scientific reports

Check for updates

OPEN

Bidirectional mendelian randomization assessment of causality between lactate levels and multiple autoimmune diseases

Hua Fan[1,2,6]✉, Hongna Song[3,6], Qizhi Fu[4,6], Ganqin Du[4], Yongjie Bai[4], Canfei Zhang[4] & Xiaofei Shi[5]✉

Bidirectional two-sample Mendelian randomization (MR) was performed to provide genetic evidence for the causal association between multiple sclerosis (MS), type 1 diabetes(T1D), rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), Crohn's disease (CD), systemic lupus erythematosus (SLE), ulcerative colitis (UC) and lactate levels. Inverse variance weighted (IVW), weighted median estimator (WME), weighted mode, and MR-Egger regression were used to assess the potential causal links. Sensitivity analysis included Cochran's Q test for heterogeneity, Steiger test of directionality for directionality, MR-Egger regression, MR pleiotropy residual sum and outlier (MR-PRESSO), and leave-one-out method. MR analysis utilized 510 SNPs associated with seven different kinds of autoimmune diseases and 11 SNPs associated with lactate levels as IVs. No significant genetic association between any autoimmune diseases and lactate levels was discovered by IVW. While IVW revealed no significant associations, exploratory analyses using WME and weighted mode methods identified nominal links between RA/IBD and lactate levels (RA: WME OR = 1.01, *P* = 0.010; weighted mode OR = 1.01, *P* = 0.008; IBD: weighted mode OR = 1.01, *P* = 0.042). These findings, though not surviving FDR correction, warrant further investigation. In reverse MR analysis, there was no significant association between lactate level exposure and any autoimmune disease outcomes. MR-Egger regression indicated potential horizontal pleiotropy in the RA-lactate analysis and Cochran's Q test suggest no absence of heterogeneity. Potential reverse causality in the analysis of SLE as outcomes and lactate levels as exposures was discovered by MR Steiger. Based on limited evidence, Our MR Analysis found a possible genetic causal association between RA and lactate level difference.

Keywords Autoimmune disease, Lactate levels, Mendelian randomization, Genetics, Single nucleotide polymorphisms

Autoimmune diseases are a diverse group of conditions characterized by immune dysfunction that result in abnormal reactivity of B cells and T cells to normal components of the host. Autoimmune diseases can affect nearly every organ system and can be classified as either systemic or organ-specific[1]. The occurrence of autoimmune diseases is almost independent of demographic characteristics, and female sex was their only common risk factors. In UK, 63.9% of new autoimmune disease patients from 2000 to 2019 were female[2]. Risk factors for various other autoimmune diseases are not consistent, and childhood, social ladder, season, and co-occurrence of autoimmune diseases may contribute separately to the development of these conditions[2]. The clinical manifestations of autoimmune diseases vary widely, from life-threatening acute organ failure to more

[1]Office of Research & Innovation, The First Affiliated Hospital, College of Clinical Medicine, Henan University of Science and Technology, No. 25, Jinghua Road, Jianxi District, Luoyang 471003, Henan, China. [2]Luoyang Key Laboratory of Neuroimmunology and innovative Drug Screening, Luoyang 471003, Henan, China. [3]Department of Neuro-auricular vertigo, The Second Affiliated Hospital of Henan, University of Science and Technology, Luoyang 471003, Henan, China. [4]Department of Neurology, The First Affiliated Hospital, College of Clinical Medicine of Henan, University of Science and Technology, Luoyang 471003, Henan, China. [5]Department of Rheumatology and Immunology, The First Affiliated Hospital, College of Clinical Medicine, Henan University of Science and Technology, No. 25, Jinghua Road, Jianxi District, Luoyang 471003, Henan, China. [6]Hua Fan, Hongna Song and Qizhi Fu contributed equally to this work. ✉email: fanhua19860208@gmail.com; xiaofeis@haust.edu.cn

subtle laboratory abnormalities[3]. It is precisely because of the diversity of autoimmune diseases that complicated their diagnosis and treatment, making it a challenge for the entire medical enterprise.

Recent research has highlighted the role of lactate, a byproduct of aerobic glycolysis, in the pathophysiology of various diseases. Lactate production is elevated under stress conditions, including trauma, infection, myocardial infarction, and heart failure, as well as in tumor cells[4–6]. At present, lactate level is considered as an indicator for the diagnosis, prognosis and efficacy evaluation of a variety of diseases in clinical practice, including a number of autoimmune diseases. Studies have reported altered lactate levels in autoimmune conditions such as multiple sclerosis (MS), type 1 diabetes (T1D), rheumatoid arthritis (RA), and inflammatory bowel disease (IBD). Lactate levels (REST) were elevated and lactate levels (MAX) were decreased in MS patients, and the blood lactate levels are different at rest and during vigorous exercise compared to healthy individuals[7]. Urinary lactate levels in patients with diabetic nephropathy (DKD) are higher than those in controls of the same age, suggesting lactate as a key element in DKD pathogenesis[8]. In RA, in vitro studies have demonstrated that hypoxic and acidic conditions in the synovial membrane lead to increased lactate levels, which, in turn, influence fibroblast and macrophage activity, IL-6 secretion, and metabolic processes via specific lactate transporters[9]. Additionally, lactate levels were found to correlate with inflammation and disease progression in IBD models[10]. Other limited but similar evidence of a relationship between autoimmune diseases and lactate levels has been found in related studies of CD[11], SLE[12], and UC[13].

While these studies suggest a potential link between lactate and autoimmune diseases, they are subject to biases inherent in observational, in vitro, or animal research. Mendelian randomization (MR) which uses genetic variation as an instrumental variable, may offer a more robust approach to assess the causal relationship between lactate levels and autoimmune diseases[14]. MR design can help minimize confounding factors due to the random distribution of genetic variants and provide stronger evidence for causality. However, the direction of causality between lactate levels and autoimmune diseases remains unclear, with conflicting findings in the existing literature. To address this gap, we conducted a bidirectional two-sample MR study using data from genome-wide association studies (GWAS) to explore the causal association between autoimmune diseases and lactate levels, incorporating a series of sensitivity analyses to strengthen our findings.

Therefore, this study aims to clarify the causal relationship between autoimmune diseases and lactate levels, potentially resolving existing discrepancies and providing novel insights into the underlying mechanisms of autoimmune pathogenesis.

Methods

Study design

This study utilized a bidirectional two-sample MR approach to investigate the causal relationship between autoimmune diseases and lactate levels. The core design and key assumptions of the present MR study were demonstrated in Fig. 1. This research was reported according to the "STrengthening the Reporting of Observational studies in Epidemiology using Mendelian Randomization" (STROBE-MR) statement[15].The methodological foundation of this investigation was constructed upon three core principles integral to MR analysis:

(1) Relevance assumption: Requires a strong connection between IVs and exposures.

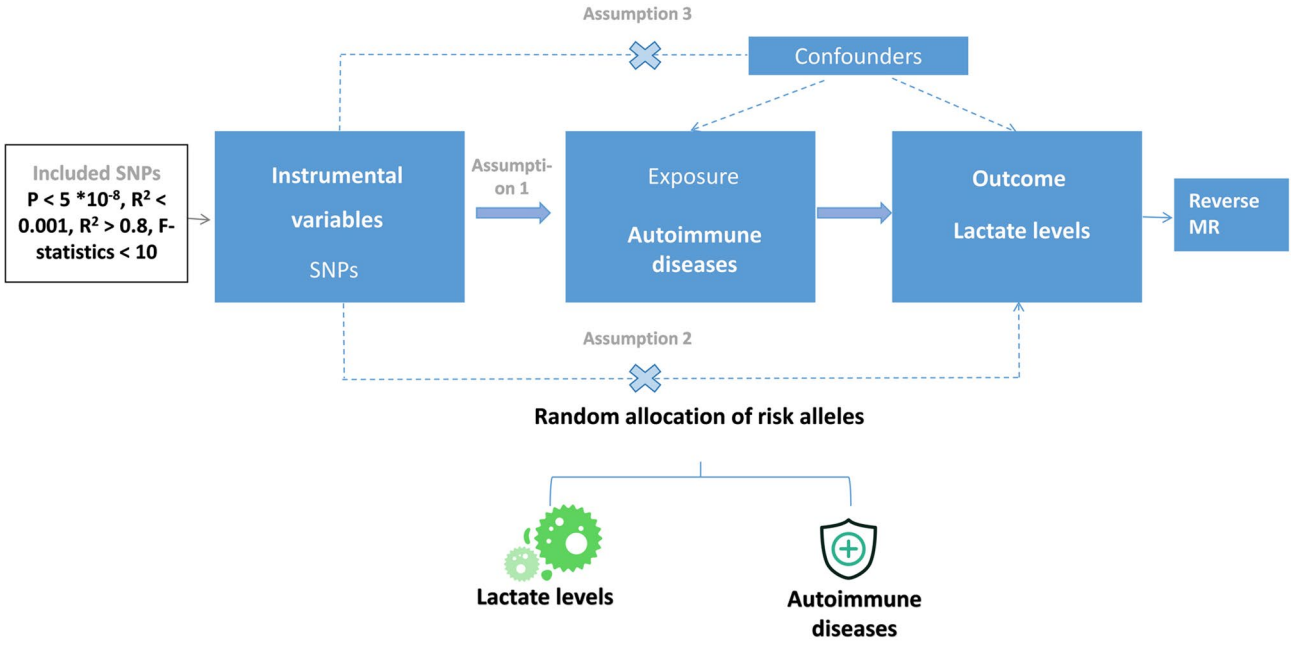

Fig. 1. Design of Experiments flowchart.

(2) Independence assumption: No existence of correlation between IVs and any variables that affected both exposure and outcome.
(3) Exclusion restriction assumption: Other than the effect on exposure, IVs did not alter outcomes by any other causal pathways.

Based on publicly available GWAS data, no additional ethical approval was required for our MR analysis.

Data source

GWAS data related to both autoimmune diseases[16] and lactate levels[17] were obtained from the IEU Open GWAS datasets (Updated to December 06, 2023). There were 11,590,399 lactate levels related SNPs from 114,806 European populations included in this MR analysis. As for autoimmune diseases, we selected 7 conditions which including MS, T1D, RA, IBD, CD, SLE, and UC. Related GWAS data were all from European populations to avoid the potential bias due to racial differences. Ethical approval and consent to participate was not required, and details of exposure and outcome data were shown in Table S1.

Instrumental variable selection

SNPs associated with the genome-wide significance of autoimmune diseases and lactate levels ($p < 5 * 10^{-8}$) and minor allele frequency (MAF) > 0.01 were screened[18]. Based on the standard of $R^2 < 0.001$ and window size = 10,000kb[19], linkage disequilibrium effect (LD) between SNPs was eliminated. When primary SNPs were unavailable in the outcome dataset, suitable SNPs with a high LD effect ($R^2 > 0.8$) were identified to replace the original IV as proxy SNPs. According to the formula $F = R^{2*} (N-2)/(1-R^2)$, this MR study calculated the F-value of each SNP in IV as the basis for assessing IV intensity. To minimize weak instrument bias, SNPs with F-statistics less than 10 were excluded during instrument selection.

MR analysis

In this MR study, four commonly used MR methods were included: random-effects IVW, weighted mode[20], WME[21], and MR-Egger. Considering the weighted average of the inverse variance of each SNP, the odds ratio (OR) and 95% confidence interval (CI) was calculated by IVW to interpret the results of MR[22]. In addition, MR-Egger, WME and weighted mode methods were used to test the robustness of the IVW analysis results[21]. This is due to the fact that MR-Egger can accurately estimate the causal effect under multi-effect bias, while WME method analyzes the causal relationship between exposure and results from another perspective, assuming the validity of half IVs.

Sensitivity analysis

Sensitivity analysis involved in this study was mainly used to detect potential pleiotropy in MR Studies. Analysis methods included Cochran's Q test, MR-Egger regression, MR pleiotropy residual sum and outlier (MR-PRESSO) and leave-one-out analysis[23,24]. When $P > 0.05$ of Cochran's Q test was obtained, the heterogeneity among IVs was considered to be low, that is, the estimates among IVs were random and had no significant impact on the IVW results. MR-Egger regression method was selected to evaluate the influence of the pleiotropy of genetic variation on the estimation of association effects. When the intercept term of MR-Egger regression approaches zero or has no statistical significance, it indicates that there was no existence of pleiotropy. Additionally, the MR pleiotropy residual sum and outlier (MR-PRESSO) method was employed to identify and adjust for outliers in the SNPs. SNPs identified as outliers with a P-value less than 0.05 were excluded from the analysis, thus mitigating the effects of pleiotropy and improving the accuracy of the causal inferences[23]. Leave one-out analysis was used to test the robustness and consistency of the results. Finally, based on MR-IVW, MR-Egger, WEM and weighted mode, reverse causality detection was performed to detect potential reverse causality with lactate levels as IVs[25].

Statistical analysis

The analysis was performed using R version 4.0.5 and the "Two-sample MR" package designed for MR studies. Graphical representations such as forest plots, scatter plots, and funnel plots were utilized to visually interpret the data. Steiger filtering was also conducted to ensure the directionality of the association between lactate levels and autoimmune diseases. To account for the inclusion of exposure variables, P-values were adjusted for multiple comparisons using the False Discovery Rate (FDR) correction method. Associations with adjusted P-values < 0.05 were considered statistically significant[26].

Results

Selection of IVs

At genome-wide association significance ($P < 5 * 10^{-8}$), our MR Analysis selected 510 autoimmune diseases related SNPs as IVs. Among them, there were 72, 90, 45, 117, 88, 62 and 36 IVs related to MS, RA, SLE, IBD, CD, UC and T1D, respectively. The mean and range of F-statistics value was 81.52 (29.81 to 1269.22) for MS, 107.49 (29.98 to 1487.90) for RA, 96.77 (29.96 to 460.93) for SLE, 70.35 (29.86 to 500.59) for IBD, 77.51 (30.14, 489.55) for CD, 70.36 (30.46 to 408.12) for UC, 181.97 (30.00 to 1406.97) for T1D. In all case of the MS, RA, and SLE related SNPs, there was 1 SNP each that did not match information in the summary data (rs9393975, rs41316148, rs28361029). Meanwhile, 2, 2, 2, 1, 2, 3, and 3 weak related IVs was eliminated from MS, RA, SLE, IBD, CD, UC and T1D related SNPs. After that, a strong correlation between IVs and exposures was protected (F-values > 10). The specific information of the selected SNPs is shown in Table S2.

In the reverse direction, 11 SNPs associated with lactate levels (mean F = 64.00, [30.81, 174.32]) met the criteria and were screened as IVs in reverse MR analysis. When using MS, IBD, CD, UC and T1D as outcomes, there were 3, 2, 2, 2, and 2 SNPs did not match information in the summary data respectively. When RA was the outcome, the reverse MR Analysis removes 1 weak instrumental variable. Detailed information of the selected SNPs is shown in Table S3.

Causal effects of autoimmune diseases on lactate levels

The results of this MR analyses examining the causal relationship between 7 genetically proxied autoimmune diseases and lactate levels were displayed. As a result, no significant genetic association between any autoimmune diseases and lactate levels was discovered by IVW (SLE (OR: 1.00; 95% CI [0.99, 1.00]; P_{FDR} = 0.303); IBD (OR: 1.01; 95% CI [1.00, 1.02]; P_{FDR} = 0.303); CD (OR: 1.00; 95% CI [0.99, 1.01]; P_{FDR} = 0.825); UC (OR: 1.01; 95% CI [1.00, 1.02]; P_{FDR} =0.366); T1D (OR: 1.00; 95% CI [0.99, 1.00]; P_{FDR} = 0.484); RA (OR: 1.00; 95% CI [1.00, 1.01]; P_{FDR} = 0.484); MS (OR: 1.00; 95% CI [0.99, 1.01]; P_{FDR} = 0.825)). While IVW analysis revealed no statistically significant associations between autoimmune diseases and lactate levels after FDR correction, exploratory analyses using WME and weighted mode methods identified nominally significant associations between IBD or RA and lactate levels. Both WME (OR: 1.01; 95% CI [1.00, 1.02]; $P = 0.01$) and weighted mode (OR: 1.01; 95% CI [1.00,1.02]; $P = 0.008$) suggested a potential association between RA and lactate levels. These findings, though not surviving multiple testing correction ($P_{FDR} > 0.05$), may, may suggest potential biological links warranting further investigation. Meanwhile, the weighted mode results nominally supported the association between IBD and lactate levels (OR: 1.01; 95% CI [1.00, 1.04]; $P = 0.042$; P_{FDR} = 0.303). Consistent with the above results, the MR regression slopes and individual causal estimates of each of the SNPs were illustrated in the scatter plot (Fig. 2) and forest plot (Figure S1). Detailed genetic association analysis data were shown in Table 1.

Causal effects of lactate levels on autoimmune diseases

To assess any reverse causality, we used lactate levels as exposure and 7 autoimmune diseases as outcome. We found no statistically significant effect of lactate levels difference on any autoimmune diseases by IVW (SLE (OR: 1.70; 95% CI [0.73, 3.92]; P_{FDR} = 0.505); IBD (OR: 1.11; 95% CI [0.78, 1.58]; P_{FDR} = 0.644); CD (OR: 1.40; 95% CI [0.88–2.23]; P_{FDR} = 0.505); UC (OR: 1.04; 95% CI [0.62–1.75]; P_{FDR} = 0.868); T1D (OR: 0.85; 95% CI [0.53–1.36]; P_{FDR} = 0.644); RA (OR: 0.83; 95% CI [0.65, 1.06]; P_{FDR} = 0.505); MS (OR: 0.84; 95% CI [0.56, 1.28]; P_{FDR} = 0.644)). The weighted model, WME, and MR-Egger gave similar causal estimates in magnitude, and the MR-Egger found no horizontal pleiotropy effect during the analysis. The visualization results of reverse MR analysis and detailed analysis results were shown in Fig. 3, Figure S2 and Table 1.

Sensitivity analyses

Directional testing conducted by MR Steiger suggests that there may be a potential reverse causality in the analysis of SLE as outcomes and lactate levels as exposures. As for rest of the analysis, bidirectional MR analysis did not reveal any causal effect of autoimmune diseases on the lactate levels difference and Steiger filtering further ensured directionality (Table 2).

The summary results of the sensitivity and pleiotropy analysis were shown in Table 3. Except when using T1D as exposure, significant heterogeneity was observed in all of other causal estimates of individual genetic variation by Cochran's Q test statistics (almost all $P > 0.05$ except T1D as exposure, $P = 0.194$). Since the IVW

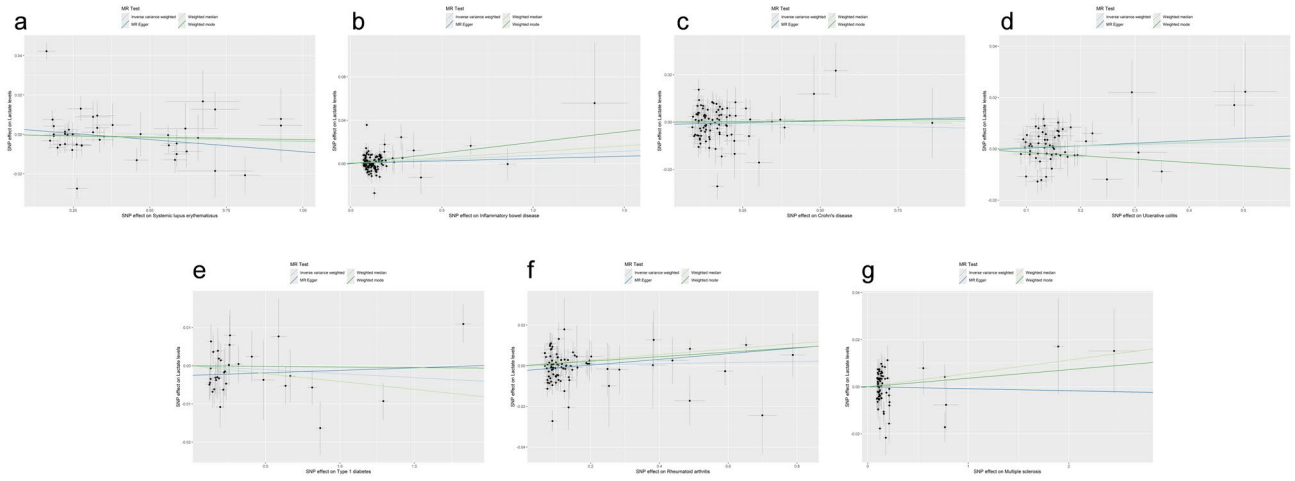

Fig. 2. (**a**). Scatter plot of causal association of SLE (exposure) on lactate levels (outcome); (**b**). Scatter plot of causal association of IBD (exposure) on lactate levels (outcome); (**c**). Scatter plot of causal association of CD (exposure) on lactate levels (outcome); (**d**). Scatter plot of causal association of UD (exposure) on lactate levels (outcome); (**e**). Scatter plot of causal association of T1D (exposure) on lactate levels (outcome); (**f**). Scatter plot of causal association of RA (exposure) on lactate levels (outcome); (**g**). Scatter plot of causal association of MS (exposure) on lactate levels (outcome);.

Exposure	Outcome	*N*.SNPs	Methods	OR (95% CI)	*P*	FDR adjusted *P*
Systemic lupus erythematosus	Lactate levels	41	IVW	1.00 (0.99–1.00)	0.081	0.303
			MR Egger	1.00 (0.99–1.01)	0.438	0.808
			Weighted median	1.00 (0.99–1.00)	0.399	0.454
			Weighted mode	1.00 (0.99–1.01)	0.849	0.916
Inflammatory bowel disease	Lactate levels	114	IVW	1.01 (1.00–1.02)	0.087	0.303
			MR Egger	1.01 (0.99–1.03)	0.356	0.808
			Weighted median	1.01 (1.00–1.02)	0.058	0.174
			Weighted mode	1.02 (1.00–1.04)	0.042	0.21
Crohn's disease	Lactate levels	85	IVW	1.00 (0.99–1.01)	0.802	0.825
			MR Egger	1.00 (0.98–1.02)	0.693	0.808
			Weighted median	1.00 (0.99–1.01)	0.946	0.947
			Weighted mode	1.00 (0.99–1.02)	0.911	0.916
Ulcerative colitis	Lactate levels	58	IVW	1.01 (1.00–1.02)	0.157	0.366
			MR Egger	1.01 (0.98–1.04)	0.602	0.808
			Weighted median	1.01 (0.99–1.02)	0.272	0.403
			Weighted mode	0.99 (0.95–1.02)	0.469	0.888
Type 1 diabetes	Lactate levels	33	IVW	1.00 (0.99–1.00)	0.285	0.484
			MR Egger	1.00 (1.00–1.01)	0.601	0.808
			Weighted median	1.00 (0.99–1.00)	0.217	0.403
			Weighted mode	1.00 (1.00–1.00)	0.891	0.916
Rheumatoid arthritis	Lactate levels	86	IVW	1.00 (1.00–1.01)	0.345	0.484
			MR Egger	1.01 (1.00–1.02)	0.022	0.154
			Weighted median	1.01 (1.00–1.02)	0.010	0.071
			Weighted mode	1.01 (1.00–1.02)	0.008	0.11
Multiple sclerosis	Lactate levels	70	IVW	1.00 (0.99–1.01)	0.825	0.825
			MR Egger	1.00 (0.99–1.01)	0.873	0.873
			Weighted median	1.01 (1.00–1.02)	0.229	0.403
			Weighted mode	1.00 (0.99–1.01)	0.438	0.888
Lactate levels	Systemic lupus erythematosus	8	IVW	1.70 (0.73–3.92)	0.216	0.505
			MR Egger	3.67 (0.26–51.62)	0.372	0.889
			Weighted median	1.56 (0.64–3.79)	0.332	0.603
			Weighted mode	1.46 (0.52–4.08)	0.493	0.689
Lactate levels	Inflammatory bowel disease	7	IVW	1.11 (0.78–1.58)	0.552	0.644
			MR Egger	1.27 (0.41–3.91)	0.695	0.889
			Weighted median	1.04 (0.74–1.48)	0.806	0.803
			Weighted mode	1.03 (0.71–1.51)	0.866	0.867
Lactate levels	Crohn's disease	7	IVW	1.40 (0.88–2.23)	0.156	0.505
			MR Egger	1.04 (0.23–4.78)	0.958	0.958
			Weighted median	1.32 (0.84–2.07)	0.235	0.603
			Weighted mode	1.27 (0.78–2.06)	0.368	0.689
Lactate levels	Ulcerative colitis	8	IVW	1.04 (0.62–1.75)	0.868	0.868
			MR Egger	1.42 (0.20–9.87)	0.735	0.889
			Weighted median	0.79 (0.45–1.38)	0.409	0.603
			Weighted mode	0.73 (0.37–1.41)	0.376	0.689
Lactate levels	Type 1 diabetes	8	IVW	0.85 (0.53–1.36)	0.491	0.644
			MR Egger	1.25 (0.31–5.12)	0.762	0.889
			Weighted median	0.91 (0.50–1.65)	0.757	0.803
			Weighted mode	1.12 (0.55–2.26)	0.766	0.867

Continued

Exposure	Outcome	*N*.SNPs	Methods	OR (95% CI)	*P*	FDR adjusted *P*
Lactate levels	Rheumatoid arthritis	9	IVW	0.83 (0.65–1.06)	0.143	0.505
			MR Egger	1.24 (0.63–2.43)	0.549	0.889
			Weighted median	0.87 (0.63–1.21)	0.419	0.603
			Weighted mode	0.84 (0.52–1.36)	0.499	0.689
Lactate levels	Multiple sclerosis	7	IVW	0.84 (0.56–1.28)	0.420	0.644
			MR Egger	0.60 (0.19–1.86)	0.418	0.889
			Weighted median	0.79 (0.47–1.33)	0.376	0.603
			Weighted mode	0.78 (0.43–1.43)	0.459	0.689

Table 1. Bidirectional MR results of causal association between lactate levels and autoimmune disease. IVW, Inverse variance weighted method.

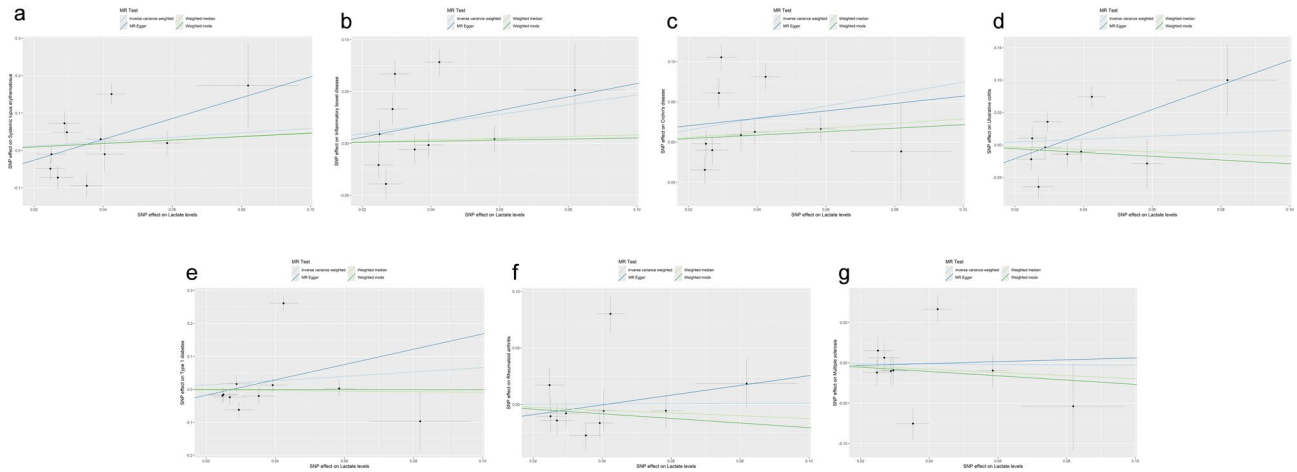

Fig. 3. (**a**). Scatter plot of causal association of lactate levels (exposure) on SLE (outcome); (**b**). Scatter plot of causal association of lactate levels (exposure) on IBD (outcome); (**c**). Scatter plot of causal association of lactate levels (exposure) on CD (outcome); (**d**). Scatter plot of causal association of lactate levels (exposure) on UD (outcome); (**e**). Scatter plot of causal association of lactate levels (exposure) on T1D (outcome); (**f**). Scatter plot of causal association of lactate levels (exposure) on RA (outcome); (**g**). Scatter plot of causal association of lactate levels (exposure) on MS (outcome).

Exposure	Outcome	SNP_r2.exposure	SNP_r2.outcome	Correct causal direction	Steiger pval
Systemic lupus erythematosus	Lactate levels	0.303261822	0.001611019	TRUE	< 0.001
Inflammatory bowel disease	Lactate levels	0.137111343	0.002179226	TRUE	< 0.001
Crohn's disease	Lactate levels	0.169153	0.001339276	TRUE	< 0.001
Ulcerative colitis	Lactate levels	0.094888357	0.000865334	TRUE	< 0.001
Type 1 diabetes	Lactate levels	0.316138109	0.00121741	TRUE	< 0.001
Rheumatoid arthritis	Lactate levels	0.14552832	0.00123998	TRUE	< 0.001
Multiple sclerosis	Lactate levels	0.046423839	0.000965938	TRUE	< 0.001
Lactate levels	Systemic lupus erythematosus	0.006131174	0.003989493	TRUE	0.086547831
Lactate levels	Inflammatory bowel disease	0.005625952	0.001430344	TRUE	< 0.001
Lactate levels	Crohn's disease	0.005625952	0.002127393	TRUE	< 0.001
Lactate levels	Ulcerative colitis	0.005625952	0.001029935	TRUE	< 0.001
Lactate levels	Type 1 diabetes	0.005625952	0.008066655	FALSE	< 0.001
Lactate levels	Rheumatoid arthritis	0.006131174	0.000617323	TRUE	< 0.001
Lactate levels	Multiple sclerosis	0.004840144	0.000302961	TRUE	< 0.001

Table 2. MR Steiger test of directionality.

Exposure	Outcome	Heterogeneity		Pleiotropy	
		Q statistic (IVW)	*P* value	MR-Egger Intercept	*P* value
Systemic lupus erythematosus	Lactate levels	178.50	< 0.001	0.003587159	0.332
Inflammatory bowel disease	Lactate levels	245.64	< 0.001	0.000471426	0.764
Crohn's disease	Lactate levels	152.59	< 0.001	-0.00098073	0.586
Ulcerative colitis	Lactate levels	93.96	0.002	-0.000698135	0.771
Type 1 diabetes	Lactate levels	38.65	0.194	-0.002511242	0.049
Rheumatoid arthritis	Lactate levels	140.46	< 0.001	-0.002477147	0.018
Multiple sclerosis	Lactate levels	110.42	0.001	1.57207E-05	0.988
Lactate levels	Systemic lupus erythematosus	52.21	< 0.001	-0.081631635	0.293
Lactate levels	Inflammatory bowel disease	70.09	< 0.001	-0.006993046	0.871
Lactate levels	Crohn's disease	61.78	< 0.001	0.011022045	0.833
Lactate levels	Ulcerative colitis	45.52	< 0.001	-0.058956279	0.244
Lactate levels	Type 1 diabetes	133.43	< 0.001	-0.064031839	0.542
Lactate levels	Rheumatoid arthritis	35.87	< 0.001	-0.017522133	0.545
Lactate levels	Multiple sclerosis	35.06	< 0.001	-0.005474229	0.903

Table 3. Sensitivity and Pleiotropy analysis of causal association between lactate levels and autoimmune disease.

results of random effects were selected as the main MR Analysis method, the influence of heterogeneity could be ignored, and the funnel plot of symmetrical distribution also proved the robustness of the results. (Table 3 and Figure S3-S4). However, the result of MR-Egger regression shows that the MR results by using RA as exposure and lactate levels as outcomes may be affected by horizontal pleiotropy (MR-Egger Intercept: – 0.0025, $P = 0.018$) (Table 3). No significant intercepts indicating directional pleiotropy were observed for other primary forward MR analyses (all $P > 0.05$).

Additionally, we applied the MR-PRESSO method, aimed at detecting and rectifying potential horizontal pleiotropy and outliers. Outliers were discovered in almost all analysis, except when IBD or RA as exposures and lactate levels as outcomes. After removing the outliers, MR-PRESSO results indicated that no genetic association was observed between any autoimmune diseases and lactate levels in any analysis directions ($P > 0.05$) (Table S4).

To further explore the potential impact of outlying genetic variants, we performed a leave-one-out analysis, systematically removing each instrumental variable and recalculating the causal estimate. However, this analysis did not demonstrate any significant changes in the overall causal estimates for any of the exposure (Figures S5-S6), indicating that the null findings were not influenced by any individual influential instrumental variable.

Discussion

To the best of our knowledge, this is the first study to combine seven different types of autoimmune diseases and lactate levels data to explore casual association between autoimmune diseases and lactate level difference using bidirectional two-sample MR method. Seven autoimmune diseases including MS, RA, SLE, IBD, CD, UC and T1D. All of these autoimmune diseases have been proved to be associated with lactate level change by previous epidemiological, in vitro, and animal studies, which was opposite with our MR analysis result. According to the bidirectional MR results, no statistically robust genetic causal association was discovered between 7 autoimmune diseases and lactate level difference in any direction after correction for multiple testing. However, exploratory analyses using WME and weighted mode methods did reveal nominally significant associations between RA and lactate levels and between IBD and lactate levels in the forward MR direction. While these findings did not survive FDR correction and require cautious interpretation, particularly given the potential pleiotropy detected in the RA-lactate analysis (discussed below), they may hint at potential weak biological links warranting further investigation with larger sample sizes or more sensitive methodologies.

Current research evidence often focuses on MS and T1D populations when exploring links with lactate. Biological plausibility exists for such links: In Multiple Sclerosis (MS), chronic inflammation within the central nervous system (CNS) creates a unique microenvironment potentially altering local lactate metabolism[7,27], and mitochondrial dysfunction, a hallmark of MS progression, can lead to lactate accumulation[28,29]. Observational studies have indeed reported higher serum lactate in progressive MS[30]. However, our bidirectional MR analysis found no robust genetic evidence supporting a causal relationship between systemic lactate levels and MS risk, or vice versa. This discrepancy might arise because MR assesses systemic circulating lactate, which may not adequately reflect localized CNS metabolic changes critical to MS pathology. Furthermore, insufficient statistical power in the available GWAS datasets or confounding in observational studies could also contribute to the differing results.

Similarly, in Type 1 Diabetes (T1D), altered glucose metabolism and potential mitochondrial dysfunction, particularly in complications like diabetic nephropathy (DKD), provide a rationale for investigating lactate[8]. Observational studies have linked factors like impaired hypoglycemic awareness or hormonal influences to lactate dynamics in T1D[31–33]. Despite these potential connections, our MR analysis did not detect a significant causal effect between genetic liability to T1D and circulating lactate levels, nor in the reverse direction. Possible

explanations include limitations in statistical power, the nature of the lactate GWAS capturing baseline systemic levels rather than dynamic responses, or non-causal explanations for previously observed associations.

Regarding Rheumatoid Arthritis (RA), the inflamed synovium is recognized as a high-lactate microenvironment where lactate plays complex roles in modulating fibroblast and macrophage function[9]. Lactate levels in synovial fluid have even been explored as a diagnostic marker[34]. Despite this established local pathophysiological link and potential biomarker utility, our MR analysis did not find robust genetic evidence for a causal relationship between RA susceptibility and systemic circulating lactate levels, or vice versa (after FDR correction). While exploratory analyses with WME and weighted mode hinted at a nominal association, this finding was not statistically robust after multiple testing correction and, critically, was potentially confounded by significant horizontal pleiotropy detected via the MR-Egger intercept test ($P = 0.018$). This suggests that systemic lactate levels, as captured by germline genetic prediction, may not be a primary causal factor for RA onset, or that the potential causal link is weak, bidirectional in a way MR cannot fully resolve with current instruments, or significantly biased by pleiotropy. The discrepancy between local synovial and systemic lactate roles warrants further investigation. It is hoped that with the accumulation of GWAS data, we can see more precise proof results in the future.

For Inflammatory Bowel Disease (IBD), including Crohn's disease (CD) and Ulcerative Colitis (UC), animal models suggest links between intestinal lactate levels, gut permeability, and local inflammation[13]. However, consistent with limited direct human evidence, our MR analysis identified no significant causal association between genetic liability to IBD, CD, or UC and systemic circulating lactate levels, nor in the reverse direction (after FDR correction). A nominal signal for the effect of IBD on lactate via weighted mode did not survive correction. The lack of robust causal findings might reflect differences between localized gut mucosal lactate metabolism and systemic levels, insufficient GWAS power, or secondary, non-causal changes in lactate during active intestinal inflammation.

In the case of SLE, no direct causal evidence linking it to lactate, and only one study has observed serum lactate levels greater than 2 mM on admission or 24 h before admission to the intensive care unit (ICU) as a major risk factor for mortality increase[12]. Congruently, our MR analysis found no significant causal association between SLE susceptibility and systemic lactate levels, or vice versa.

Overall, the predominantly null causal findings from our comprehensive MR analysis contrast with some observational or mechanistic hypotheses linking lactate to these autoimmune diseases. This underscores the importance of using genetically informed methods to probe causality. The discrepancies observed could stem from several factors inherent to MR studies and the complexity of both lactate metabolism and autoimmune pathophysiology. Lactate dynamics are influenced by numerous factors beyond baseline genetic predisposition, including exercise, medication, diet, and crucially, the dynamic nature of autoimmune diseases themselves, with fluctuations between active flares and remission[7,35]. Our MR approach, based on GWAS of general disease susceptibility, cannot capture these temporal variations or stage-specific effects.

Several limitations should be acknowledged when interpreting our findings. First, while MR minimizes traditional confounding, residual confounding or unaccounted pleiotropy cannot be entirely excluded, although sensitivity analyses were largely reassuring. However, the significant MR-Egger intercept detected for the association between RA and lactate levels specifically indicates potential directional pleiotropy, requiring significant caution when interpreting any link involving RA. Second, due to our strict threshold limits, many of the genetic burdens of autoimmune disease and lactate levels were excluded during the IVs selection phase, which may lead to a failure outcome. Third, the use of summary-level GWAS data precluded stratification by clinical heterogeneity, such as disease activity (flare vs. remission) or severity. Since lactate levels can fluctuate with disease state, averaging effects across diverse patient subgroups might mask true associations present only in specific clinical contexts. Finally, our analyses were restricted to individuals of European ancestry, limiting the generalizability of the findings to other populations. Future research in diverse cohorts and studies incorporating detailed clinical data or utilizing individual-level data are needed to address these limitations and further clarify the complex relationship between lactate metabolism and autoimmune diseases.

In summary, we performed a bidirectional two-sample MR analysis for evidence of a putative causal relationship between autoimmune diseases and lactate levels by applying several genetic information methods. No causal association between them was discovered, with limited evidence points to possible effects of RA and IBD on lactate levels. Further studies are needed to explore the roles of these autoimmune diseases in lactate levels.

Data availability

All data generated or analyzed during this study are included in this article and supplementary information files.

Received: 24 October 2024; Accepted: 13 May 2025
Published online: 21 May 2025

References

1. Pisetsky, D. S. Pathogenesis of autoimmune disease. *Nat. Rev. Nephrol.* **19**, 509–524 (2023).
2. Conrad, N. et al. Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort study of 22 million individuals in the UK. *Lancet* **401**, 1878–1890 (2023).
3. Kumar, S., Kaushik, D. & Sharma, S. K. Autoimmune disorders: types, symptoms, and risk factors. In: (eds Raza, K. & Singh, S.) Artificial Intelligence and Autoimmune Diseases: Applications in the Diagnosis, Prognosis, and Therapeutics. Singapore: Springer Nature Singapore; 3–31. (2024).
4. Li, M. et al. DDIT3 directs a dual mechanism to balance Glycolysis and oxidative phosphorylation during glutamine deprivation. *Adv. Sci. (Weinh).* **8**, e2003732 (2021).

5. Chen, Z., Liu, M., Li, L. & Chen, L. Involvement of the Warburg effect in non-tumor diseases processes. *J. Cell. Physiol.* **233**, 2839–2849 (2018).
6. Dabral, S. et al. A RASSF1A-HIF1α loop drives Warburg effect in cancer and pulmonary hypertension. *Nat. Commun.* **10**, 2130 (2019).
7. Cerexhe, L., Easton, C., Macdonald, E., Renfrew, L. & Sculthorpe, N. Blood lactate concentrations during rest and exercise in people with multiple sclerosis: A systematic review and meta-analysis. *Mult Scler. Relat. Disord.* **57**, 103454 (2022).
8. Darshi, M. et al. Glycolytic lactate in diabetic kidney disease. *JCI Insight* **9**, (2024).
9. Pucino, V. et al. Differential effect of lactate on synovial fibroblast and macrophage effector functions. *Front. Immunol.* **14**, 1183825 (2023).
10. Carvalho, L. et al. Reactive oxygen species deficiency due to Ncf1-Mutation leads to development of adenocarcinoma and metabolomic and lipidomic remodeling in a new mouse model of dextran sulfate Sodium-Induced colitis. *Front. Immunol.* **9**, 701 (2018).
11. Cai, J., Chen, H., Weng, M., Jiang, S. & Gao, J. Diagnostic and Clinical Significance of Serum Levels of D-Lactate and Diamine Oxidase in Patients with Crohn's Disease. *Gastroenterol. Res. Pract.*. 8536952 (2019). (2019).
12. Ñamendys-Silva, S., Reyes-Ruiz, M., Rivero-Sigarroa, E. & Cherit, G. D. Pronóstico de Pacientes Con lupus eritematoso generalizado En Una Unidad de Cuidados intensivos. *Gac. Med. Mex.* **154**, 468–472 (2018).
13. Cui, D. J. et al. Gallincin ameliorates colitis-associated inflammation and barrier function in mice based on network Pharmacology prediction. *J. Int. Med. Res.* **48**, 300060520951023 (2020).
14. Little, M. Mendelian randomization: methods for using genetic variants in causal Estimation. *J. Royal Stat. Soc. Ser. A: Stat. Soc.* **181**, 549–550 (2018).
15. Bell, S. et al. A genome-wide meta-analysis yields 46 new loci associating with biomarkers of iron homeostasis. *Commun. Biol.* **4**, 156 (2021).
16. Multiple sclerosis genomic. Map implicates peripheral immune cells and microglia in susceptibility. *Science* **365**, (2019).
17. Richardson, T. G. et al. Characterising metabolomic signatures of lipid-modifying therapies through drug target Mendelian randomisation. *PLoS Biol.* **20**, e3001547 (2022).
18. Li, Y., Miao, Y. & Zhang, Q. Causal associations of obstructive sleep apnea with cardiovascular disease: a Mendelian randomization study. *Sleep* **46**, (2023).
19. Larsson, S. C., Butterworth, A. S. & Burgess, S. Mendelian randomization for cardiovascular diseases: principles and applications. *Eur. Heart J.* **44**, 4913–4924 (2023).
20. Hartwig, F. P., Davey Smith, G. & Bowden, J. Robust inference in summary data Mendelian randomization via the zero modal Pleiotropy assumption. *Int. J. Epidemiol.* **46**, 1985–1998 (2017).
21. Bowden, J., Davey Smith, G., Haycock, P. C. & Burgess, S. Consistent Estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet. Epidemiol.* **40**, 304–314 (2016).
22. Bowden, J., Davey Smith, G. & Burgess, S. Mendelian randomization with invalid instruments: effect Estimation and bias detection through Egger regression. *Int. J. Epidemiol.* **44**, 512–525 (2015).
23. Verbanck, M., Chen, C. Y., Neale, B. & Do, R. Detection of widespread horizontal Pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat. Genet.* **50**, 693–698 (2018).
24. Greco, M. F., Minelli, C., Sheehan, N. A. & Thompson, J. R. Detecting Pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat. Med.* **34**, 2926–2940 (2015).
25. Davey Smith, G. & Hemani, G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum. Mol. Genet.* **23**, R89–98 (2014).
26. Hemani, G., Tilling, K. & Davey Smith, G. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet.* **13**, e1007081 (2017).
27. Bittner, S., Pape, K., Klotz, L. & Zipp, F. Implications of immunometabolism for smouldering MS pathology and therapy. *Nat. Rev. Neurol.* **19**, 477–488 (2023).
28. Woo, M. S., Engler, J. B. & Friese, M. A. The neuropathobiology of multiple sclerosis. *Nat. Rev. Neurosci.* **25**, 493–513 (2024).
29. Wasyluk, W. & Zwolak, A. Metabolic alterations in Sepsis. *J Clin. Med* **10**, (2021).
30. Esmael, A., Talaat, M., Egila, H. & Eltoukhy, K. Mitochondrial dysfunction and serum lactate as a biomarker for the progression and disability in MS and its correlation with the radiological findings. *Neurol. Res.* **43**, 582–590 (2021).
31. De Ridder, F. et al. Trends of glucose, lactate and ketones during anaerobic and aerobic exercise in subjects with type 1 diabetes: the ACTION-1 study. *Diabetes Metab. Res. Rev.* **38**, e3537 (2022).
32. Wiegers, E. C. et al. Effect of Exercise-Induced lactate elevation on brain lactate levels during hypoglycemia in patients with type 1 diabetes and impaired awareness of hypoglycemia. *Diabetes* **66**, 3105–3110 (2017).
33. Zhao, L. et al. Effects of fibroblast growth factor 21 on lactate uptake and usage in mice with Diabetes-Associated cognitive decline. *Mol. Neurobiol.* **59**, 5656–5672 (2022).
34. Patel, J., Tran, Q. K., Martinez, S., Wright, H. & Pourmand, A. J. S. E. J. E. Utility of serum lactate on differential diagnosis of seizure-like activity: A systematic review and meta-analysis. **102**, 134–142 (2022).
35. Albanese, M. et al. Cerebrospinal fluid lactate is associated with multiple sclerosis disease progression. *J. Neuroinflammation.* **13**, 36 (2016).

Author contributions

Hongna Song, Qizhi Fu and Ganqin Du carried out the studies, participated in collecting data, and drafted the manuscript. Hua Fan and Xiaofei Shi performed the statistical analysis and participated in its design. Yongjie Bai and Canfei Zhang participated in acquisition, analysis, or interpretation of data and draft the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by Joint Fund of Henan Provincial Science and Technology R&D Project (No. 242103810034), Henan Academy of Innovations in Medical Science "Three Hundreds" Plan (S20240092), Henan Provincial Young and Middle-aged Health Science and Technology Innovation Leading Talent Training Program (No. LJRC2024019) and Heluo Young Talents Support Program (2024HLTJ08).

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

This article is a mendelian randomization study. The data for this study were obtained from publicly available databases and published literature data and does not require ethical approval and written informed consent.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-02507-9.

Correspondence and requests for materials should be addressed to H.F. or X.S.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.