Assessing the causality of IFN-γ and IFN-γ receptor 1/2 with systemic lupus erythematosus risk using genetic data

Xiao-Dong Li  
Second Hospital of Shanxi Medical University

Kai-Xin Yao  
Ministry of Education, Key Laboratory of Cellular Physiology at Shanxi Medical University

Jia-Wei Hao  
Ministry of Education, Key Laboratory of Cellular Physiology at Shanxi Medical University

Yin-Qi Long  
Ministry of Education, Key Laboratory of Cellular Physiology at Shanxi Medical University

Lu-Lin Qiao  
Ministry of Education, Key Laboratory of Cellular Physiology at Shanxi Medical University

Ya-Ru Zhang  
Ministry of Education, Key Laboratory of Cellular Physiology at Shanxi Medical University

Ke-Xin Ma  
Ministry of Education, Key Laboratory of Cellular Physiology at Shanxi Medical University

Sheng-Xiao Zhang  
Second Hospital of Shanxi Medical University

Xiao-Feng Li  (lxf_9859@sxmu.edu.cn)  
Second Hospital of Shanxi Medical University

Research Article

Keywords: systemic lupus erythematosus, mendelian randomization, interferon-gamma (IFN-γ), interferon-gamma receptor 1 (IFN-γR1), interferon-gamma receptor 2 (IFN-γR2), single nucleotide polymorphism, risk factor

Posted Date: April 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2776347/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

The interferon-gamma (IFN-γ) signaling pathway is activated in Systemic lupus erythematosus (SLE). This study aims to assess the causal association between IFN-γ, IFN-γR1, and IFN-γR2 and SLE within a bidirectional Mendelian-randomization design.

Methods

Genetic instruments of exposure to IFN-γ, IFN-γR1, and IFN-γR2 were derived from the large genome-wide association study (GWAS), including 3,301 sample size. Instrumental variables for SLE were selected from another independent GWAS analysis comprising 7,219 cases and 15,991 controls with European ancestry. Bidirectional two-sample MR was performed using inverse variance weighting (IVW), MR-Egger regression, and weighted median methods. A series of sensitivity analyses were conducted to assess the robustness of the results.

Results

The IVW showed IFN-γ had a positive causal association with the risk of SLE [OR 1.24 (95% CI 0.85, 2.26), P = 0.018]. IFN-γR2 was found to have a negative correlation with the onset of SLE [OR 0.85 (95% CI 0.73, 0.99), P = 0.034]. However, no genetic association was detected between IFN-γR1 and SLE [OR 0.97 (95% CI 0.79, 1.19), P = 0.768]. Evidence from bidirectional MR did not support reverse causality. Weighted median regression also showed directionally similar estimates.

Conclusion

Higher levels of IFN-γ or lower levels of IFN-γR2 are significantly associated with an increased risk of SLE, providing insights into the pathogenesis of SLE.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that causes an increase in autoantibodies and immune complexes, characterized by the abnormal immune system that produces autoantibodies[1]. The pathogenesis of SLE is related to genetic and environmental factors and immune abnormalities[2–6]. IFN-γ has been reported to mediate the development of SLE and is a risk factor for SLE[7].

Interferon (IFN-γ) is a crucial cytokine associated with developing autoimmune diseases produced by T lymphocytes, macrophages, mucosal epithelial cells, or natural killer cells[8, 9]. The functional IFN-γ sensor consists of two subunits: IFN-γ receptor α (IFN-γR1) and IFN-γ receptor β (IFN-γR2). When in contact with the IFN-γ receptor (IFN-γR1/2), IFN-γ activates the Janus kinase (JAK) signaling sensor and transcription protein (STAT) pathway, leading to changes in the body's immune system, regulating various immune cells and ultimately mediating the development of systemic lupus erythematosus[10, 11]. It is reported that the IFN-γ signaling pathway is activated in SLE patients[11]. In addition, studies have found cross-interference between IFN-γ and MHC molecules. IFN-γ activates the transcription of class I and II MHC molecules, which contributes
to the development and severity of SLE[12]. In the mouse lupus model, Ozmen et al. found that treating NZB/W mice with soluble mouse IFN-γ receptors found chronic lupus lesions in mice could be inhibited, which illustrates the importance of receptors for the development of SLE. Although elevated IFN-γ levels are associated with SLE [1, 13], this association may be driven by reverse causation, confounding, and selection bias (i.e., selective survival before recruitment). Elucidating the role of IFN-γ and IFN-γR in SLE may help prevent the incidence of SLE and help develop new therapeutic targets.

Mendelian randomization (MR) is a genetic epidemiology approach that assesses the casual association between outcomes and exposures[14]. Genetic variants significantly related to exposure are selected as instrumental variables (IVs) to infer the causality[15]. The IVs that affect the exposure affect the results proportionally if the exposure is causal. Compared with traditional observational studies, MR analysis can overcome confounding factors, loss of follow-up, time-consuming and other difficulties in conventional studies. Therefore, in this study, we used a two-sample MR analysis MR to investigate the causal relationship between the three exposures (IFN-γ, IFN-γR1, and IFN-γR2) and SLE.

**Materials And Methods**

**Study Design**

The overall design used for this work is illustrated in Fig. 1. We first conducted forward MR analyses to investigate the effects of IFN-γ, IFN-γR1, and IFN-γR2 on SLE risk using data. Briefly, IFN-γ, IFN-γR1, and IFN-γR2 served as the exposure, while SLE served as the outcome. Single-nucleotide polymorphisms (SNPs) significantly associated with IFN-γ, IFN-γR1, and IFN-γR2 were selected as IVs based on strict inclusion and exclusion criteria. A series of sensitivity analyses were performed for significant associations. Subsequently, we performed reverse MR analyses to examine whether the genetic liability to SLE influences levels of IFN-γ, IFN-γR1, and IFN-γR2. There are three core assumptions for selecting IVs in the MR analysis[16]: (i) the genetic variants used to proxy for the exposure are robustly associated with the exposure. (ii) there is no confounding of the selected IVs with the outcome. (iii) the IVs should affect the outcome risk only through exposure, not other pathways.

**Data Sources**

In terms of the exposure, Genetic instruments of exposure to IFN-γ, IFN-γR1, and IFN-γR2 were derived from the large genome-wide association study (GWAS), which included a total of 3,301 sample size. They create and interrogate a genetic atlas of the human plasma proteome, using an expanded version of an aptamer-based multiplex protein assay (SOMAscan) to quantify 3,622 plasma proteins in 3,301 healthy participants from the INTERVAL study[17].

As for the outcome, Genetic variants for SLE were obtained from a GWAS including 23,210 individuals (7,219 SLE patients and 15,991 controls) of European ancestry[18].

To eliminate population stratification bias, all SNPs and their accompanying summary data were retrieved from studies that solely included populations of European ancestry. All the data adopted in this current study are publicly available in the GWAS summary datasets.
Selection of single-nucleotide polymorphisms

The following steps were used to select IVs to guarantee the authenticity and accuracy of the conclusions on the causal association of IFN-γ, IFN-γR1, and IFN-γR2 with SLE risk. First, the genetic variants significantly associated with the exposure (P < 5 × 10^{-6}) were considered IVs. Second, one of the principles of the MR approach is that there is no linkage disequilibrium (LD) among the included IVs since strong LD might result in biased results. The corresponding LD was screened (R^2 < 0.001 and clumping distance = 10,000kb) to avoid unnecessary excursions. Third, the minor allele frequency (MAF) threshold of variants of interest was 0.01. Fourth, extracting data for the above-selected SNPs from the outcome trait GWAS summary. Fifth, to guarantee that the effect alleles belong to the same allele, we harmonized the exposure and outcome datasets to eliminate ambiguous SNPs with non-concordant alleles and SNPs with intermediate allele frequencies. To avoid distortion of strand orientation or allele coding, we deleted palindromic SNPs (e.g., with A/T or G/C alleles). Finally, the F-statistic (F > 10) from a regression of the exposure on the variant is used to guarantee the slight possibility of weak instrumental variable bias[19] (Supplementary Table S1). Correspondingly, P < 5 × 10^{-8} was used as a threshold to obtain SNPs that were highly correlated with SLE as IVs and IFN-γ, IFN-γR1, and IFN-γR2 levels as an outcome. We performed the same methods to screen SNPs associated with SLE (Supplementary Table S2). These SNPs were used to perform reverse MR analysis in each data set. We utilized these carefully chosen SNPs as the final genetic IVs for the subsequent MR analysis[20, 21].

Mendelian Randomization analysis

Inverse-variance weighted (IVW) was applied to derive an overall weighted estimate of the potential causal effect by calculating the MR-derived odds ratio (OR) of SLE risk for IFN-γ, IFN-γR1, and IFN-γR2, which integrates the Wald ratio estimates of each SNP by meta-analysis (β coefficient of SNPs for SLE divided by β coefficient of SNPs for IFN-γ, IFN-γR1, and IFN-γR2) to obtain the overall effect of IFN-γ, IFN-γR1, and IFN-γR2 on SLE[22]. The IVW method is most powerful when all IVs are valid. However, when horizontal pleiotropy is present, it may lead to biased inference[23]. Accordingly, MR-Egger and weighted median enhance the IVW estimates because they present more reliable but less efficient estimates over a broader range of scenarios[24]. The weighted median method estimates the causal effect from the median of the weighted empirical density function of SNP-outcome/SNP-exposure ratio estimates, which provides valid estimates when ≥ 50% of the information is contributed from valid SNPs[25].

Sensitivity Analyses

Sensitivity analyses with different assumptions were performed to make the result more reliable, including pleiotropy and heterogeneity. The MR-Egger allows for directional pleiotropy by introducing an intercept in the weighted regression model. A significant MR-Egger intercept indicates the presence of directional pleiotropy[24]. MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) analyses were performed to identify and correct for potential outliers, which also helped to avoid potential horizontal pleiotropy[26]. The funnel plots visualize MR analyses and look for asymmetry as a sign of pleiotropy[27]. We used the IVW method and MR-Egger regression to detect heterogeneity. The heterogeneities were quantified by Cochran's Q statistic; a P value of < 0.05 would be regarded as significant heterogeneity. We also performed a “leave-one-out” sensitivity analysis to identify potentially influential SNPs. Forest plots were used to visualize the results from leave-one-
out analyses to evaluate the stability of effect sizes which recalculate the causal estimates from IVW by dropping out one SNP at a time to verify if the estimates were biased or driven by an outlier.

All statistical analyses were conducted using R. The IVW, weighted median, and MR-Egger regression methods were performed using the “TwoSampleMR” package. The MR-PRESSO test was performed using the “MRPRESSO” package. All the analyses with P < 0.05 were considered statistically significant.

Results

Selection of single-nucleotide polymorphisms

A total of 26 SNPs, including 9 SNPs associated with IFN-γ (rs115861866, rs138094598, rs141059739, rs1440480, rs2363910, rs7459901, rs75268621, rs7567468), 8 SNPs associated with IFN-γR1 (rs7080536, rs11770018, rs117480750, rs73044907, rs117748849, rs62245853, rs12034435, rs79708242) and 9 SNPs associated with IFN-γR2 (rs111566682, rs117210563, rs140878030, rs17709867, rs4540249, rs72639485, rs7852203, rs9586564, rs2065525), were selected by filtering the threshold of significance (P < 5 × 10⁻⁶), false discovery rate (FDR) < 5%, and LD (r² < 0.001). None of the genetic variants was palindromic with intermediate allele frequencies. Correspondingly, 40 harmonized SNPs of SLE for IFN-γ, IFN-γR1, and IFN-γR2 SNPs associated with SLE were screened (Supplementary Table S3). Accordingly, 66 SNPs were used to perform bi-directional MR analysis in each data set (Supplementary Table S4).

Mendelian Randomization analysis

The results of IVW estimates showed that higher levels of IFN-γ were associated with an increased risk of SLE [OR 1.24 (95% CI 0.85, 2.26), P = 0.018]. The results of the weighted median analysis supported our findings in the IVW analysis [OR 1.07 (95% CI 0.86, 1.33), P = 0.55]. In the IVW analysis, IFN-γR2 levels were inversely associated with the risk of SLE [OR 0.85 (95% CI 0.73, 0.99), P = 0.034] after correction for multiple comparisons. In addition, the weighted median [OR 0.89 (95% CI 0.73, 1.09), P = 0.260] also showed consistent results. MR-Egger produced consistent direction of effect estimates, strengthening the confidence toward true association. Therefore, we concluded that the results of the MR analysis might illustrate the potential causal association between increased IFN-γ levels and increased risk of SLE, which was opposite to the potential causal association of IFN-γR2 levels on the risk of SLE.

On the contrary, there is a lack of evidence to suggest an association between genetic predisposition to SLE and IFN-γR1. As the primary estimator, the multiplicative random effect IVW model showed that genetic predisposition to IFN-γR1 was not associated with the risk of SLE (OR = 0.97, 95% CI: 0.79 1.19, p = 0.768). A null association was also observed using the MR-Egger (OR = 0.80, 95% CI: 0.55 1.14, p = 0.264). Consistently, little evidence of causal effects of IFN-γR1 levels on SLE was provided in weighted median analysis (OR = 0.84, 95% CI: 0.70 1.02, p = 0.085). (Fig. 2) (Table 1)
Table 1

Mendelian randomization of IFN-γ, IFN-γR1, and IFN-γR2 levels on the risk of SLE.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>MR method</th>
<th>No. of SNPs</th>
<th>Association</th>
<th>Heterogeneity</th>
<th>Egger regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ levels</td>
<td>MR Egger</td>
<td>9</td>
<td>0.32887, 1.38940, 0.228</td>
<td>10.69006, 0.153</td>
<td>-0.02271, 0.626</td>
</tr>
<tr>
<td></td>
<td>IVW</td>
<td>9</td>
<td>0.21126, 1.23523, 0.018</td>
<td>11.08669, 0.197</td>
<td></td>
</tr>
<tr>
<td>IFN-γR1 levels</td>
<td>MR Egger</td>
<td>8</td>
<td>-0.22757, 0.79647, 0.264</td>
<td>11.34511, 0.078</td>
<td>0.05509, 0.252</td>
</tr>
<tr>
<td></td>
<td>IVW</td>
<td>8</td>
<td>-0.03064, 0.96982, 0.768</td>
<td>14.37791, 0.045</td>
<td></td>
</tr>
<tr>
<td>IFN-γR2 levels</td>
<td>MR Egger</td>
<td>11</td>
<td>-0.11252, 0.89358, 0.612</td>
<td>5.99048, 0.541</td>
<td>-0.00859, 0.813</td>
</tr>
<tr>
<td></td>
<td>IVW</td>
<td>11</td>
<td>-0.16118, 0.85114, 0.034</td>
<td>6.05086, 0.642</td>
<td></td>
</tr>
</tbody>
</table>

Odds ratios (ORs) estimate the relationship between systemic lupus erythematosus risk and IFN-γ, IFN-γR1, and IFN-γR2. OR < 1: genetic variants with higher/lower physiological serum IFN-γ, IFN-γR1, and IFN-γR2 levels are associated with decreased risk of SLE and vice versa. MR: Mendelian randomization; IVW, inverse variance weighted analysis; SNP: single-nucleotide polymorphism; No. of SNPs: number of single nucleotide polymorphisms; beta: beta coefficients; OR: Odds ratios; pval: p-value.

### Sensitivity Analyses

Horizontal pleiotropy between IVs and outcome was evaluated by MR-Egger regression, and the results indicated no evidence for a significant intercept. MR-PRESSO analysis identified no outlying SNP in associations between IFN-γ, IFN-γR1, IFN-γR2, and SLE, suggesting that the observed associations might not be affected by pleiotropy. For heterogeneity, Q statistics of the IVW test and the MR-Egger regression demonstrated no significant heterogeneity except for IFN-γR1 on SLE (MR-Egger, \( P = 0.078 \) and IVW, \( P = 0.045 \)).

In addition, the leave-one-out sensitivity analysis showed stable results of MR analysis. (Fig. 2) (Table 1)

### Reverse Analysis MR

To investigate the causal association of SLE on IFN-γ, IFN-γR1, and IFN-γR2, we implemented the reverse MR analysis by using SLE as exposure with IFN-γ, IFN-γR1 and IFN-γR2 levels as the outcome. We obtained SNPs that were highly correlated with SLE as IVs. The results of the IVW showed that there was no significant causal association of SLE on IFN-γ levels (β = -0.006, \( P = 0.730 \)), IFN-γR1 levels (β = 0.004, \( P = 0.801 \)), and IFN-γR2 levels (β = -0.007, \( P = 0.656 \)). No significant pleiotropy among the SNPs (\( P > 0.05 \)) was observed by performing MR-Egger. Mild heterogeneity was detected of IFN-γ (MR-Egger, \( P = 0.031 \) and IVW, \( P = 0.037 \)), indicating slight bias
caused by a few SNPs. The results of the funnel plot and leave-one-out sensitivity analysis showed that the association of SLE on IFN-\(\gamma\), IFN-\(\gamma\)R1, and IFN-\(\gamma\)R2 levels was not remarkably affected by any individual SNP. (Fig. 3)(Table 2)

Table 2

<table>
<thead>
<tr>
<th>outcome</th>
<th>MR method</th>
<th>No. of SNPs</th>
<th>Association</th>
<th>Heterogeneity</th>
<th>Egger regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td></td>
<td>(\beta)</td>
<td>S.E.</td>
<td>pval</td>
</tr>
<tr>
<td>IFN-(\gamma) Levels</td>
<td>MR Egger</td>
<td>43</td>
<td>-0.01973</td>
<td>0.03685</td>
<td>0.596</td>
</tr>
<tr>
<td></td>
<td>IVW</td>
<td>43</td>
<td>-0.00615</td>
<td>0.01779</td>
<td>0.730</td>
</tr>
<tr>
<td></td>
<td>Weighted median</td>
<td>43</td>
<td>-0.01483</td>
<td>0.02312</td>
<td>0.521</td>
</tr>
<tr>
<td>IFN-(\gamma)R1 levels</td>
<td>MR Egger</td>
<td>43</td>
<td>-0.03478</td>
<td>0.03397</td>
<td>0.312</td>
</tr>
<tr>
<td></td>
<td>IVW</td>
<td>43</td>
<td>0.00421</td>
<td>0.01673</td>
<td>0.801</td>
</tr>
<tr>
<td></td>
<td>Weighted median</td>
<td>43</td>
<td>-0.00818</td>
<td>0.02163</td>
<td>0.705</td>
</tr>
<tr>
<td>IFN-(\gamma)R2 levels</td>
<td>MR Egger</td>
<td>43</td>
<td>0.01420</td>
<td>0.03040</td>
<td>0.643</td>
</tr>
<tr>
<td></td>
<td>IVW</td>
<td>43</td>
<td>-0.00660</td>
<td>0.01483</td>
<td>0.656</td>
</tr>
<tr>
<td></td>
<td>Weighted median</td>
<td>43</td>
<td>0.00266</td>
<td>0.02000</td>
<td>0.894</td>
</tr>
</tbody>
</table>

The \(\beta\)-coefficients represent the log odds ratio (OR) of SLE risk for each additional effect allele \((\beta < 0, \text{OR} < 1; \beta = 0, \text{OR} = 1; \beta > 0, \text{OR} > 1)\). \(\beta < 0\): genetic variants with physiological serum IFN-\(\gamma\), IFN-\(\gamma\)R1, and IFN-\(\gamma\)R2 levels are associated with reduced risk of SLE and vice versa. MR: Mendelian randomization; SNP: single-nucleotide polymorphism; No. of SNPs: number of single nucleotide polymorphisms; IVW, inverse variance weighted analysis; beta: beta coefficients; se: standard error; pval: p-value.

Discussion

We elucidate the causal relationship between IFN-\(\gamma\) or IFN-\(\gamma\)R and SLE through two-sample MR analysis in both directions. After eliminating complex confounders, genetic evidence suggests that higher IFN-\(\gamma\) levels or lower IFN-\(\gamma\)R2 levels were significantly associated with an increased risk of SLE. IFN-\(\gamma\)R1 has no apparent causal relationship with SLE. To clarify the causal relationship, we performed reverse MR, and the results showed no significant relationship between SLE risk and IFN-\(\gamma\) or IFN-\(\gamma\)R levels.

Patients with SLE are known to have significantly higher levels of IFN-\(\gamma\) mRNA and protein than healthy people and higher than normal levels of mRNA produced by IFN-induced gene type II (IRF1, GBP1, CXCL9, CXCL10, and SERPING1) [28] [29]. Thomason et al. showed that IFN-\(\gamma\) activation could indicate the disease activity of SLE patients[30]. In addition, the response to ustekinumab treatment in SLE patients was related to the
suppression of serum IFN-γ levels[31]. Furthermore, Jacob CO et al. found that in (NZB)/(NZW)F1 mice, good efficacy was observed in vivo with IFN-γ monoclonal antibodies in lupus nephritis[32]. These studies show that elevated IFN-γ levels are associated with an increased risk of SLE, confirming our analysis. However, Hron JD et al. found that IFN-RII deficiency protected MRL/LPR mice from severe autoimmune-related lymphadenopathy, autoantibodies, and kidney disease[33]. This contradicts our results that reduced IFN-γR2 levels are associated with an increased risk of SLE.

In conducting the analysis, the mechanism of exposure to the results needs to be clarified. IFN-γ works through a signaling pathway that binds to the IFN-γR, which is expressed on most cells and activates JAK1 and JAK2, causing STAT1 to phosphorylate, and then attaches to the IFN-γ activation site (GAS) for gene transcription to complete the related functions[34]. Understanding the process of the IFN-γ signaling pathway can help explain IFN-γ and IFN-γR as the causes of exposure, clarify the development path of the disease, and provide theoretical support for future clinical treatment. The pathogenesis of SLE is complex, and the entire pathogenesis cascade is mediated by immune disorders. Immune signatures include loss of immune self-tolerance and enhanced T and B cell responses. And IFN-γ is a major pro-inflammatory cytokine capable of regulating the function of several important immune system cells, including B cells and T cells, contributing significantly to the development of SLE[35, 36].

First, it causes disordered regulation of T cells. In the pathogenesis of SLE, imbalances in Th1 and Th2 cells are common. IFN-γ signaling can greatly inhibit the differentiation of CD4+ T cells into Th2, which leads to an imbalance of Th1 and Th2 cells[37]. Second, B cells are an inflammatory mediator that produces pathogenic antibodies to enhance the inflammatory response, directly damaging tissues and cells [38]. IFN-γ signaling stimulates T cells and antigen-presenting cells (APCs) to produce B-lymphocyte-stimulating factor (BLyS), which is used to differentiate and survive B-cell[39–42]. Third, Due to its role in maintaining peripheral immune tolerance, defects in Treg cell function or quantity are thought to be part of the pathogenesis of SLE[43]. Recent studies have shown the ability of IFN-γ to directly inhibit the function of Treg cells, leading to loss of autoimmune tolerance and mediating the development of SLE disease[44–46].

The IFN-γ signaling pathway is critical in both innate and adaptive immunity, and in this regard, IFN-γR is required for these IFN-γ biological activities and signaling. Based on past studies, H Nakashima et al. found amino acid polymorphisms (Val14Met) within IFN-γR1, and the frequency of the Met14 allele in SLE patients was significantly higher than in healthy controls. Amino acid polymorphisms (Gln64Arg) were found in IFN-γR2. The most significant risk of SLE development was detected in individuals with the IFNGR1 Met14/Val14 genotype and the IFNGR2 Gln64/Gln64 genotype, indicating that it plays a vital role in SLE susceptibility[47]. Yao Xu et al. found that the IFN-γR2 Arg64/Arg64 genotype reduced the risk of SLE (P = 0.047)[48]. The study illustrates that reduced IFN-γR2 levels are associated with an increased risk of SLE. Our results show that IFN-γR2 is associated with SLE, while IFN-γR1 is not related to SLE, which will guide the study of the mechanism of SLE in the later stages and provide better therapeutic targets for clinical treatment.

The strength of our study is the MR study design, which mitigates unobserved confounding and reverses causation by utilizing genetic variation as a proxy for IFN-γ and IFN-γR. In addition to the method’s advantages, our study extends previous MR studies on IFN-γ and IFN-γR versus SLE. First, we used bidirectional MR, and we found evidence from only 1 direction, IFN-γ, and IFN-γR2 showing a potential causal relationship with SLE,
which does not appear to have such a relationship with IFN-γ and IFN-γR. Second, most articles study IFN-α, but IFN-γ also plays a large role in SLE, and the two are even used together in some pathways. Our study not only studies IFN-γ but also its receptors, which provide a more rigorous understanding of the risk factors for SLE, which is affected not only by IFN-γ but also by IFN-γR2. Moreover, we used summary-level data from many SLE and controls from the latest GWAS population, which increased the statistical power of causal estimates.

However, some limitations should be mentioned. First, the patients we recruited were all European, so causality in other populations still needs to be improved. We need more data to prove causality between other people. Second, we cannot rule out the possibility that horizontal pleiotropy affects our results, even if we take steps to identify and rule out anomalous variables. Third, the few SNPs with heterogeneity in our reverse Mendelian randomization experiment caused less bias. However, the correlation of SLE to IFN-γ, IFN-γR1, and IFN-γR2 levels was not significantly affected by any single SNP.

**Conclusion**

The MR study showed a causal relationship between IFN-γ, IFN-γR2, and SLE. Higher levels of IFN-γ are associated with an increased risk of SLE. In contrast, lower levels of IFN-γR2 were associated with an increased risk of SLE, while IFN-γR1 had no causal relationship with SLE. It shows that IFN-γR plays a role in SLE mainly IFN-γR2, and IFN-γR2 is a potential therapeutic target for the treatment of SLE.

**Declarations**

**Acknowledgments**

Data about the IFN-γ-related GWAS data was obtained from the INTERVAL study Proteomics- GWAS data. We thank all involved investigators for sharing their data. We want to acknowledge the participants and investigators of the Integrative Epidemiology Unit (IEU) GWAS database.

**Author Contributions**

Study design and manuscript writing: XDL. KXY. And JWH. Data extraction, quality assessment, analysis and interpretation of data: XDL. KXY. And JWH. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Li had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Funding**

Sheng-xiao Zhang (China National Funds for Distinguished Young Scientists)

**Availability of data and materials**

The datasets generated and analysed during the current study are available in the IEU open gwas project [https://gwas.mrcieu.ac.uk/], and the GWAS ID are prot-a-1428, prot-a-1430, prot-a-1432, ebi-a-GCST003156, respectively.
Ethics approval and consent to participate

not applicable.

Consent for publication

not applicable.

Competing interests

None.

References


Figures

Figure 1

Schematic diagram of bidirectional two-sample MR study on the association between IFN-γ, IFN-γR1, IFN-γR2, and SLE

(A-B): The genetic variant is closely associated with exposure. The IVs should influence the risk of outcomes through risk factors only, not any alternative pathways. The genetic variation used as IV should not be associated with confounding factors. IFN-γ, Interferon-gamma; IFN-γR1, Interferon gamma receptor 1; IFN-γR2,
Interferon gamma receptor 2; SLE, systemic lupus erythematosus; MR, Mendelian randomization; SNP, single nucleotide polymorphism.

**Figure 2**

MR test, pleiotropic effect analysis, heterogeneity of IFN-γ, IFN-γR1, and IFN-γR2 levels, and the risk of SLE (A-C): Scatter plots of genetic associations with IFN-γ, IFN-γR1, and IFN-γR2 against the genetic associations with SLE. Plots of effect sizes for SNP IFN-γ, IFN-γR1, and IFN-γR2 associations (x-axis, SD units) and SNP-SLE associations (y-axis, log OR) against standard error lines. The slope of each bar indicates the causal
association of each method. The blue line is the inverse-variance variance weighted estimates, the green line indicates weighted median estimates, and the dark blue line indicates MR-Egger estimates. (D-F): Heterogeneity of genetic associations with IFN-γ, IFN-γR1, and IFN-γR2 with SLE assessed by funnel plot. The blue line represents the inverse-variance weighted estimate, and the dark blue line represents the MR-Egger estimate. (G-I): Leave-one-out sensitivity analysis of single SNP of IFN-γ, IFN-γR1, and IFN-γR2 for SLE. Leave-one-out sensitivity analysis to determine if individual SNPs disproportionately affect the association of SLE. Each black point in the forest plot represents the MR analysis (using IVW), excluding that particular SNP. The overall analysis, including all SNPs, is also shown for comparison. (A, D, G: IFN-γ; B, E, H: IFN-γR1; C, F, I: IFN-γR2)
MR test, pleiotropic effect analysis, heterogeneity of the risk of SLE, and IFN-γ, IFN-γR1, and IFN-γR2 levels

(A-C): Scatter plots of SLE against the genetic associations with IFN-γ, IFN-γR1, and IFN-γR2. Plots of effect sizes for SNP-SLE association (x-axis, SD units) and the SNP IFN-γ, IFN-γR1, and IFN-γR2 associations (y-axis, log OR) against standard error bars. The slopes of each line indicate the causal association for each method. The blue line is the inverse-variance variance weighted estimates, the green line indicates weighted median estimates, and the dark blue line indicates MR-Egger estimates. (D-F): Heterogeneity of genetic associations with SLE with IFN-γ, IFN-γR1, and IFN-γR2 assessed by funnel plot. The blue line represents the inverse-variance weighted estimate, and the dark blue line represents the MR-Egger estimate. (G-I): Leave-one-out sensitivity analysis of single SNP of SLE for IFN-γ, IFN-γR1, and IFN-γR2. Leave-one-out sensitivity analysis to determine if individual SNPs disproportionately affect the association of IFN-γ, IFN-γR1, and IFN-γR2. Each black point in the forest plot represents the MR analysis (using IVW), excluding that particular SNP. The overall analysis, including all SNPs, is also shown for comparison. (A, D, G: IFN-γ; B, E, H: IFN-γR1; C, F, I: IFN-γR2)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx