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Causal association between *Helicobacter pylori* infection and Sjogren's syndrome: a bidirectional Mendelian randomization analysis

Dinglu Cui[1], Rongxian An[1], Lei Li[1], Long Jiang[1], Chunshan Jiang[2*] and Jingchun Jin[1*]

Abstract

Background The results of observational studies indicate a potential link between *Helicobacter pylori* infection and Sjogren's syndrome (SS), but the causal relationship between them remains unknown. This study applied Mendelian randomization (MR) to evaluate this relationship.

Method Genome-wide association study (GWAS) summary statistics on *H. pylori* infection [sample size=8735 (EBI, https://gwas.mrcieu.ac.uk/)] and SS [sample size=368,028 (cases=2495, controls=365533) (FinnGen, https://r9.finngen.fi/)] were analyzed. We used bidirectional MR to evaluate the association between *H. pylori* infection and SS and identify causation. The major MR analysis method was inverse-variance weighted (IVW) MR, supplemented by MR–Egger and weighted median approaches. In addition, the stability and reliability of the results were tested using the retention method, heterogeneity test, and horizontal gene pleiotropy test.

Results Evidence of the impact of *H. pylori* infection on SS risk was found in the IVW results [odds ratio (OR)=1.6705; 95% confidence interval (CI)=1.0966 to 2.5446; P=0.0168]. Evidence of the impact of SS on *H. pylori* infection risk was also found (OR=1.0158; 95% CI=1.0033 to 1.0285; P=0.0128).

Conclusion The results of MR analysis support a causal association between *H. pylori* infection and SS and indicate that SS can lead to a greater risk of *H. pylori* infection. Our research will support the development of novel approaches for continued *H. pylori* and SS-related research and therapy that consider the genetic relationship between *H. pylori* infection and SS.

Keywords Mendelian randomization, *Helicobacter pylori* infection, Sjogren's syndrome, Causal inference

Introduction

Helicobacter pylori (*H. pylori*) is a spiral-shaped gram-negative bacterium that frequently colonizes multiple sites in the body, such as the stomach and duodenum. According to the literature, the global prevalence of *H. pylori* infection in adults declined from 50–55% to 43% from 2014–2020, mostly attributed to improvements in living standards and hygiene conditions [1]. The current rates range from 50.8% in developing countries to 34.7% in developed countries [2]. *H. pylori* is clinically

*Correspondence:
Chunshan Jiang
jcs0915@163.com
Jingchun Jin
jingchun680928@163.com
[1] Department of Rheumatology, Yanbian University Hospital, 1327 Juzi Street, Yanji 133000, China
[2] Yanbian University Hospital, 1327 Juzi Street, Yanji 133000, China

associated with various gastrointestinal diseases, such as gastric tumors, autoimmune gastritis, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastrointestinal ulcers [3]. In addition, there is a correlation between *H. pylori* infection and the occurrence of extragastrointestinal immune diseases such as rheumatic immune disease and autoimmune thyroid disease [4].

Sjogren's syndrome (SS) is a chronic inflammatory autoimmune disease characterized by lymphocyte proliferation and progressive damage to the exocrine glands. In addition to impaired function of the salivary and lacrimal glands, the clinical manifestations of SS may include multiple system and organ involvement, serum autoantibodies and hyperimmunoglobulinaemia [5]. The combined prevalence of SS in the global population is 60.82/100000, with women affected approximately ten times as often as men [6]. The prevalence of SS in Europe is approximately 0.23% [7]. At present, the exact aetiology and pathogenesis of SS are not clear, but it is generally believed that immune dysfunction is caused by various factors, such as genetic variants, viral infection, and abnormal sex hormone levels.

To explore the possible correlation between *H. pylori* infection and SS, many scholars have investigated the relationship between *H. pylori* and *H. pylori*-related antibodies in SS patients. Studies have shown that the average titre of anti-*H. pylori* serum antibodies in SS patients is significantly greater than that in SLE and RA patients [8]. Another study analyzed the levels of anti-*H. pylori* antibodies in 43 patients with SS and 95 controls, and reported that the levels of these antibodies were significantly increased in SS patients (34% vs. 10.5%, P=0.0001) [9]. Thus, the results of this study revealed that the infection rate of *H. pylori* infection in the SS group was significantly greater than that in the healthy population. Although many studies have shown that *H. pylori* infection promotes SS, other studies of the relationship between *H. pylori* infection and SS have reached the opposite conclusion. Theander et al. [10] reported that *H. pylori* seropositivity is not associated with the presence of immunological markers for SS, such as circulating autoantibodies or lip biopsies with abnormal focal scores. Interestingly, Ram et al. [11] reported that *H. pylori* infection rates are lower in patients with SS, suggesting a protective role, that is, that *H. pylori* infection is negatively correlated with the occurrence and development of SS.

The relationship between *H. pylori* infection and SS has been controversial. Furthermore, conclusions about causality cannot be drawn solely from the results of observational studies, as cohort and cross-sectional studies may have limitations such as limited sample sizes, difference in the racial compositions of cohorts, and the presence of other confounding factors.

Mendelian randomization (MR) is a technique that uses genetic variation as an instrumental variable (IV) to assess whether the observed associations between exposure factors and outcomes are consistent with causal effects [12]. MR tests three assumptions: 1) genetic variation is associated with risk factors; 2) genetic variation is not associated with confounders; and 3) genetic variation affects outcomes only through risk factors [13]. Since genetic variation is not influenced by other factors, such as the external environment and social behavior, it is a stable exposure factor over time. Therefore, in observational studies, analytical bias can be minimized by avoiding the influence of confounders and reverse causality on correlation effects through MR methods. In recent years, MR methods have been widely used in studies assessing the causal relationship between exposure and outcome [14].

In clinical practice and research, a certain correlation between *H. pylori* infection and SS has been identified, but the causal relationship between *H. pylori* infection and SS remains unknown. Considering these findings, this study aimed to investigate the causal relationship between *H. pylori* infection and SS, using the data from a large-scale GWAS in a bidirectional MR design.

Materials and methods

Ethics

This study was reported according to the STROBE-MR guidelines [15], with data collected from public databases. No ethical approval was required for this study.

Study design

In this study, a bidirectional MR design was used to investigate the causal association between *H. pylori* infection and SS [14]. SNPs are used as IVs for MR studies to evaluate the causal effect of exposure variables [16]. The overall setup for the current MR study is shown in Fig. 1. The validity of MR analysis is subject to three core assumptions: ① relevance; ② independence; and ③ exclusion-restriction [13].

Data sources for anti-*Helicobacter pylori* IgG seropositivity and SS

Summary GWAS data for *H. pylori* infection were obtained from the publicly available European Bioinformatics Institute (EBI) database. Moreover, the genetic association of SS was available from FinnGen, comprising 2495 European cases and 365533 European controls. Detailed information about the GWAS data contained in this study is provided in Table 1.

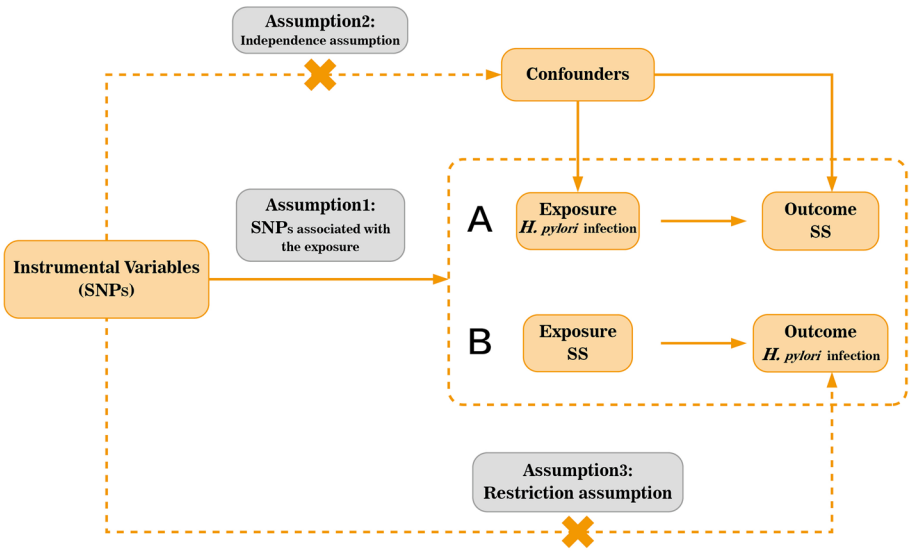

Fig. 1 Overall design of Mendelian randomization analysis. **A** Causal estimation of *H. pylori* infection on SS. **B** Causal estimation of SS on *H. pylori* infection. *H. pylori*:*Helicobacter pylori*; SS:Sjogren's syndrome; SNPs:single nucleotide polymorphisms

Table 1 Description of GWASs used for each phenotype

Phenotype	Data sources	Sample size	SNPs (*n*)	Ancestry
H. pylori infection	EBI	8735	9,170,312	European
SS	FinnGen	368,028 (2495cases; 365533controls)	20,170,011	European

GWAS Genome-wide association studies, *H pylori Helicobacter pylori*, *SS* Sjogren's syndrome, *SNPs* Single nucleotide polymorphisms

Selection of genetic variants as instrumental variables(IVs)

In this study, MR analysis was performed on the principle that SNPs must be significantly correlated with exposure, and the loci of SNPs that were significantly associated with *H. pylori* infection or SS were selected according to a genome-wide threshold of $P<5\times10^{-8}$. Unfortunately, only a small number of SNPs were obtained for the IVs of *H. pylori* infection. To explore more relationships between *H. pylori* and SS and obtain more comprehensive results, we selected SNPs with a less strict significance of $P<5\times10^{-6}$ as suggested by previous studies [17, 18], and selected them as a second IV set to find more potential causal associations [19, 20].

Moreover, linkage disequilibrium (LD) analysis was performed to ensure independence between SNPs (LD, r2<0.001, clumping distance>10,000 kb) [13]. To ensure that the effect alleles belonged to the same allele, the exposure and outcome datasets were harmonized to eliminate SNPs with intermediate allele frequencies and ambiguous SNPs with mismatched alleles. Furthermore, to avoid the influence of weak IVs on the causal effect, the F-statistic of each selected IV had to be greater than 10. Finally, to ensure that IVs could affect the outcome only through exposure, SNPs associated with confounders were manually eliminated in PhenoScanner [21].

Statistical analysis

A two-sample MR method was used to evaluate the potential causal relationship between *H. pylori* infection and SS. Before the MR analysis, a pleiotropy residual sum and outlier (PRESSO) method was adopted to evaluate the horizontal polytropy of the data, which was corrected before effect assessment, to ensure the reliability of the MR analysis.

The inverse-variance weighted (IVW) method was the primarily method used in the MR analysis, as it provides consistent estimates of exposure–outcome associations when the IVs are not pleiotropic [22].

Cochran's Q statistic was applied to assess the heterogeneity across individual SNPs [23]. When the P value of Cochran's Q test was greater than 0.05, there was no heterogeneity among the SNPs. MR–Egger and the weighted median were used in complementary analyses [24, 25]. Furthermore, sensitivity analysis was performed using the leave-one-out method, and the degree of influence of each SNP on causality was carefully evaluated after removing the final included SNPs one by one. All data analysis and statistical plots generation in this study were performed via the TwoSampleMR package (0.5.7), version R 4.3.1. Forest plots were created using the "forestplot" R package (version 3.1.1). The statistical results are

expressed as odds ratios (ORs) and 95% confidence intervals (95% CIs), and $P<0.05$ was considered to indicate statistical significance.

Results

Instrumental variable selection

Using a genome-wide threshold of $P<5\times10^{-6}$, rigorous screening was performed as previously described, resulting in the identification of 17 SNPs mediating the causal associations between *H. pylori* infection and SS. Moreover, 10 SNPs were ultimately obtained ($P<5\times10^{-8}$) for MR analysis of the causal association between SS and *H. pylori* infection. The F statistics of all IVs were greater than 10, suggesting that these IVs could generally be considered to provide sufficient information for MR studies (Supplementary Tables 1-3).

Causal effects of *H. pylori* infection on SS risk

The causal estimates of this MR analysis are shown in Table 2 and Fig. 2. The ORs of *H. pylori* infection associated with SS for the three methods (IVW, weighted median, and MR–Egger) were 1.6705 (95% CI: 1.0966-2.5446, P=0.0168), 1.9298 (95% CI: 1.0331-3.6047; P=0.0391), and 1.1493 (95% CI: 0.3035-4.3513; P=0.8403), respectively. The results of our study revealed that genetically predicted *H. pylori* infection was significantly associated with the risk of SS (IVW $P<0.05$).

Causal effects of SS on *H. pylori* infection risk

The reverse MR results were presented in Table 3 and Figure 3. The ORs of SS associated with *H. pylori* infection for the three methods (IVW, weighted median, and MR-Egger) were 1.0158 (95% CI: 1.0033–1.0285, P=0.0128), 1.0182 (95% CI: 1.0013–1.0353; P=0.0341), and 1.0256 (95% CI: 0.9924–1.0600; P=0.1697), respectively. The results demonstrated that genetically predicted SS led to a greater risk of *H. pylori* infection (IVW $P<0.05$).

Heterogeneity and sensitivity tests

Heterogeneity refers to the variability observed in the causal estimates obtained for each SNP. Low heterogeneity suggests increased reliability of MR estimates. In our study, the Cochran's Q test demonstrated no evidence of heterogeneity among the IV estimates based on the individual variants (Tables 2, 3). Therefore, based on Cochran's Q test, we applied fixed effect IVW. Moreover, the leave-one-out analysis did not identify any SNP outliers, suggesting that our results were stable (Figs. 2C, 3C). In addition, the funnel plot displayed no evidence of asymmetry, indicating the absence of directional horizontal pleiotropy (Fig. 4).

Discussion

In this study, a bidirectional MR design was utilized to investigate the causal association between *H. pylori* infection and SS. To our knowledge, this study is the first to use MR methods for this purpose. Our data of this study revealed that there was a significant causal association between *H. pylori* infection and SS.

In recent years, studies have shown a correlation between *H. pylori* infection and SS. Studies have also revealed that SS patients have increased levels of anti-*H. pylori* serum antibodies than age-matched controls or patients with other connective tissue diseases [8, 9]. Similar results have been obtained in studies on anti-*H. pylori* antibodies in Italian SS patients (OR=15.67, 95% CI: 4.5–54.8; $P < 0.001$) [26].

Owing to the high degree of sequence homology between *H. pylori* and human heat shock protein (HSP), Aragona et al. hypothesized that *H. pylori* infection may trigger an autoimmune response to its HSP and proposed that the HSP60 produced by *H. pylori* may play a role in the pathogenesis of SS [27]. Thus, these results indicate that the hypothetical role of HSP60 in the development of the immune response in both primary SS and secondary SS seems to be closely linked to the prevalence of *H. pylori* infection.

SS patients commonly experience blood-related symptoms, including leukopenia, thrombocytopenia, and in some cases even severe thrombocytopenia. Autoimmune factors play a dominant role in the pathogenesis of thrombocytopenia caused by SS [28]. In 1998, Gasparin et al. [29] reported that *H. pylori* infection is associated with the occurrence of autoimmune thrombocytopenia.

Table 2 Causal effects of *H. pylori* infection on SS risk according to MR analysis

Exposure	Outcome	SNPs (n)	MR method	OR	95% CI	*P*-value	Cochran Q Statistic	Heterogeneity *P*-value
H.pylori infection	SS	17	IVW	1.6705	(1.0966, 2.5446)	0.0168	19.209	0.2579
			Weighted median	1.9298	(1.0331, 3.6047)	0.0391		
			MR-Egger (P for pleiotropy=0.5648)	1.1493	(0.3035, 4.3513)	0.8403	18.775	0.2240
			Weighted mode	2.6847	(0.8791, 8.1986)	0.1021		

H. pylori Helicobacter pylori, *SS* Sjogren's syndrome, *SNPs* Single nucleotide polymorphisms

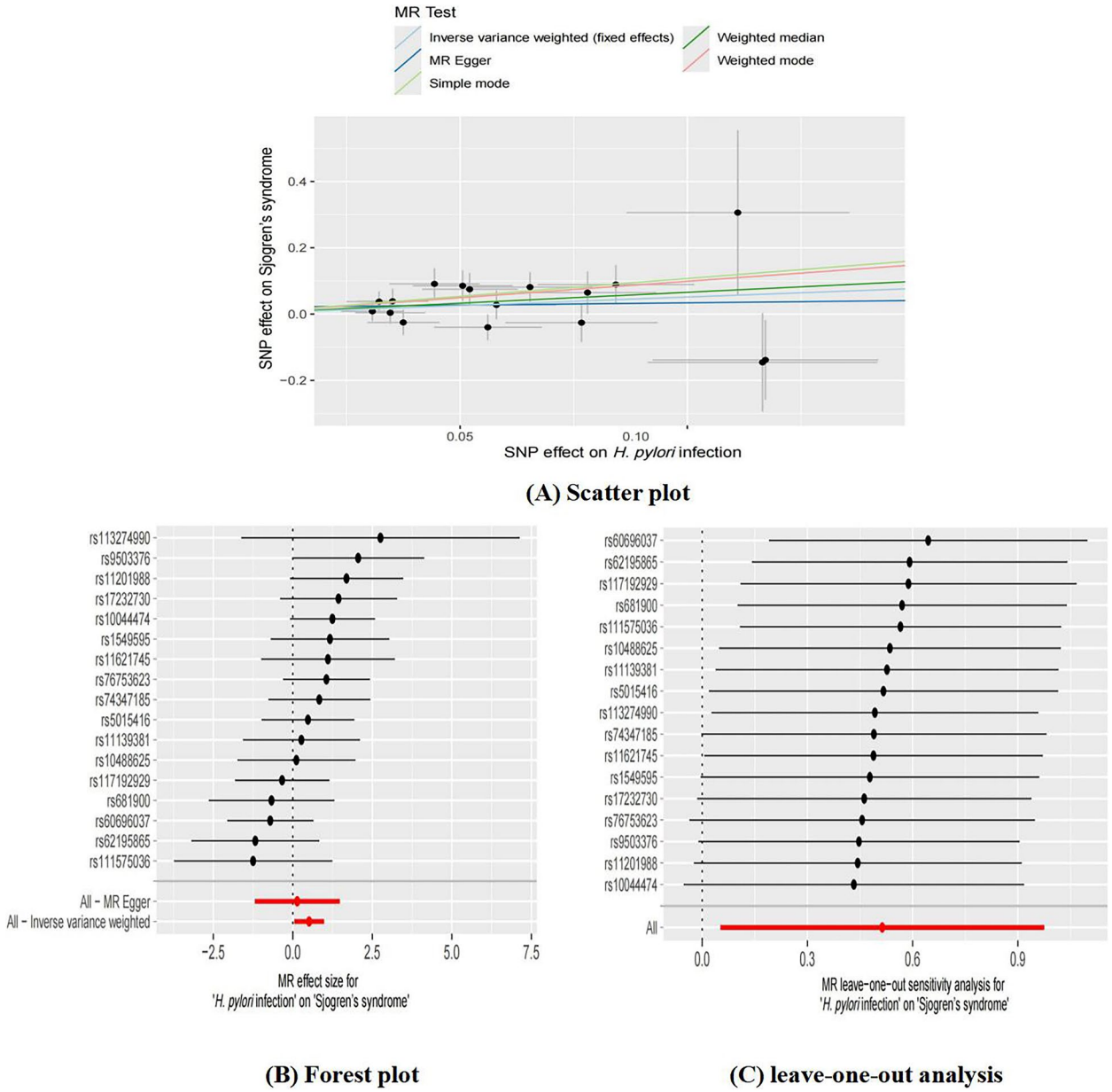

Fig. 2 The causal effects of *H. pylori* infection on SS in different MR methods

Table 3 Causal effects of SS on *H. pylori* infection risk according to MR analysis

Exposure	Outcome	SNPs (n)	MR method	OR	95% CI	*P*-value	Cochran Q Statistic	Heterogeneity *P*-value
SS	H.pylori infection	10	IVW	1.0158	(1.0033, 1.0285)	0.0128	12.698	0.1767
			Weighted median	1.0182	(1.0013, 1.0353)	0.0341		
			MR-Egger (P for pleiotropy=0.5370)	1.0256	(0.9924, 1.0600)	0.1697	12.071	0.1480
			Weighted mode	1.0179	(1.0011, 1.0349)	0.0650		

H. pylori Helicobacter pylori, *SS* Sjogren's syndrome, *SNPs* Single nucleotide polymorphisms

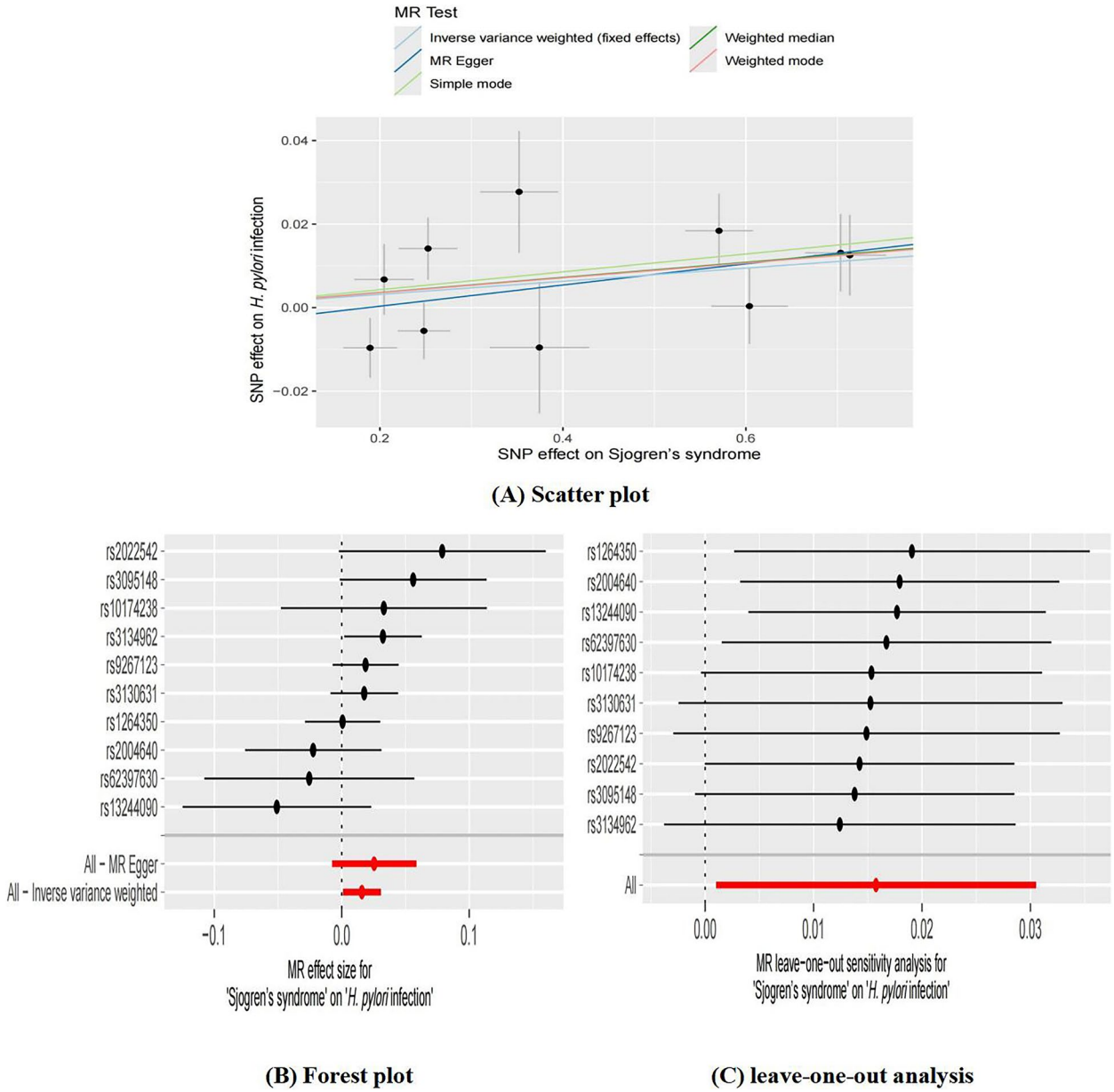

Fig. 3 The causal effects of SS on *H. pylori* infection in different MR methods

At present, the mechanism by which *H. pylori* infection leads to thrombocytopenia in SS patients is not yet clear. Kurata et al. suggested that there may be cross-reactivity between *H. pylori* and a certain platelet antigen component [30]. After *H. pylori* infection, certain components of the body are induced to transform into platelet cross antigens that are recognized by the body's immune system, suggesting that *H. pylori* infection may be associated with the occurrence of thrombocytopenia in SS patients.

Compared with normal individuals, SS patients are more likely to be infected with *H. pylori* [31]. A meta-analysis revealed that 1958 participants (including 619 patients with SS) from nine studies met the inclusion criteria. The total infection rate of *H. pylori* was 53.83% (1054/1958). The study revealed that patients with SS had a significantly greater *H. pylori* infection rate than did control patients (OR=1.19, 95% CI: 1.01-1.41, *P*=0.033) [32].El Miedany et al. conducted relevant studies to determine the presence of clinical markers related to *H. pylori* infection in SS patients and their significance for the treatment of such patients [33]. The results revealed that certain risk factors, including age, disease duration,

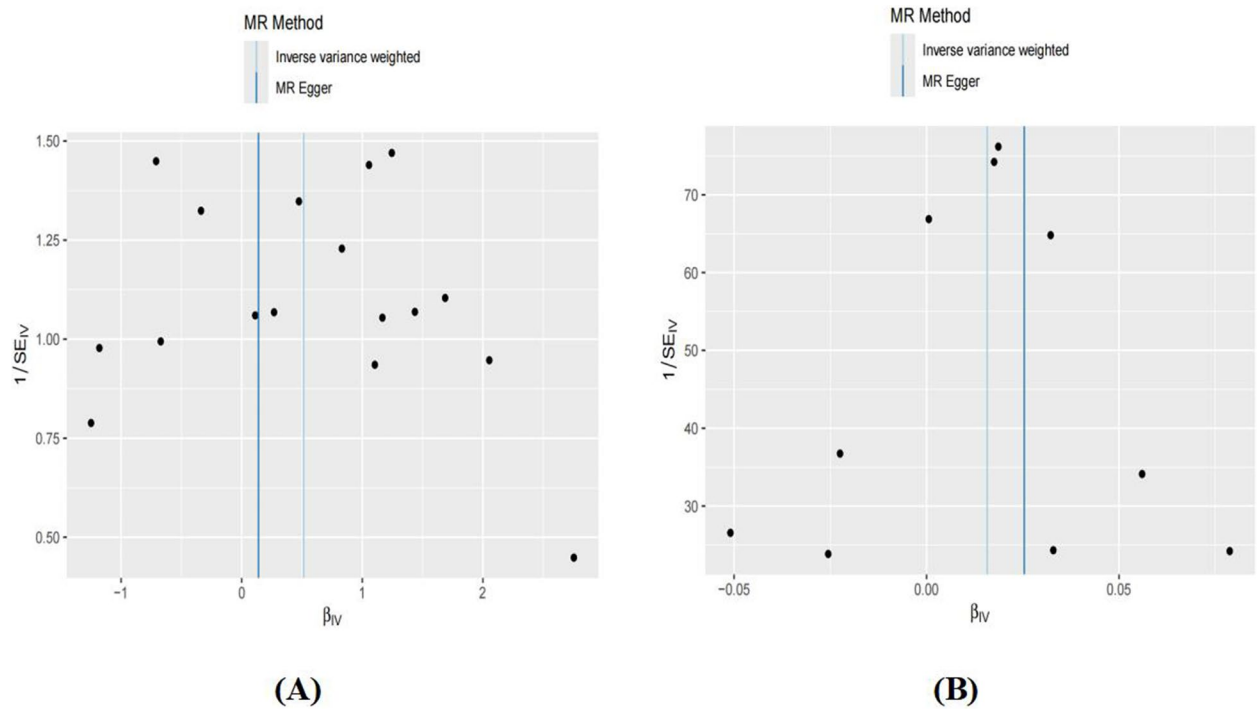

Fig. 4 Funnel plot. **A** The causal effects of *H. pylori* infection on SS. **B** The causal effects of SS on *H. pylori* infection

overall disease severity and C-reactive protein (CRP) levels, may be significantly associated with *H. pylori* infection in SS patients.

The role of *H. pylori* infection in the pathogenesis of immune diseases is not yet clear. Possible mechanisms include the activation of superantigens or polyclonal lymphocytes, molecular antigen imitation, epitope transmission, and bystander activation, all of which are believed to be related to immune dysregulation during infection [34]. The *H. pylori* strain encoding cytotoxin-associated gene A (CagA) has an enhanced ability to stimulate the secretion of proinflammatory cytokines, resulting in tissue injury, polarity, and host cell proliferation, thereby regulating the host immune response [35]. Therefore, *H. pylori* infection may be one factor that can trigger rheumatic immune disease.

Infection, including viral and bacterial infection, is considered a risk factor for SS. To date, some studies have suggested that dysbiosis of the oral microbiota may induce the occurrence and development of SS by promoting abnormal B lymphocytes activation and differentiation, leading to many lymphocytes infiltrating the salivary glands [36]. Given the presence of *H. pylori* in the host's oral cavity, it is also believed that SS may be associated with *H. pylori* infection.

The persistent presence of *H. pylori* in the gastric mucosa leads to chronic immune system activation, resulting in sustained cytokine signaling; infiltration of neutrophils, macrophages and lymphocytes into the gastric mucosa; and the production of antibodies and effector T cells [37]. Studies have shown that *H. pylori* infection induces a helper T-cell 1 (Th1) response, leading to the production of interleukin-2 (IL-2) and interferon-γ (IFN-γ) [38]. The IL-2 content in the lacrimal glands of SS patients is significantly greater than that in individuals without SS, indicating that IL-2 plays a major role in the pathological changes in lacrimal gland tissue and may be one of the main factors causing degeneration of lacrimal gland cells [39]. Other studies have shown that the salivary glands of SS patients are infiltrated with many plasmacytoid dendritic cells (pDCs), which mainly secrete IFN-γ, and that IFN-γ in the salivary glands of SS patients can induce dysfunction in salivary gland secretion [40].

H. pylori infection is associated with SS, also due to the common histological findings, such as exocrine gland destruction, lymphocyte infiltration and CD8+ T-cell activation [41, 42]. Irani et al. confirmed through immunohistochemistry that the level of *H. pylori* in patients with inflammatory lesions of the oral mucosa is greater than that in healthy individuals [43]. Moreover, *H. pylori* may interact with the surface of epithelial cells, directly causing cell damage or producing proinflammatory mediators [10]. SS patients with persistent oral lesions are more likely to be infected with *H.*

pylori. Furthermore, a recent meta-analysis reported that of a total of 224 patients diagnosed with SS, 94 (41.96%) were infected with *H. pylori*. Multivariate analysis demonstrated that hypergammaglobulinemia could be an independent risk factor for *H. pylori* infection in patients with SS [44].

In summary, the *H. pylori* infection rate in SS patients was significantly higher than that in healthy individuals. Possible risk factors include age, duration of disease, overall disease severity, CRP, and hyperglobulinemia, etc. Moreover, *H. pylori* infection will facilitate the progress of SS. *H. pylori* infection causes chronic immune system activation, which produces a sustained cytokine signal that attacks the lacrimal glands, salivary glands, platelets, and more. Besides, the relationship between *H. pylori* infection and SS was explored in this study from a genetic perspective, and the results revealed a bidirectional causal relationship. Therefore, in the clinical management of SS, it is necessary to strengthen screening for *H. pylori* infection. In addition, *H. pylori* infection can exacerbate SS, and if necessary, anti-*H. pylori* treatment should be added to the treatment for SS. However, whether *H. pylori* eradication treatment can improve the clinical symptoms of SS still requires further rigorous large-scale, multicentre investigations of interfering factors.

Our MR study has several strengths. First, to our knowledge, this study is the first to use MR methods to assess the causal effects between *H. pylori* infection and SS. Second, MR explores the causal relationship between exposure and outcome through genetic data, which are unaffected by causal inversion and confounding factors. Third, MR uses genetic variation as IVs to mimic the design of randomized controlled trials. MR falls between observational studies and intervention trials, providing information on public health interventions in situations where randomized controlled trials may not be feasible.

However, this analysis also has several limitations. First, the majority of the samples used are from European populations. Although the use of a single population to study causal relationships can minimize population stratification bias, the findings of this study may not be applicable to other populations. Unfortunately, developing countries generally have higher rates of *H. pylori* infection than European countries; thus these results should be confirmed in other populations. Second, the diagnosis of *H. pylori* infection in the datasets was based on serum IgG antibodies testing. Finally, to ensure the inclusion of a sufficient number of SNPs that contribute to *H. pylori* infection, we used a more relaxed value ($P<5x10^{-6}$) for the SNP selection cutoff. Previous studies have recommended this strategy [17] with the caveat that it might result in a slight bias in IVs.

Conclusion

This study explored the causal relationship between *H. pylori* infection and SS at the genetic level through two-sample MR analysis utilizing publicly available databases and large-scale GWAS. These results indicate that *H. pylori* infection increases the risk of developing SS and that SS can lead to a greater risk of *H. pylori* infection. However, the mechanism underlying *H. pylori* infection and SS are not clear. Data from GWAS with larger sample sizes are still needed to validate this relationship in the future. Most importantly, this study provides novel approaches for continued research and therapy for *H. pylori* and SS that consider the genetic relationship between *H. pylori* infection and SS.

Abbreviations

SS	Sjogren's syndrome
H.pylori	*Helicobacter pylori*
MALT	Mucosa associated lymphoid tissue
SLE	Systemic lupus erythematosus
RA	Rheumatoid arthritis
MR	Mendelian randomization
IV	Instrumental variable
GWAS	Genome-wide association studies
SNPs	Single nucleotide polymorphisms
EBI	European Bioinformatics Institute
LD	Linkage disequilibrium
PRESSO	Pleiotropy residual sum and outlier
IVW	Inverse variance weighted
OR	Odds ratio
CI	Confidence interval
HSP	Human heat shock protein
CRP	C-reactive protein
Th1	Helper T cell 1
IL-2	Interleukin-2
IFN-γ	Interferon-γ
pDCs	Plasmacytoid dendritic cells
CagA	Cytotoxin-associated gene A

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Authors' contributions

Dinglu Cui: Data curation, Software, Writing – original draft. Rongxian An: Writing – original draft. Lei Li: Writing – original draft. Long Jiang: Writing – original draft. Chunshan Jiang: Writing – review & editing. Jingchun Jin: Supervision, Writing – review & editing.

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Availability of data and materials

The datasets generated during analysis in the current study are available in the European Bioinformatics Institute (EBI) database[https://gwas.mrcieu.ac.uk/], and the FinnGen consortium[https://r9.finngen.fi/].

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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