran's Q test; Q-*P* value (RA on SLE) > 0.05; Q-*P* value (SLE on RA) < 0.05.

variance weighted (IVW) models. No significant heterogeneity was found for the effect of RA on SLE (ME: value = 0.21; IVW: Q-*P* value = 0.30) (**Table 5**). However, significant heterogeneity was observed for the effect of SLE on RA (ME: Q-*P* value = 2.30×10^{-5}; IVW: Q-*P* value = 5.59×10^{-6}) (**Table 5**). Funnel plots demonstrated a symmetrical distribution of instrumental variables for the impact of RA on SLE, but not for the impact of SLE on RA (**Figure 3**), consistent with the heterogeneity findings. The MRE-IVW method was prioritized for the SLE on RA analysis due to the presence of significant heterogeneity coupled with funnel plot asymmetry, as this method accounts for between-instrument variability and provides more conservative estimates. The MRE-IVW analysis yielded an OR of 1.061 (95% CI 0.99-1.14; *P* = 0.100) indicating no statistically significant causal effect of SLE on RA. This finding was the most reliable estimate.

Pleiotropy was assessed using the ME intercept test, which indicated no evidence of directional pleiotropy in the instrumental variables employed to explore the causal relationship between RA and SLE (**Table 6**). Additionally, a leave-one-out analysis was performed to evaluate the influence of individual SNPs on the overall causal estimation by systematically excluding each SNP and reiterating the MR analyses. The results remained consistent even after sequential removal of each SNP, as illustrated in **Figure 4**.

Relevant indicator evaluation in RA and SLE

We analyzed serum TNF-α, a pivotal inflammatory cytokine implicated in the pathophysiology of both RA and SLE [30, 31]. As anticipated, patients with RA or SLE exhibited markedly elevated circulating TNF-α compared with healthy controls (**Figure 5A**), reaffirming the immune-mediated inflammatory nature of both diseases. We next investigated two biomarkers - IRF5 and STAT4 - that have previously been linked to SLE and RA [32-34]. qRT-PCR analysis on peripheral blood showed that both IRF5 and STAT4 mRNA expression levels were significantly up-regulated in individuals with SLE and RA relative to healthy controls (**Figure 5B** and **5C**). Integrating these findings with our MR analysis, the convergence of elevated TNF-α, IRF5, and STAT4 points to a common inflammatory network underlying RA and SLE, despite the absence of a direct causal relationship between the two conditions at the genetic level.

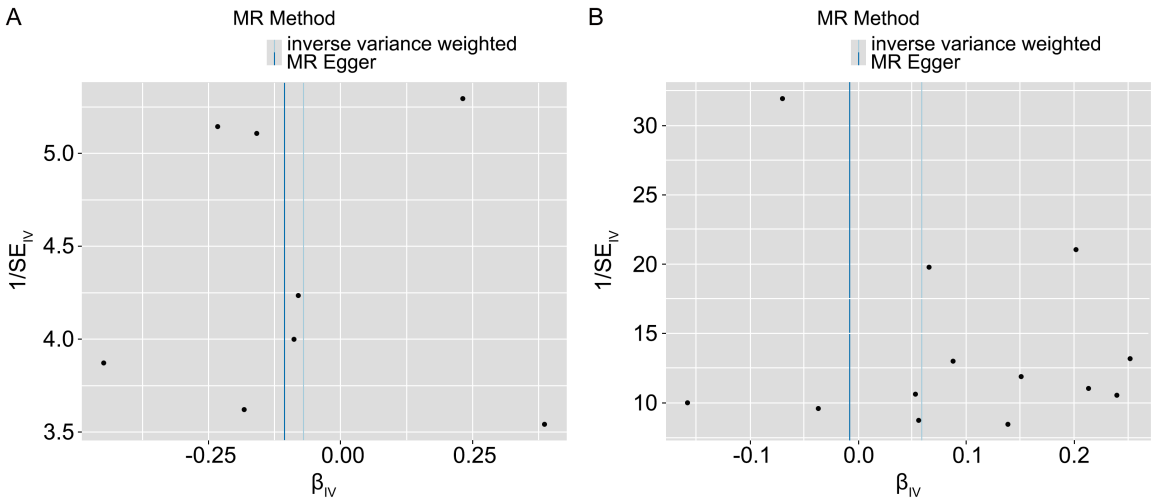

Figure 3. Funnel plot analysis between SLE and RA. A. Funnel plot for RA on SLE demonstrating no heterogeneity. B. Funnel plot for SLE on RA indicating the presence of heterogeneity. RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SE, standard error; IV, instrumental variable; β, beta coefficient.

Table 6. Pleiotropy tests in univariable MR analysis

Exposure	Outcome	MR-Egger-intercept	SE	*P* value
RA	SLE	5.12E-03	0.038	0.898
SLE	RA	0.028	0.023	0.254

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SE, standard error; MR, Mendelian randomization; P > 0.05.

Discussion

This study evaluated the potential bidirectional direct causal relationship between RA and SLE using MR framework. No significant evidence of a direct genetic causal association was identified in either direction. These findings suggested that the observed clinical overlap between RA and SLE was unlikely to be explained by a unidirectional genetic causal pathway.

RA and SLE share several clinical manifestations, including joint pain, swelling, systemic inflammation, and multi-organ involvement [35, 36]. Sequential or overlapping presentations have been reported in some patients, and the coexistence of both conditions is termed 'Rhupus' [37, 38]. Furthermore, overlapping genetic susceptibility loci have been described, indicating a potential connection between the two diseases [39, 40]. However, pathogenic and biological mechanisms are different. SLE is characterized by dysregulated autoantibody production and complement activity, while RA is driven largely by synovial inflammation mediated by cytokines and macrophages [41, 42]. These distinct mechanisms may explain why no direct genetic causal relationship was detected.

Several interpretations may be considered. First, shared genetic susceptibility loci may increase overall autoimmune predisposition without establishing a direct causal pathway between the two diseases [43]. Many of these loci encode broadly acting immunomodulatory molecules rather than disease-specific effectors. These gene mutations may simultaneously increase the body's susceptibility to multiple autoimmune reactions, but do not establish a unidirectional and inevitable causal relationship between RA and SLE. Second, autoimmune diseases arise from complex interactions between genetic background and environmental exposure [3]. Environmental triggers may differentially influence disease expression in individuals with similar genetic backgrounds [44]. For example, individuals carrying specific HLA genotypes are associated with RA in the context of smoking [45], while ultraviolet exposure has been linked to SLE under the same genotype [46]. Such gene-environment interaction may lead to temporal overlap in the same individual, without implying genetic causation. Epigenetic regulation may further mediate the interaction between environmental exposure and immune activation [47]. Persistent immune dysregulation may facilitate autoimmune res-

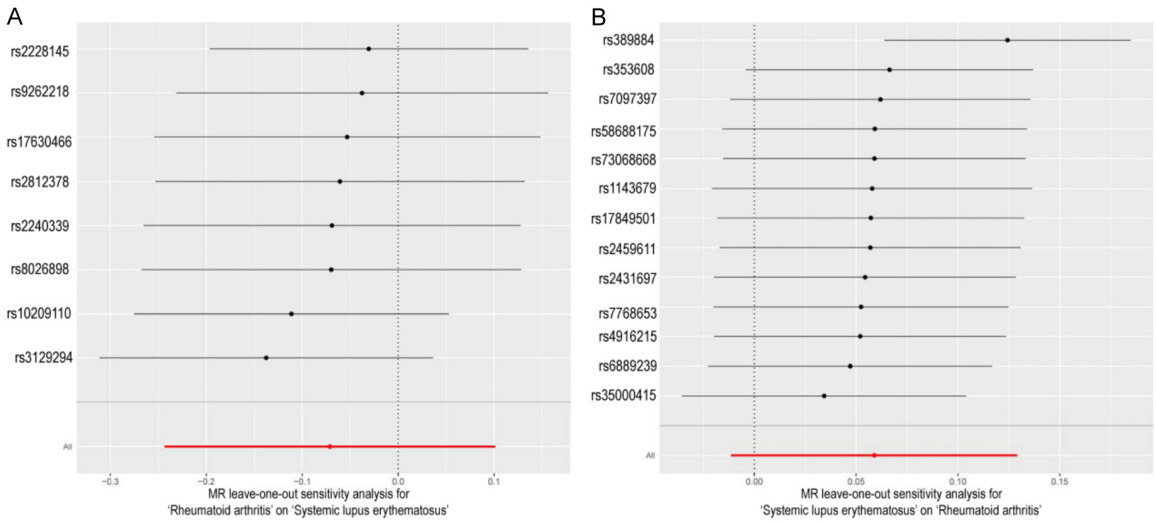

Figure 4. Leave-one-out sensitivity analysis between SLE and RA showing the stability of the results. A. Leave-one-out plot for RA on SLE. B. Leave-one-out plot for SLE on RA. SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; MR, Mendelian randomization.

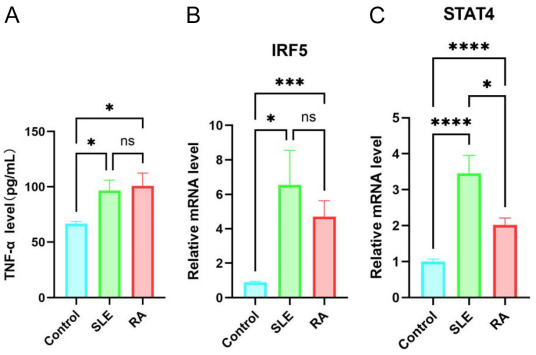

Figure 5. Biomarker detections in SLE and RA. A. Serum TNF-α levels in healthy control (n = 10), SLE (n = 11) and RA (n = 11) groups. B. Relative mRNA levels of IRF5 in healthy control (n = 11), SLE (n = 10) and RA (n = 13) groups. C. Relative mRNA levels of STAT4 in healthy control (n = 13), SLE (n = 13) and RA (n = 11) groups. ns, not significant; *P < 0.05; ***P < 0.001; ****P < 0.0001. P, *P*-value; NS, not significant; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; TNF-α, tumor necrosis factor-α; IRF5, interferon regulatory factor 5; STAT4, signal transducer and activator of transcription 4; mRNA, messenger RNA.

ponses against different self-antigens, potentially contributing to overlapping phenotypes such as Rhupus syndrome. These observations support the hypothesis that Rhupus represents a distinct immune endotype rather than a linear progression from one disease to another. Such patients may carry a specific genetic background (such as a specific HLA haplotype combination) that predisposes their immune system to produce a broad autoimmune response attacking both joints and multiple organs [48]. Thus, the interaction between multiple genes and various factors likely contributes to the development of both diseases [49]. From a clinical perspective, the absence of a direct genetic causal relationship suggests that RA does not inherently confer genetic risk for SLE and vice versa. Thus, Rhupus syndrome is a unique disease endotype, rather than a simple superposition of two diseases. Therefore, routine management should remain disease-specific, while recognizing that shared susceptibility and environmental factors may contribute to overlapping clinical features. Taken together, this study provided a theoretical basis for more precise risk assessment, diagnostic stratification, and personalized treatment.

The application of bidirectional two-sample MR analysis strengthens causal inference by reducing confounding and reverse causation [50]. Besides, MR was not used to analyze individual samples but the whole population, supporting the statistical power of the results.

Several limitations of this research should be acknowledged. The analysis was primarily based on individuals of European ancestry, which may limit generalizability to other populations with different genetic architectures, population-specific allele frequencies, and linkage disequilibrium patterns, and disease prevalence may influence causal estimates. For example,

the frequency of the risk allele T of STAT4 rs7574865 is higher in Asian populations than in European populations [51]. The prevalence of SLE is not only higher in Asian populations, but the condition is often more severe [52]. Moreover, there are differences in the linkage disequilibrium structure. The instrumental variable SNP that is effective in the European population may be invalid in other populations due to changes in the strength of association with the pathogenic site [53], thus weakening the accuracy of the analysis. In addition, gene-environment interactions are not fully captured by standard MR approaches. Finally, the size of GWAS datasets used in this study was small, and causal effect estimates in MR analysis usually increase with larger sample sizes. Larger GWAS datasets and more diverse ancestry representation would improve statistical power and external validity.

In conclusion, no direct bidirectional genetic causal relationship between RA and SLE was identified. The clinical overlap between the two diseases is more likely attributable to shared genetic susceptibility, environmental influences, and complex immune regulatory mechanisms rather than a simple unidirectional causal pathway. Further study of the interaction between several genes and environmental factors may be helpful in discussing the potential correlation between RA and SLE.

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Disclosure of conflict of interest

None.

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