Unveiling the Mystery: Investigating the Debate Surrounding Helicobacter pylori Infection and Multiple Sclerosis Using Mendelian Randomization Analysis

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Research Article

Keywords: Causal relationship, Single nucleotide polymorphism, Genetically predicted, GWAS, Autoimmune disease

Posted Date: May 23rd, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4406946/v1

Additional Declarations: No competing interests reported.

Abstract

Background

Many studies have explored the association between Helicobacter pylori (H. pylori) infection and multiple sclerosis (MS), but there is significant controversy in the results, and a unified conclusion has not yet been reached.

Methods

In order to calculate the causal relationship between H. pylori and MS, we employed a two-sample bidirectional Mendelian randomization (MR) analysis. Genetic instruments for MS from FinnGen were used as the training cohort, and the MS dataset from the International Multiple Sclerosis Genetics Consortium was used as the validation cohort. Additionally, genome-wide association study (GWAS) data for 7 H. pylori antibody levels were obtained from previous literature. MR analysis was primarily carried out using the Inverse Variance Weighted (IVW) method, supported by four other validation approaches, to explore the association between H. pylori infection and MS.

Results

After our comprehensive investigation, no significant causal relationship between H. pylori infection and MS was found in either the training or validation cohorts (IVW, $P > 0.05$). Similarly, the reverse MR analysis also yielded negative results (IVW, $P > 0.05$).

Conclusions

Our analysis revealed no causal relationship between H. pylori infection and MS.

1.0 Introduction

Multiple sclerosis (MS) is a common cause of adult disability, characterized by chronic inflammatory demyelination and neurodegeneration within the central nervous system [1]. MS typically manifests during the productive stages of individuals' lives, when they are planning families and establishing careers, thereby exerting a significant impact on affected individuals, their families, and society [2]. Global estimates indicate that MS currently affects 2.8 million adults worldwide [2]. The complex etiology and pathogenesis of MS result from the interplay of genetic and environmental factors. Factors such as Epstein-Barr virus, sunlight (UV exposure), smoking, vitamin D, along with individual genetic backgrounds, play crucial roles in the causal pathways leading to the onset of MS [3]. Identifying the risk factors for multiple sclerosis contributes to a better understanding of its pathogenesis and enables the provision of care and treatment strategies for patients and healthcare professionals.

Helicobacter pylori (H. pylori) is a Gram-negative bacterium that colonizes the human gastric mucosa, leading to gastritis in approximately half of the global population [4]. Currently, it is recognized that H. pylori infection is closely associated with gastritis, duodenal ulcers, gastric cancer, and other gastrointestinal issues [5]. H. pylori can also release various toxins and effector proteins, including cytotoxin-associated gene A (CagA), outer membrane vesicles (OMV), outer inflammatory protein A (OipA), vacuolating toxin gene A (VacA), heat shock protein A (HtrA), outer membrane protein (OMP), and neutrophil-activating protein A (NepA) [6]. Among these, CagA is a virulence factor associated with disease severity, capable of influencing multiple cellular processes. Additionally, VacA has diverse functions, ranging from inducing cell apoptosis to modulating the immune system [7]. Both of these factors impact cell morphology and immune cells, potentially leading to elevated levels of autoimmune antibodies [7].

The infectious agent induces autoimmunity through two distinct mechanisms. Initially, it elicits homologous antigen-specific signals via molecular mimicry or the mobilization of endogenous antigens. Simultaneously, it incites inflammation, generating antigen-specific signals that bolster the immune response through a phenomenon known as the adjuvant effect [8]. Presently, H. pylori infection has been linked to a range of autoimmune diseases, such as inflammatory bowel disease, autoimmune metabolic disorders, autoimmune liver diseases, systemic lupus erythematosus, and others [9]. However, there is significant controversy surrounding whether H. pylori infection, as one of the most common environmental factors associated with MS, is causally linked to the disease. Some studies indicate that H. pylori infection could worsen MS as a risk factor [10, 11], while others propose the opposite, suggesting that H. pylori infection might actually act as a protective factor, reducing the risk of developing MS [12–15]. Additionally, certain research suggests that there may be no direct causal relationship between H. pylori infection and MS [16, 17]In recent years, conflicting conclusions have emerged from two meta-analyses. Delaram Arjmandi et al. posit that active H. pylori infection could be a risk factor for the development of MS [18], while Sangharsha Thapa et al. argue against a causal association between H. pylori infection and MS [19]. Therefore, exploring the causal relationship between H. pylori infection and multiple sclerosis remains a focal point of our research, as it impacts the treatment strategies for MS patients.

Currently, the causal relationship between H. pylori infection and multiple sclerosis is limited to observational studies, which have inherent limitations such as unmeasured or imprecisely measured confounders, reverse causation, and other sources of bias. To address these limitations, leveraging data from genome-wide association studies (GWAS) for Mendelian randomization (MR) has emerged as a promising approach for evaluating causal relationships in assumed exposure-outcome pathways [20]. Essentially, MR acts as a natural randomized trial, utilizing the random allocation of genetic variants at conception to partition individuals into different subgroups, akin to a placebo group and an intervention group in a randomized controlled trial. This method can assess potential causal relationships between risk factors (H. pylori infection) and disease outcomes (such as MS), while ensuring that confounding variables are also random [21]. In our study, we have curated the latest GWAS summaries on H. pylori infection and MS. Utilizing a two-sample MR analysis, our central objective is to elucidate the causal relationship between these factors. This pursuit is pivotal in unraveling the pathogenesis of MS and pinpointing prospective therapeutic targets.

2.0 Methods

2.1 Study design

In this study, all data were sourced from publicly available databases and received approval from the relevant research institution's review board, obviating the need for ethical committee review.

Our research harnessed single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to execute a comprehensive bidirectional MR analysis [22], investigating the causal relationship between H. pylori infection and MS. Furthermore, MR analysis must adhere to three key assumptions: (1) SNPs are associated with the exposure; (2) SNPs are independent of confounding factors in the exposure-outcome relationship; (3) SNPs influence the outcome solely through the exposure [23].

2.2 GWAS summary data sources

2.2.1 Multiple Sclerosis

The genetic association with MS was derived from two independent GWAS datasets. One dataset was obtained from the FinnGen database as the training cohort, comprising 2,182 MS patients and 373,987 controls (https://storage.googleapis.com/finngen-public-data-r10/summary_stats/finngen_R10_G6_MS.gz). Furthermore, for

validation purposes, we utilized summary statistics for MS from the largest GWAS dataset of the International Multiple Sclerosis Genetics Consortium (IMSGC). This dataset included 115,803 individuals of European ancestry, with 47,429 cases and 68,374 controls [24]. The data from the IMSGC can be found on the IEU OpenGWAS project (mrcieu.ac.uk) (Table 1).

Table 1
Details of the genome-wide association studies and datasets used in our analyses

Phenotypes	Cases/ controls	Consortium/Author	PubMed ID	Data Download Link
MS in training cohort	2,182/373,987	FinnGen consortium	-	https://storage.googleapis.com/finngen-public-data-r10/summary_stats/finngen_R10_G6_MS.gz.
MS in validation cohort	47,429/68,374	IMSGC	31604244	https://www.ebi.ac.uk/gwas/; ID, ieu-b-18.
Anti-H. pylori IgG seropositivity	8,735	UK Biobank	33204752	https://www.ebi.ac.uk/gwas/; Accession numbers, GCST90006910.
H. pylori CagA antibody levels	985	UK Biobank	33204752	https://www.ebi.ac.uk/gwas/; Accession numbers, GCST90006911.
H. pylori Catalase antibody levels	1,558	UK Biobank	33204752	https://www.ebi.ac.uk/gwas/; Accession numbers, GCST90006912.
H. pylori GroEL antibody levels	2,716	UK Biobank	33204752	https://www.ebi.ac.uk/gwas/; Accession numbers, GCST90006913.
H. pylori OMP antibody levels	2,640	UK Biobank	33204752	https://www.ebi.ac.uk/gwas/; Accession numbers, GCST90006914.
H. pylori UREA antibody levels	2,251	UK Biobank	33204752	https://www.ebi.ac.uk/gwas/; Accession numbers, GCST90006915.
H. pylori VacA antibody levels	1,571	UK Biobank	33204752	https://www.ebi.ac.uk/gwas/; Accession numbers, GCST90006916.

2.2.2 H. pylori Infection

H. pylori infection was characterized by measuring serum-specific antibodies targeting H. pylori proteins, utilizing data from seven distinct antibody measurements [25–29]. Our dataset originates from a research paper that utilized the UK Biobank cohort, which contains information on 13 pathogens and delineates 46 phenotypes. From this dataset, we specifically extracted 7 data points relevant to H. pylori antibody levels [30]. Specifically, these 7 phenotypes encompass levels of IgG (n = 8,735), CagA (n = 985), VacA (n = 1,571), UREA (n = 2,251), Catalase (n = 1,558), OMP (n = 2,640), and GroEL (n = 2,716) antibodies against H. pylori. The dataset includes 16,404 individuals of European ancestry, comprising both males and females. Detailed information regarding the aforementioned data is provided in Table 1.

2.3 IVs selection and data harmonization

In our study, we conducted a rigorous screening of SNPs to maintain high standards. For the selection of IVs for MS, we specifically included only SNPs with genome-wide significance ($P < 5 \times 10^{-8}$) for further investigation. However, in the screening process for SNPs related to H. pylori antibody levels, due to the limited availability of IVs, we utilized a p-value threshold of 5E-06 for IV selection to ensure an adequate number of IVs and enhance statistical power. It is important to note that this threshold is commonly employed in MR analysis [31]. Moreover, in our analysis, we excluded palindromic and ambiguous SNPs as IVs [32]. Subsequently, we grouped SNPs based on linkage disequilibrium using a window size of 10,000 kb and an r^2 threshold of less than 0.001. Additionally, we calculated the F-statistic to assess the variance explained by each exposure SNP, using the formula [(N - K − 1) / K] / [R^2 / (1 - R^2)], where K represents the number of genetic instruments and N denotes the sample size [33]. To ensure the reliability and consistency of our results, we removed weak IVs with an F value below 10. Furthermore, we conducted a literature review to evaluate all established phenotypes associated with the genetic tools considered in our study, manually excluding SNPs linked to confounding factors.

2.4 Statistical analysis

For our meticulous and comprehensive analysis, we performed MR analysis using R software (version 4.2.0, http://www.r-project.org) in combination with the "Two-Sample MR" package (version 0.5.6) [34].

2.5 Primary analysis

To investigate the causal relationship between H. pylori infection and MS, we employed a two-sample MR analysis method, using GWAS data on H. pylori antibody levels from seven sources as exposure variables and GWAS data on MS from the training cohort as the outcome variable.

In the current MR analysis, we utilized a variety of techniques including Inverse Variance Weighted (IVW), MR-Egger, Weighted Median, Weighted Mode, and Simple Mode for analysis. A significance level of $P < 0.05$ indicates a positive result. Specifically, IVW, as the primary method of study, combines meta-analysis strategies with the Wald estimate of each SNP. In the absence of horizontal pleiotropy, IVW results are unbiased [35]. Based on the Instrument Strength Independent of Direct Effect (InSIDE) assumption, MR-Egger regression evaluates horizontal pleiotropy through the intercept term. Consistency with IVW is indicated if the intercept term is zero, suggesting no horizontal pleiotropy [36]. Weighted Median technique accurately assesses causal relationships when up to 50% of the instruments are invalid [37]. Studies have shown that, in cases violating the InSIDE assumption, Weighted Mode estimation demonstrates stronger causal effect identification, lower bias, and lower Type I error rates compared to MR-Egger regression [37]. Additionally, although the Simple Mode method has lower precision, it can reduce bias [37]. These techniques are crucial for ensuring the reliability of study results.

2.6 Reverse MR Analysis

To explore the potential reverse causal relationship between H. pylori infection and MS, this study conducted a reverse MR analysis, with MS as the exposure and H. pylori antibody levels as the outcome. The aforementioned techniques were also utilized for the analysis, and the robustness of the findings was further validated by incorporating data from a separate validation cohort.

2.7 Sensitivity analysis

Due to variations in experimental conditions, study populations, and SNPs, heterogeneity can arise in two-sample MR analysis, potentially introducing bias in estimating causal effects. Therefore, this study conducted heterogeneity tests using the IVW and MR-Egger methods. Cochrane's Q statistic was employed to evaluate the heterogeneity of genetic

instruments, with a P-value > 0.05 indicating non-significant heterogeneity [38]. Furthermore, a key assumption in MR analysis is that the IV influences the outcome solely through the exposure, necessitating an examination of potential horizontal pleiotropy between the exposure and outcome [39]. The study utilized the MR-Egger intercept method to assess the presence of pleiotropy. A P-value > 0.05 indicates minimal or negligible potential for pleiotropy in causal analysis, allowing for its exclusion. Finally, outliers in the IVW analysis were identified and adjusted using the MR-PRESSO test [40], while the leave-one-out analysis was employed to determine the genetic causal effects of individual SNPs on the exposure-outcome relationship [41].

3.0 Result

The schematic representation of the study design can be found in Fig. 1.

3.1 Association of genetically predicted levels of H. pylori antibodies and MS in the training cohort

In the training cohort, we initiated our analysis by considering genetically predicted levels of H. pylori antibodies as the primary exposures. Following a meticulous SNP selection process, we employed five distinct methodologies to investigate their potential associations with MS. After conducting a comprehensive analysis, we did not identify any causal relationship between Anti-H. pylori IgG seropositivity (IVW method: odds ratio (OR) 1.035; 95% Confidence Interval (CI) 0.931–1.151; P = 0.525), H. pylori CagA antibody levels (IVW method: OR 1.016; 95% CI 0.896–1.152; P = 0.804), H. pylori Catalase antibody levels (IVW method: OR 1.015; 95% CI 0.925–1.113; P = 0.757), H. pylori GroEL antibody levels (IVW method: OR 1.050; 95% CI 0.851–1.296; P = 0.649), H. pylori OMP antibody levels (IVW method: OR 1.132; 95% CI 0.944–1.357; P = 0.182), H. pylori UREA antibody levels (IVW method: OR 0.941; 95% CI 0.787–1.124; P = 0.500), and H. pylori VacA antibody levels (IVW method: OR 1.016; 95% CI 0.904–1.141; P = 0.793) and MS (Fig. 2). In this stage of MR analysis, Detailed documentation of the SNP features associated with H. pylori antibody levels and MS can be found in Supplementary Table S1, while the comprehensive MR analysis results are documented in Table S2.

Furthermore, it is noteworthy that during the MR analysis between H. pylori CagA antibody levels and MS, we identified and subsequently excluded the outlier rs9270475. Similarly, in the MR analysis involving H. pylori VacA antibody levels and MS, we also excluded the outlier rs372744619. Subsequent sensitivity analysis revealed the absence of horizontal pleiotropy in all the aforementioned analyses (Table S9A). Furthermore, heterogeneity was observed only in the MR analysis concerning H. pylori UREA and H. pylori VacA antibody levels with MS (Table S9A).

3.2 Reverse MR analysis in the training cohort

To explore the potential for reverse causality, we conducted a reverse MR analysis, where we considered MS as the exposure and H. pylori antibody levels in the training cohort as the outcome.

Similarly, in the reverse MR analysis, we found a potential causal relationship between MS and H. pylori OMP antibody levels (IVW, $P < 0.05$), but this was later excluded due to heterogeneity identified in the sensitivity analysis. Furthermore, we did not observe any causal relationship between MS and the remaining H. pylori antibody levels (Fig. 3). Detailed records of SNP features associated with MS and H. pylori antibody levels can be found in Supplementary Table S3. Additionally, comprehensive MR analysis results are presented in Supplementary Table S4. Notably, in the reverse MR analysis of MS and H. pylori catalase antibody levels, rs9268807 was identified as an outlier and thus excluded from the analysis. Moreover, no horizontal pleiotropy was observed in the aforementioned reverse MR analysis. Apart from the MR analysis of MS and H. pylori OMP antibody levels, no heterogeneity was observed in other reverse MR analyses (Table S9A).

3.3 Conducting bidirectional MR analysis in the validation cohort.

In order to validate the robustness of our findings, we acquired an additional set of summary statistics for MS from the IEU OpenGWAS project for further validation analysis [24].

The consistent approach with our previous research methodology was to utilize genetically predicted levels of H. pylori antibodies as the exposures and MS dataset from the validation cohort as the outcome for analysis. In line with our earlier findings, we found no causal relationship between the aforementioned H. pylori antibody levels and MS (Fig. 4). In the MR analysis involving H. pylori OMP antibody levels and MS, we excluded the outliers rs6899857 and rs703135 (Table S5). Notably, due to a limited number of SNPs, regular MR analysis could not be conducted for H. pylori CagA antibody levels and H. pylori OMP antibody levels (Table S6). In subsequent sensitivity analyses, only the MR analysis between Anti-H. pylori IgG seropositivity and MS showed heterogeneity, while the remaining results passed tests for horizontal pleiotropy, heterogeneity (Table S9B).

Following this, we conducted reverse MR analyses in the validation cohort. Overall, the results consistently indicated no causal relationship between MS and H. pylori antibody levels (Fig. 5). In the reverse MR analysis of H. pylori OMP antibody levels and MS, we excluded the outlier rs71542422. Apart from the presence of horizontal pleiotropy in the reverse MR analysis between H. pylori Catalase antibody levels and H. pylori GroEL antibody levels with MS, the rest of the results met the assumptions of MR analysis (Table S7, S8).

3.4 Summary of results

This study utilized 7 types of H. pylori antibody levels to reflect H. pylori infection and employed a two-sample bidirectional MR analysis to confirm the lack of causal relationship between H. pylori infection and MS, a conclusion supported by the validation cohort. Finally, using a "leave-one-out" sensitivity analysis approach, we found that systematically excluding each SNP did not significantly alter the model's effect estimates or qualitative conclusions (Figures S1, S2, S3, S4).

4.0 Discussion

This study aimed to investigate the causal relationship between H. pylori infection and MS using a two-sample bidirectional MR analysis, with the objective of providing a more definitive answer to the ongoing controversy. Consistent with previous research, H. pylori infection in this study was characterized by measuring serum-specific antibodies targeting H. pylori proteins, utilizing data from seven distinct antibody measurements [25–29]. Following rigorous control for heterogeneity and horizontal pleiotropy in our MR analysis, all results consistently indicated the absence of a causal relationship between the two. This conclusion was further validated in the verification dataset. To our knowledge, this study is the first comprehensive MR analysis comparing the relationship between H. pylori infection and multiple sclerosis.

The current mainstream hypothesis regarding H. pylori infection potentially leading to MS suggests that H. pylori may act as a superantigen through its proteins, thereby reducing immune self-tolerance and serving as an inductive factor for neuroinflammatory diseases [42]. Furthermore, superantigens are associated with extensive immune dysregulation and chaotic activation of polyclonal T cells, potentially leading to mimicry of central nervous system antigens, akin to the presentation of multiple sclerosis [43]. On the other hand, an alternative viewpoint posits that H. pylori infection could be a protective factor against MS. This perspective argues that infections in early life are crucial for priming the immune system, thereby aiding in preventing allergies and autoimmune diseases later in life. There are speculations that H. pylori infection is associated with alterations in chemokines of regulatory T cells and integrin receptors,

potentially resulting in fewer T cells crossing the blood-brain barrier into the central nervous system [44]. Additionally, research indicates that H. pylori antigens are believed to enhance the production of interleukin-10, an anti-inflammatory peptide, aligning with a potential protective role in MS [45].

The presence of numerous confounding factors often leads to inconsistent research findings. Among these, the choice of different methods for diagnosing H. pylori infection can have a significant impact on the conclusions drawn. In a recent meta-analysis, it was found that when using ELISA as the diagnostic method, the prevalence of H. pylori infection in MS patients is lower [19]. However, in regions where the prevalence of H. pylori infection is below 20%, there is a higher risk of false positive results with ELISA-positive serum tests. Other methods such as Western Blot, Immunofluorescence, and commercial latex agglutination tests have not shown differences between the two conditions [19]. Additionally, factors such as regional variations, study populations, sample sizes, statistical differences, and technical limitations can all contribute to biases in research results. The data used in this study was sourced from the Luminex 100 platform (Luminex Corporation, Austin, TX, USA) for testing serum samples. This technology quantifies standardized values of antibodies in samples by measuring the fluorescence emitted by the analyte-capture agent complexes [30]. By utilizing genetic variation as IVs, MR can help mitigate the impact of unmeasured confounders or biases. Using this method to explore the relationship between H. pylori infection and MS can enhance the credibility of the findings.

Our analysis showcases a blend of strengths and limitations. A notable strength of our study is the utilization of seven distinct levels of H. pylori antibodies to investigate their bidirectional correlations with MS. We augmented our analysis by incorporating an additional MS dataset for validation, yielding consistent results. Moreover, adhering to the three fundamental assumptions of MR, all outcomes remained harmonious. Nevertheless, our analysis is not without constraints. Primarily, the focus of our genetic analysis on patients of European descent may restrict the generalizability of our research findings to other ethnicities. Furthermore, a subset of our findings reveals instances of heterogeneity and horizontal pleiotropy. In future endeavors, it may be imperative to conduct studies with larger sample sizes and across multiple regions to validate our conclusions.

5.0 Conclusions

This study, utilizing multiple datasets, demonstrates no causal relationship between H. pylori infection and MS. Further validation through studies with larger sample sizes may be warranted in the future.

Abbreviations

MS	Multiple Sclerosis
H. pylori	Helicobacter Pylori
CagA	Cytotoxin-associated Gene A
OMV	Outer membrane Vesicles
OipA	Outer Inflammatory Protein A
VacA	Vacuolating Toxin Gene A
HtrA	Heat Shock Protein A
OMP	Outer Membrane Protein

NepA	Neutrophil-activating Protein A
MR	Mendelian Randomization
GWAS	Genome-wide Association Studies
SNPs	Single Nucleotide Polymorphisms
IVs	Instrumental Variables
IMSGC	International Multiple Sclerosis Genetics Consortium
IVW	Inverse Variance Weighted
InSIDE	Instrument Strength Independent of Direct Effect

Declarations

Acknowledgements

Not applicable

Authors' Contributions

J. Z and D. Z were involved in the study concept and design; K. W, Y. X and C. C collected data and conducted analyses; J. Z and H. W wrote the draft of the article; D. Z revised the manuscript and had primary responsibility for final content. All authors reviewed and approved the final version of the manuscript.

Fundings

This study was funded by the Jiangsu University Medical Education Collaborative Innovation Fund Project (No. JDY2023018) and the Medical Research Project of Jiangsu Health Commission (No. Z2021010).

Availability of data and materials

The data can be accessed in the supplementary files. Additionally, data are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was based on publicly available summary data and required no ethics approval or participant consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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Figures

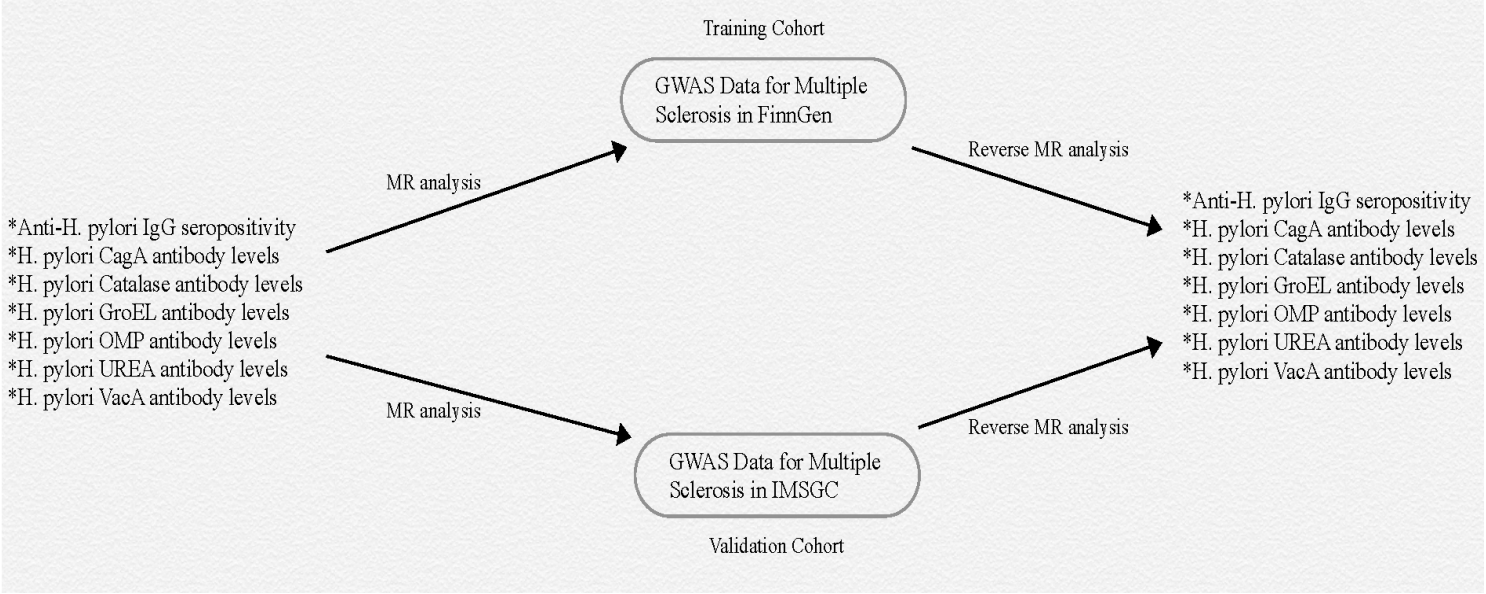

Figure 1

The schematic representation of the study design. Note: H. pylori

, Helicobacter pylori; MR, Mendelian randomization; GWAS, genome-wide association study; IMSGC, International Multiple Sclerosis Genetics Consortium.

Exposure	Outcome	method	nsnp	pval	OR (95% CI)
Anti–H. pylori IgG seropositivity	MS	MR Egger	10	0.177	1.255 (0.929 – 1.696)
		Weighted median	10	0.146	1.112 (0.964 – 1.284)
		IVW	10	0.525	1.035 (0.931 – 1.151)
		Simple mode	10	0.311	1.120 (0.911 – 1.377)
		Weighted mode	10	0.163	1.132 (0.965 – 1.329)
H. pylori CagA antibody levels	MS	MR Egger	5	0.930	1.025 (0.615 – 1.708)
		Weighted median	5	0.999	1.000 (0.851 – 1.175)
		IVW	5	0.804	1.016 (0.896 – 1.152)
		Simple mode	5	0.589	0.938 (0.756 – 1.163)
		Weighted mode	5	0.581	0.934 (0.747 – 1.167)
H. pylori Catalase antibody levels	MS	MR Egger	9	0.679	0.964 (0.816 – 1.138)
		Weighted median	9	0.593	1.034 (0.915 – 1.168)
		IVW	9	0.757	1.015 (0.925 – 1.113)
		Simple mode	9	0.467	1.071 (0.899 – 1.275)
		Weighted mode	9	0.650	1.036 (0.894 – 1.201)
H. pylori GroEL antibody levels	MS	MR Egger	6	0.580	0.869 (0.550 – 1.372)
		Weighted median	6	0.815	1.032 (0.795 – 1.339)
		IVW	6	0.649	1.050 (0.851 – 1.296)
		Simple mode	6	0.931	1.018 (0.692 – 1.497)
		Weighted mode	6	0.938	0.983 (0.647 – 1.492)
H. pylori OMP antibody levels	MS	MR Egger	7	0.506	1.158 (0.775 – 1.731)
		Weighted median	7	0.600	1.066 (0.840 – 1.352)
		IVW	7	0.182	1.132 (0.944 – 1.357)
		Simple mode	7	0.867	1.030 (0.736 – 1.443)
		Weighted mode	7	0.869	1.030 (0.731 – 1.452)
*H. pylori UREA antibody levels	MS	MR Egger	10	0.895	1.036 (0.622 – 1.727)
		Weighted median	10	0.554	0.942 (0.772 – 1.149)
		IVW	10	0.500	0.941 (0.787 – 1.124)
		Simple mode	10	0.303	0.820 (0.575 – 1.170)
		Weighted mode	10	0.518	0.911 (0.694 – 1.195)
*H. pylori VacA antibody levels	MS	MR Egger	15	0.763	0.962 (0.754 – 1.228)
		Weighted median	15	0.220	1.083 (0.953 – 1.230)
		IVW	15	0.793	1.016 (0.904 – 1.141)
		Simple mode	15	0.309	1.108 (0.916 – 1.339)
		Weighted mode	15	0.156	1.123 (0.965 – 1.307)

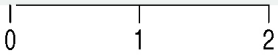

Figure 2

The MR analysis between H. pylori infection and MS in the training cohort. Note: MR, Mendelian randomization; H. pylori, Helicobacter pylori; MS, Multiple Sclerosis; nSNPs, Number of Single Nucleotide Polymorphisms; OR, odds ratio; CI, Confidence Interval; IVW, Inverse variance weighted; *, The results exhibit heterogeneity.

Exposure	Outcome	method	nsnp	pval	OR (95% CI)
MS	Anti–H. pylori IgG seropositivity	MR Egger	11	0.535	0.951 (0.816 – 1.108)
		Weighted median	11	0.227	0.940 (0.851 – 1.039)
		IVW	11	0.416	0.966 (0.887 – 1.051)
		Simple mode	11	0.453	1.082 (0.888 – 1.318)
		Weighted mode	11	0.286	0.939 (0.841 – 1.048)
MS	H. pylori CagA antibody levels	MR Egger	11	0.130	0.856 (0.714 – 1.027)
		Weighted median	11	0.237	0.923 (0.809 – 1.054)
		IVW	11	0.395	0.955 (0.859 – 1.062)
		Simple mode	11	0.643	0.943 (0.740 – 1.201)
		Weighted mode	11	0.277	0.918 (0.793 – 1.062)
MS	H. pylori Catalase antibody levels	MR Egger	10	0.509	1.137 (0.790 – 1.637)
		Weighted median	10	0.319	1.081 (0.927 – 1.261)
		IVW	10	0.638	1.038 (0.888 – 1.213)
		Simple mode	10	0.529	1.073 (0.869 – 1.326)
		Weighted mode	10	0.425	1.089 (0.891 – 1.332)
MS	H. pylori GroEL antibody levels	MR Egger	11	0.895	0.992 (0.877 – 1.121)
		Weighted median	11	0.713	0.985 (0.907 – 1.069)
		IVW	11	0.883	1.005 (0.939 – 1.076)
		Simple mode	11	0.546	0.950 (0.810 – 1.115)
		Weighted mode	11	0.630	0.979 (0.898 – 1.066)
*MS	H. pylori OMP antibody levels	MR Egger	11	0.052	0.830 (0.705 – 0.977)
		Weighted median	11	<0.001	0.841 (0.775 – 0.914)
		IVW	11	0.022	0.894 (0.812 – 0.984)
		Simple mode	11	0.152	0.888 (0.763 – 1.032)
		Weighted mode	11	0.002	0.839 (0.771 – 0.914)
MS	H. pylori UREA antibody levels	MR Egger	11	0.613	1.033 (0.916 – 1.165)
		Weighted median	11	0.918	0.995 (0.910 – 1.089)
		IVW	11	0.287	0.963 (0.898 – 1.032)
		Simple mode	11	0.981	0.998 (0.858 – 1.161)
		Weighted mode	11	0.936	0.997 (0.918 – 1.082)
MS	H. pylori VacA antibody levels	MR Egger	11	0.168	0.897 (0.778 – 1.034)
		Weighted median	11	0.366	0.954 (0.863 – 1.056)
		IVW	11	0.824	0.991 (0.912 – 1.076)
		Simple mode	11	0.872	1.016 (0.844 – 1.223)
		Weighted mode	11	0.469	0.960 (0.864 – 1.067)

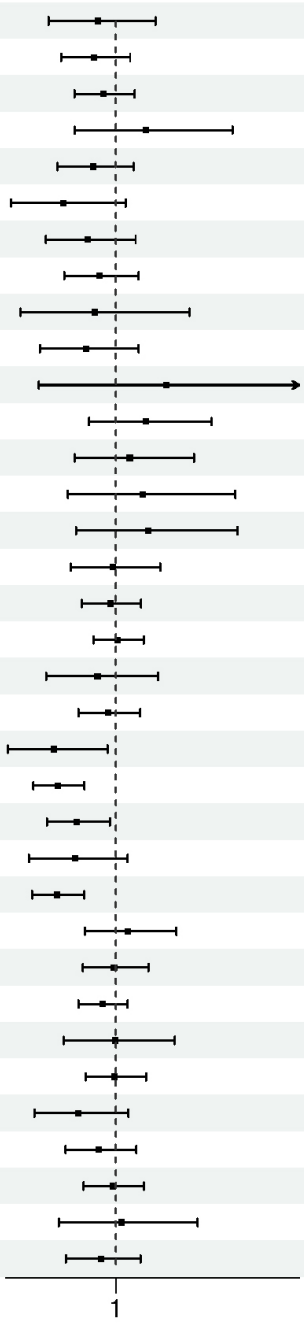

Figure 3

The reverse MR analysis between H. pylori infection and MS in the training cohort. Note: MR, Mendelian randomization; H. pylori, Helicobacter pylori; MS, Multiple Sclerosis; nSNPs, Number of Single Nucleotide Polymorphisms; OR, odds ratio; CI, Confidence Interval; IVW, Inverse variance weighted; *, The results exhibit heterogeneity.

Exposure	Outcome	method	nsnp	pval	OR (95% CI)
*Anti–H. pylori IgG seropositivity	MS	MR Egger	4	0.770	1.134 (0.543 – 2.368)
		Weighted median	4	0.510	0.941 (0.785 – 1.128)
		IVW	4	0.951	0.994 (0.813 – 1.214)
		Simple mode	4	0.407	0.868 (0.651 – 1.158)
		Weighted mode	4	0.455	0.856 (0.599 – 1.222)
H. pylori Catalase antibody levels	MS	MR Egger	3	0.336	0.790 (0.603 – 1.034)
		Weighted median	3	0.718	0.970 (0.825 – 1.142)
		IVW	3	0.512	0.953 (0.826 – 1.100)
		Simple mode	3	0.683	1.051 (0.854 – 1.295)
		Weighted mode	3	0.802	1.032 (0.833 – 1.277)
H. pylori GroEL antibody levels	MS	MR Egger	3	0.323	0.046 (0.002 – 1.315)
		Weighted median	3	0.805	1.025 (0.842 – 1.249)
		IVW	3	0.974	0.997 (0.830 – 1.198)
		Simple mode	3	0.705	1.059 (0.820 – 1.368)
		Weighted mode	3	0.714	1.054 (0.824 – 1.349)
H. pylori UREA antibody levels	MS	MR Egger	3	0.646	1.456 (0.445 – 4.764)
		Weighted median	3	0.535	0.946 (0.794 – 1.127)
		IVW	3	0.906	0.989 (0.819 – 1.194)
		Simple mode	3	0.538	0.922 (0.742 – 1.145)
		Weighted mode	3	0.547	0.930 (0.763 – 1.134)
H. pylori VacA antibody levels	MS	MR Egger	4	0.718	1.093 (0.719 – 1.663)
		Weighted median	4	0.490	1.052 (0.911 – 1.214)
		IVW	4	0.385	1.050 (0.940 – 1.174)
		Simple mode	4	0.407	1.088 (0.916 – 1.291)
		Weighted mode	4	0.915	1.009 (0.874 – 1.164)

Figure 4

The MR analysis between H. pylori infection and MS in the validation cohort. Note: MR, Mendelian randomization; H. pylori, Helicobacter pylori; MS, Multiple Sclerosis; nSNPs, Number of Single Nucleotide Polymorphisms; OR, odds ratio; CI, Confidence Interval; IVW, Inverse variance weighted; *, The results exhibit heterogeneity.

Exposure	Outcome	method	nsnp	pval	OR (95% CI)
MS	Anti–H. pylori IgG seropositivity	MR Egger	62	0.242	0.924 (0.811 – 1.053)
		Weighted median	62	0.989	1.001 (0.908 – 1.102)
		IVW	62	0.320	1.032 (0.969 – 1.100)
		Simple mode	62	0.562	1.060 (0.872 – 1.288)
		Weighted mode	62	0.795	0.986 (0.885 – 1.098)
MS	H. pylori CagA antibody levels	MR Egger	62	0.109	0.872 (0.739 – 1.028)
		Weighted median	62	0.259	0.921 (0.799 – 1.062)
		IVW	62	0.913	0.995 (0.915 – 1.083)
		Simple mode	62	0.847	0.967 (0.689 – 1.358)
		Weighted mode	62	0.351	0.908 (0.742 – 1.111)
^MS	H. pylori Catalase antibody levels	MR Egger	62	0.001	0.789 (0.695 – 0.896)
		Weighted median	62	0.020	0.881 (0.792 – 0.981)
		IVW	62	0.196	0.958 (0.898 – 1.022)
		Simple mode	62	0.130	0.834 (0.661 – 1.052)
		Weighted mode	62	0.016	0.834 (0.722 – 0.962)
*^MS	H. pylori GroEL antibody levels	MR Egger	63	0.009	0.852 (0.759 – 0.957)
		Weighted median	63	0.159	0.943 (0.869 – 1.023)
		IVW	63	0.665	0.987 (0.932 – 1.046)
		Simple mode	63	0.568	0.952 (0.805 – 1.126)
		Weighted mode	63	0.063	0.907 (0.819 – 1.004)
MS	H. pylori OMP antibody levels	MR Egger	61	0.697	0.972 (0.841 – 1.123)
		Weighted median	61	0.035	1.091 (1.006 – 1.183)
		IVW	61	0.513	1.019 (0.962 – 1.080)
		Simple mode	61	0.224	1.138 (0.926 – 1.398)
		Weighted mode	61	0.181	1.130 (0.947 – 1.348)
MS	H. pylori UREA antibody levels	MR Egger	62	0.524	0.963 (0.857 – 1.081)
		Weighted median	62	0.852	0.992 (0.913 – 1.078)
		IVW	62	0.686	1.011 (0.957 – 1.069)
		Simple mode	62	0.484	1.070 (0.886 – 1.292)
		Weighted mode	62	0.995	1.000 (0.889 – 1.124)
MS	H. pylori VacA antibody levels	MR Egger	62	0.534	0.958 (0.839 – 1.095)
		Weighted median	62	0.833	1.011 (0.916 – 1.115)
		IVW	62	0.433	1.027 (0.961 – 1.096)
		Simple mode	62	0.054	1.214 (1.001 – 1.474)
		Weighted mode	62	0.796	0.984 (0.872 – 1.111)

Figure 5

The reverse MR analysis between H. pylori infection and MS in the validation cohort. Note: MR, Mendelian randomization; H. pylori, Helicobacter pylori; MS, Multiple Sclerosis; nSNPs, Number of Single Nucleotide Polymorphisms; OR, odds ratio; CI, Confidence Interval; IVW, Inverse variance weighted; *, The results exhibit heterogeneity; ^, The results show the presence of horizontal pleiotropy.

Supplementary Files

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