RESEARCH ARTICLE

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Helicobacter Pylori Infection is Not Causally Associated with Colorectal Cancer: A Two-Sample Mendelian Randomization Study

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Abstract

Background: Some observational studies have indicated an association between Helicobacter pylori (*H. pylori*) infection and colorectal cancer (CRC). Nevertheless, the causal relationship between *H. pylori* infection and CRC remains to be evaluated. **Methods:** A two-sample Mendelian randomization (MR) analysis was conducted to investigate whether *H. pylori* infection is causally associated with CRC in the European population. We chose anti–*H. pylori* IgG levels as the exposure, CRC as the outcome, and genetic variants strongly linked to anti–*H. pylori* IgG levels ($P < 1\times10^{-5}$) as the instrumental variables (IVs). Data were obtained from publicly available genetic summary data, specifically the OpenGWAS database. Inverse variance weighted (IVW), weighted median, weighted mode, and MR Egger were used for MR analyses, where IVW analysis was identified as the primary method for our study. MR-Egger regression methods were used to assess horizontal pleiotropy. **Results:** No causal association between anti–*H. pylori* IgG levels and CRC was found in IVW (β, –0.0002; 95% CI, –0.0016 to 0.0012; P = 0.7945), weighted median (β, 0.0006; 95% CI, –0.0010 to 0.0022; P = 0.4456), weighted mode (β, 0.0012; 95% CI, –0.0019 to 0.0043; P = 0.4688), and MR-Egger (β, –0.0025; 95% CI, –0.0052 to 0.0002; P = 0.0886). Sensitivity analyses did not show any evidence of heterogeneity (P = 0.1500) or horizontal pleiotropy (P = 0.0776) among the IVs. **Conclusions:** *H. pylori* infection does not have a significant effect on the risk of CRC in the European population.

Keywords: *Helicobacter pylori*- causal association- colorectal cancer- Mendelian randomization

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Introduction

Colorectal cancer (CRC) is a prevalent malignant tumor with the third highest incidence [1]. Considering that it poses a significant medical burden, it is important to explore the modifiable risk factors of CRC so as to reduce its burden [2]. Smoking, alcohol consumption, and obesity are widely recognized as absolute risk factors for CRC [3]. However, it is unclear whether there is an association between *H. pylori* infection and the risk of CRC.

The prevalence of *H. pylori* infection ranges from 24% to 75% [4]. It is well-established that *H. pylori* infection is associated with the development of gastric cancer [5]. However, the association between *H. pylori* infection and CRC is not sufficiently clear. Some observational studies have also found an elevated risk of CRC with *H. pylori* infection [6, 7], but other studies have not confirmed such an association [8, 9]. Research has revealed a significant genetic association between *H. pylori* infection and CRC. Utilizing a genome-wide case-control association study design, the study demonstrated that genetic predisposition to *H. pylori* infection may be influenced by specific variants within the DCC (Deleted in Colorectal Cancer) gene [10]. Observational studies are inherently biased and incapable of correcting for unknown confounders, which may affect their inference of causal associations [11]. Thus, alternative approaches are necessary to overcome these limitations.

Mendelian randomization (MR) is a method that uses evaluation of genetic variation to establish the causal relationship between exposure and outcome, thereby reducing the effect of confounding factors [12]. There have been numerous MR studies investigating risk factors for CRC, including body mass index, height, and so on [13, 14]. However, only one MR study has been conducted to assess the causal relationship between *H. pylori* infection and CRC [15]. Therefore, we employed two-sample MR to evaluate the potential causal association between *H. pylori* infection and CRC.

Materials and Methods

As all the data used in this study are publicly available, ethical approval is not required for this study.

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Data sources and selection of genetic variants

We searched the MR-Base database (https://www.mrbase.org/), which summarizes a large amount of genome-wide association studies (GWAS). The exposure data were obtained from the GWAS summary data for anti–*H. pylori* IgG levels in the European population (n = 4,683). We conducted a two-sample MR study using the genetic variants associated with anti–*H. pylori* IgG levels as the instrumental variables (IVs). Since genetic variants identified by GWAS for anti–*H. pylori* IgG levels rarely reach genome-wide significance levels ($P < 5\times10^{-8}$), we selected exposure data with $P < 1\times10^{-5}$ to obtain more correlation results [16]. We used 20 single-nucleotide polymorphisms (SNPs) associated with anti–*H. pylori* IgG levels extracted from the GWAS as the IVs. The outcome data were extracted from the GWAS summary data, which included 5,657 CRC cases and 372,016 control cases.

Statistical analysis for MR

The primary approach for MR analysis was the inverse-variance weighted (IVW) method. While weighted median, weighted mode, and MR-Egger were used as complementary methods. MR-Egger regression methods were used to assess horizontal pleiotropy [17]. To detect heterogeneity, we employed Cochran's Q statistic for the MR-IVW and MR-Egger analyses. Leave-one-out sensitivity analysis was employed to evaluate the influence of individual SNPs on the results. This involved sequentially removing one SNP at a time to determine if a single SNP had a significant horizontal pleiotropic effect. $P < 0.05$ was considered statistically significant. The MR-Base platform was used to conduct the MR analyses [18].

Results

IVs for MR

We identified 20 SNPs from GWAS on anti–*H. pylori* IgG levels that met all the necessary criteria to establish independent causal links to anti–*H. pylori* IgG levels at the genome-wide level (Table 1, Figure 1). Out of the 20 SNPs analyzed, eleven were positively correlated with colorectal cancer and nine were negatively correlated, although only two (rs2169557 and rs7912386) of them were statistically significant.

Results of MR

The IVW method demonstrated no causal relationship

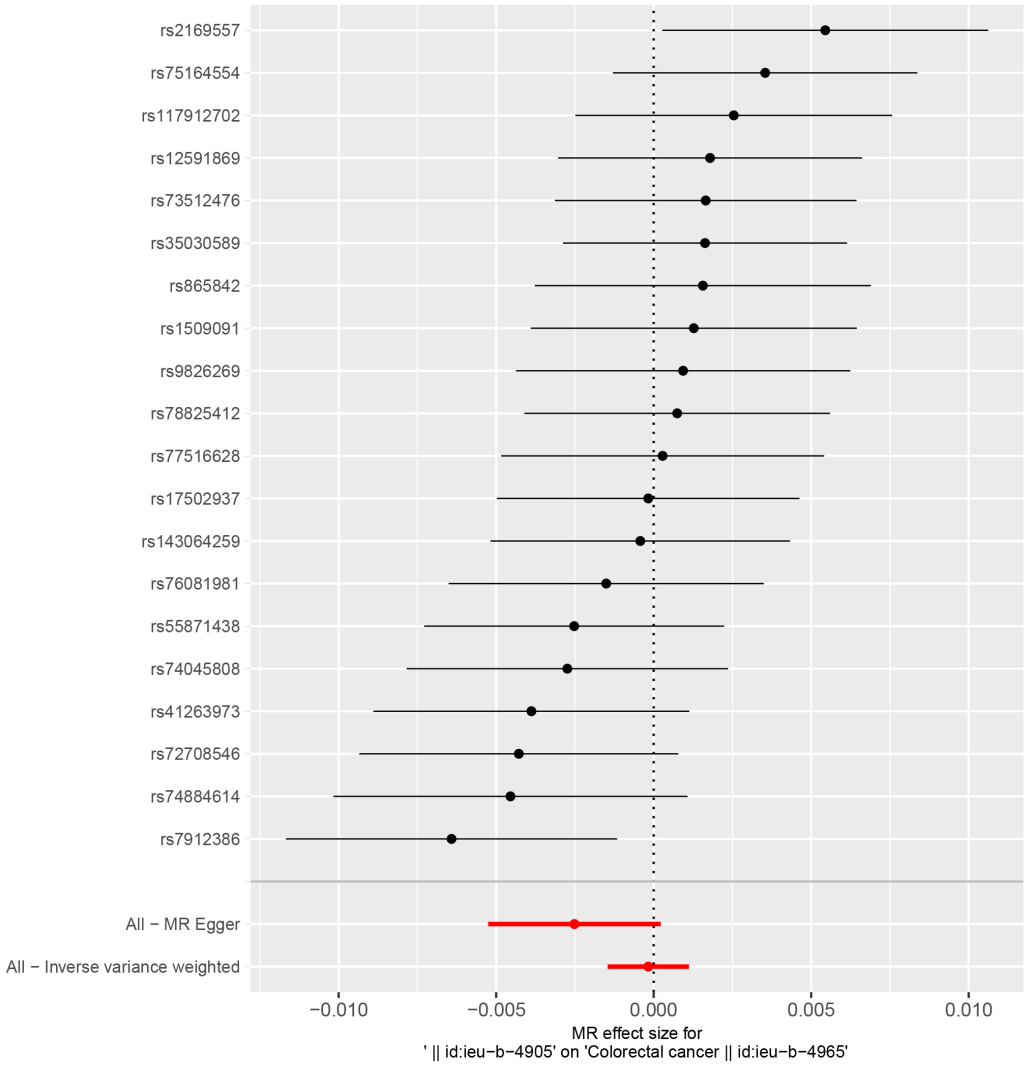

Figure 1. Forest Plot of the Causal Effects of Anti–*H. pylori* IgG Levels on CRC.

Table 1. Mendelian Randomization (MR) Estimates from each Method Used for Assessing the Causal Effect of anti–*H. pylori* IgG Levels on the Risk of Colorectal Cancer

MR method	Number of SNPs	β	SE	Association P value
Inverse variance weighted	20	−0.0002	0.0007	0.7945
MR Egger	20	−0.0025	0.0014	0.0886
Weighted median	20	0.0006	0.0008	0.4456
Weighted mode	20	0.0012	0.0016	0.4688

SNPs, single-nucleotide polymorphisms; SE, standard error.

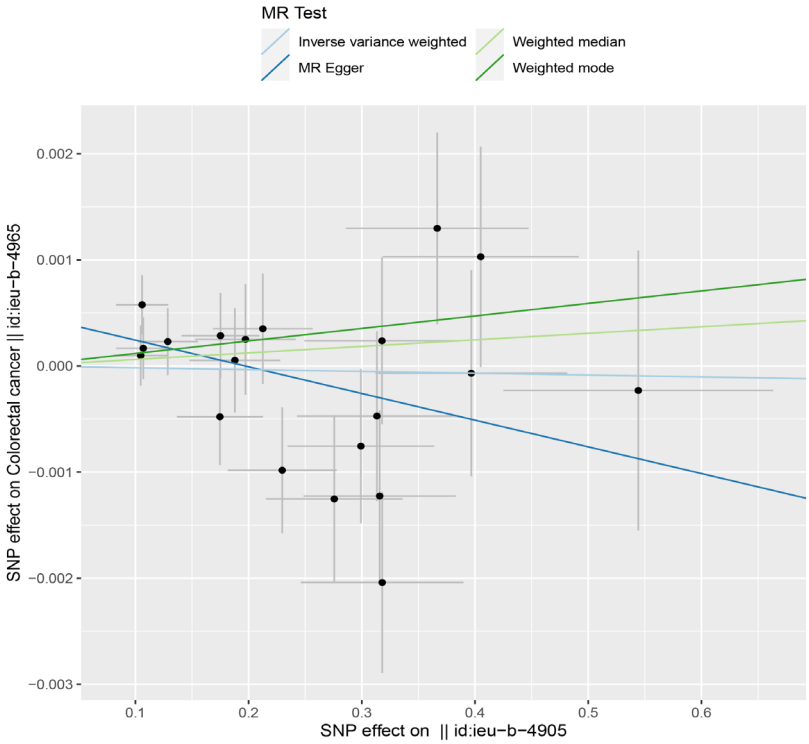

Figure 2. Scatter Plots of the Genetic Associations of anti–*H. pylori* IgG Levels with the Genetic Associations of CRC.

between anti–*H. pylori* IgG levels and colorectal cancer (β = − 0.0002, SE = 0.0007, P = 0.7945; Table 1, Figure 1). The same results were obtained for the other three methods weighted median (β, 0.0006; 95% CI, –0.0010 to 0.0022; P = 0.4456), weighted mode (β, 0.0012; 95% CI, –0.0019 to 0.0043; P = 0.4688), and MR-Egger (β, –0.0025; 95% CI, –0.0052 to 0.0002; P = 0.0886) as shown in Figure 2.The MR-Egger intercept test P value was 0.0776, proving the absence of pleiotropy.

Heterogeneity and sensitivity analysis

The Cochran's Q test indicated no heterogeneity among the SNPs (Table 2), which was supported by the funnel plot (Figure 3). Results from the "leave-one-out" analysis revealed that no individual SNP influenced the causal inferences (Figure 4).

Discussion

To the best of our knowledge, this was the second MR study to assess the causal relationship between *H. pylori* infection and CRC. We used four different methods (IVW, weighted median, weighted mode, and MR-Egger) for MR analysis. Our results showed no causal association between anti–*H. pylori* IgG levels and CRC in the European population.

Although the results of our study are in line with those of the recently published study [15], there are differences between the two studies. First, we used an analytical platform (https://www.mrbase.org/) for MR to avoid possible human calculation errors. Second, the two studies used different datasets, although both datasets were sourced from European populations. The two studies obtained the same results using different datasets, indicating that the results may be reliable.

Table 2. Results of Heterogeneity Detection between SNPs

MR method	Cochran Q statistic	df	I^2	Heterogeneity P value
Inverse-variance weighted	25.33	19	0.2499	0.1500
MR Egger	21.20	18	0.1509	0.2693

MR, Mendelian randomization; SNPs, single-nucleotide polymorphisms.

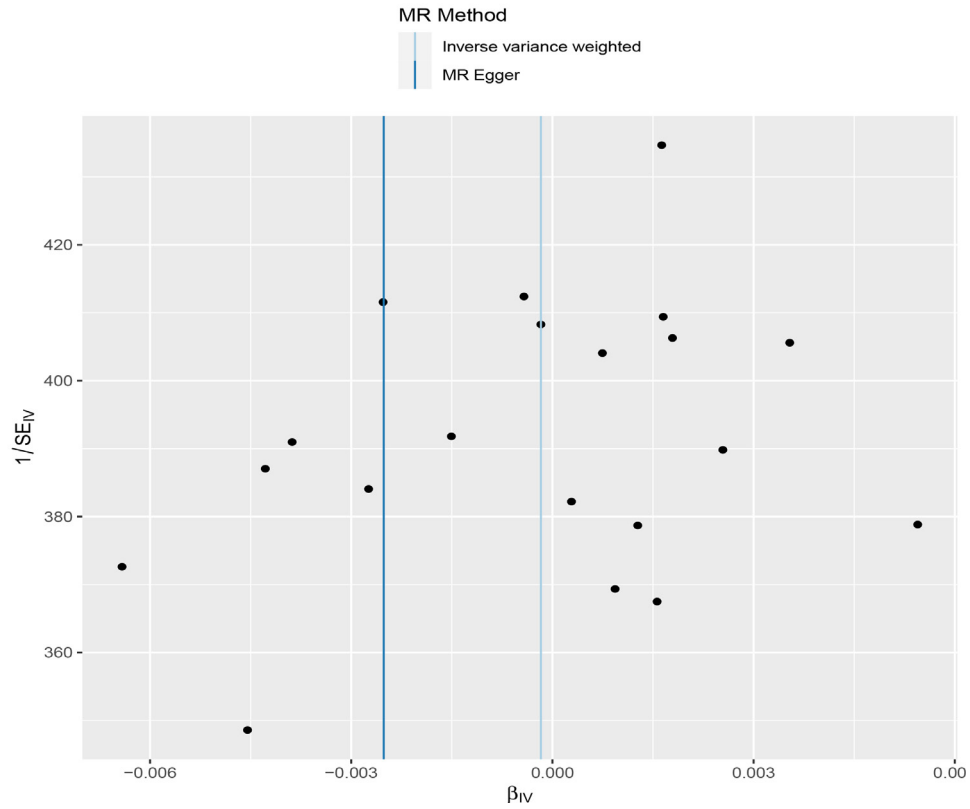

Figure 3. Funnel Plots for Assessing Heterogeneity

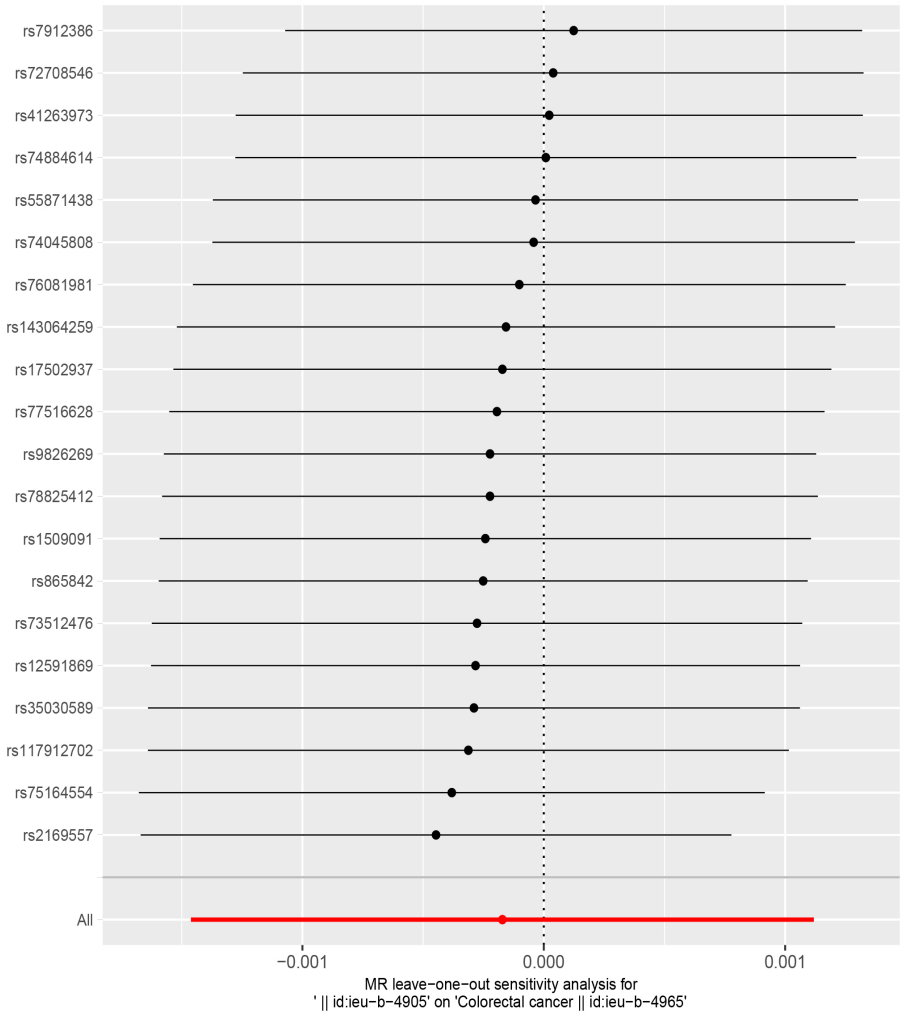

Figure 4. Leave-One-Out Sensitivity Analysis of SNPs

There are several ways to diagnose *H. pylori* infection, including serological and non-serological methods. In a meta-analysis of observational studies, heterogeneity analysis revealed varying associations between serological and non-serological diagnoses of *H. pylori* infection and CRC [19]. The association with CRC was slightly stronger for non-serological methods than for serological methods, because even people infected with *H. pylori* occasionally test seronegative, especially in the elderly [20]. Geography can also influence this association. Namely, one study showed that *H. pylori* infection was linked to a higher risk of CRC in Western countries (OR, 1.34; 95% CI, 1.14 to 1.57; P < 0.001), but not in East Asian countries (OR, 1.16; 95% CI, 1.88 to 1.54; P = 0.297) [21]. In our study, anti–*H. pylori* IgG serological test was used for diagnosing the infection. IgG detection demonstrates reliable results in initial infection screening, with a sensitivity and specificity that reach 99% and 96%, respectively [22, 23]. By including only European populations, our study avoided the influence of regional factors, thereby revealing associations that are closer to reality.

Although our study did not demonstrate a causal association between *H. pylori* and CRC, it is possible that *H. pylori* influences the progression of CRC. Some studies have shown elevated plasma gastrin levels in patients with CRC [24, 25], and hypergastrinemia increases the risk of CRC [26]. *H. pylori* infection can lead to increased levels of gastrin, particularly in cases of chronic, long-term infections [27]. Furthermore, if *H. pylori* infection is eradicated, serum levels of gastrin decrease [28]. Therefore, it may be inferred that *H. pylori* infection increases the risk of CRC, and is potentially mediated by hypergastrinemia. Another study, however, concluded that *H. pylori*, rather than gastrin, was associated with the development of CRC [29]. Therefore, the possible biological mechanisms remain unclear.

Our study has several advantages. First, this was a two-sample MR study. The level of evidence provided by the MR lies between that of randomized controlled trials and observational studies, and MR studies often provide more reliable evidence than a conventional observational study [30]. Second, if our results are confirmed, it may not be necessary to aggressively remove *H. pylori* as part of CRC prevention, as is done in gastric cancer prevention. Nevertheless, there is a limitation to our study. Serological tests do not differentiate between current and previous infections. Even if *H. pylori* has been eradicated, antibodies may persist for years [31]. Possible false-positive results may underestimate the relationship between *H. pylori* infection and CRC.

This MR study does not provide evidence that *H. pylori* infection significantly influences the risk of CRC in a European population.

Author Contribution Statement

Shuanhu Wang conceived the study, extracted and analyzed the data, and drafted the manuscript. Yakui Liu extracted the data. Yi Shi analyzed the data. Mulin Liu participated in the study design. All authors read and approved the final manuscript.

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If it was approved by any scientific Body/ if it is part of an approved student thesis

No, it was not approved by any scientific body, nor is it part of an approved student thesis.

Any conflict of interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Ethical approval and consent to participate were waived in our study because they were obtained from previous original studies.

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