Causal Association of Folic Acid Supplementary Therapy and Gastric Ulcer: A Mendelian Randomization Study

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Research Article

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Abstract

Background.

The incidence of gastric ulcer (GU) remains high worldwide with limited prevention. While promising animal experiments have suggested a potential preventive role of folic acid (FA) in the development of gastric ulcers, the lack of robust clinical evidence has hindered its widespread implementation as a preventative measure. Therefore, this research aims to determine the relationship between FA supplementation and GU genetically by Mendelian randomization (MR) approach, in order to establish a foundation for developing more effective preventative strategies for this condition.

Methods.

Genome-wide association studies (GWAS) investigating the association between folic acid or folate supplementation and gastric ulcers were sourced from the UK Biobank. The primary methods for Mendelian randomization analysis were the inverse variance-weighted (IVW) methods, including fixed-effect and random-effect IVW models. Other methods used to test the robustness of the results included simple model and median, weighted model and median, as well as penalized weighted median.

Results.

MR analysis was performed to investigate the causal effect of FA adjuvant therapy on GU. Seven single nucleotide polymorphisms (SNPs) of genetic loci associated with FA adjuvant therapy were identified. The random-effect and fixed-effect IVW models revealed that genetically predicted FA complementary therapy was significantly related to the reduction of GU risk (OR, 0.870; 95% CI, 0.826–0.917, p < 0.001; OR, 0.870; 95% CI, 0.825–0.918, p < 0.001). Similar results were also observed using simple mode (OR, 0.826; 95% CI, 0.724–0.943, p = 0.030), Weighted mode (OR, 0.828; 95% CI, 0.728–0.941, p = 0.028), simple median method (OR, 0.835; 95% CI, 0.773–0.901, p < 0.001), weighted median (OR, 0.854; 95% CI, 0.794–0.919, p < 0.001) and penalised weighted median (OR, 0.849; 95% CI, 0.789–0.914, p < 0.001). The association between FA supplementary therapy and GU was not considerably driven by any individual SNP according to the leave-one-out sensitivity analysis.

Conclusions.

This MR study provides evidence from a genetic perspective that FA supplementation may decrease the risk of gastric ulcer. Clinicians should prioritize the role of FA in preventing gastric ulcers among patients.

Clinical Perspective

What Is New?
Previous evidence from basic research supports FA supplementation as a protective factor for gastric ulcers. Nevertheless, observational studies have not recognized a causal effect of FA supplementation on gastric ulcers. This MR Study provides genetic evidence of a causal relationship between FA supplementation and gastric ulcers.

**What Are the Clinical Implications?**

These findings confirm the causally decreased risk of gastric ulcer induced by FA supplementary therapy. According to the evidence, clinicians and researchers should attach great importance to the protective role of FA in the prevention and treatment of gastric ulcers. Exploration of the underlying mechanism will provide useful guidance for the protection of gastric ulcer.

**Introduction**

Peptic ulcers are a common type of chronic digestive disease, with an estimated 4 million cases occurring worldwide annually. The prevalence of peptic ulcers in the general population is estimated to be between 5–10% [1, 2], and gastric ulcer is one of the most common types. GU are characterized by natural relief and recurrence, with a high five-year recurrence rate of up to 24.3% [3]. The high incidence of GU is attributed to a series of induced factors, such as *Helicobacter pylori* (*Hp*) infections, abuse of non-steroidal anti-inflammatory drugs (NSAID), alcoholism, and smoking. [4]. Despite developing various medicines for the prevention and treatment of GU including Proton-Pump Inhibitors (PPIs), Histamine-2 Receptor Antagonists (H2RAs) as well as Prostaglandin Analogues [5, 6], the incidence of GU remains high.

Folic acid, also known as folate, is a common B-family vitamin, which exists in all kinds of vegetables, fruits, beans, and other grains [7]. As an essential nutrient that cannot be made by humans, folate plays a crucial role in DNA and RNA synthesis and is involved in protein metabolism [8, 9]. Consequently, it is frequently added to foods as a dietary supplement in the form of folic acid and sold as a supplement [10]. In fact, this form is better than when absorbed from foods. Folate deficiency has been reported to be closely associated with the occurrence of numerous diseases, such as anemia [11], neural tube defects and congenital heart disease [12], atherosclerosis [13], adverse pregnancy outcomes [14] and cancer [15, 16]. However, the potential impact of folic acid on gastric mucosa has received little attention. Several studies have demonstrated that pretreatment with folic acid can effectively prevent the formation of gastric ulcers [17–21]. Nevertheless, most of these studies have been limited to the animal and cellular level and lack of clinical and genetic evidence, which limits their reliability.

MR is a genetic epidemiology method that evaluates the causal association between genetically determined exposure and disease.[22–24]. Early access to the results of MR studies before initiating randomized controlled trials (RCTs) can save time, effort, and research funding, and allow for more informed study design [25, 26].
Therefore, this study aims to investigate the potential causal effect of FA supplementary therapy on GU from a genetic perspective using MR analysis.

**Methods**

**Study Design**

We conducted an MR analysis to investigate the potential causal effects of genetically predict FA adjuvant therapy on GU. The MR design is a method for testing whether exposure has a causal relationship with the development of diseases in which genetic variations are considered instrumental variables. This method can overcome unmeasurable confounding factors and make stronger causality inferences [27]. The design of MR is based on three underlying hypotheses: (1) the genetic variants are closely associated with the exposure; (2) the genetic variants are independent of other confounding factors; (3) the genetic variants are only related to the results of investigated exposure [28]. The brief process of this work is displayed in Fig. 2.

**Data Source and Methods**

Summary-level data on the correlations of FA supplements were obtained from a large-scale genome-wide association study database (GWAS) (https://gwas.mrcieu.ac.uk/datasets/ukb-b-3563/; ICD: “ukb-b-3563”). Prior to MR analysis, single nucleotide polymorphisms (SNPs) were rigorously screened to ensure the quality. Firstly, we gathered all SNPs using linkage disequilibrium clumping (r2 < 0.01 within windows 1000 kb for variants in the gene locus). Moreover, we retained SNPs linked to the opportune exposure at the genome-wide significance threshold (p < 5 × 10⁻⁸). Finally, a total of 7 independent SNPs were genome-wide significant with FA supplements and were applied to the analysis (Table 1). We conducted a comprehensive search of risk factors for GU from previously published literature. After searching the 7 SNPs mentioned above on the PhenoScanner V2 web (http://www.phenoscanner.medschl.cam.ac.uk/), the results showed that none of these SNPs were related to GU risk factors. Consequently, the ultimate MR analysis included all 7 SNPs. Summary statistics for the relation between the 7 FA supplements-related SNPs and GU derived from the GWAS database (https://gwas.mrcieu.ac.uk/datasets/ukb-d-k25/; ICD: “ukb-d-k25”). Studies providing data for these GWAS meta-analyses were ethically approved by the relevant institutional review committees. In the present research, we only used the aggregated data of these studies; hence, there is no need for additional ethical approval.
Statistical analysis

Seven MR analysis methods were used in this study. Among them, IVW was the primary method for analyzing the outcomes and could provide robust estimates of causality even if heterogeneity exists. All instrumental variables were required to meet the MR assumptions in the IVW methods, and the other methods were used for additional sensitivity analyses. A consistent causal assessment could be offered by the weighted median estimator while more than half of the tool variables were effective. In addition, IVW approaches with MR Egger intercept and Cochran's Q statistics were used to evaluate the pleiotropy and heterogeneity of individual SNPs. As long as there is no significant difference between the intercept and 0 (p > 0.05), it is considered that the pleiotropic effects do not exist. Cochran's Q value was applied to assess the heterogeneity. IVW method with the random-effects model was adopted as the main outcome when the p value of Cochran's Q was less than 0.05; otherwise, the fixed-effects model was adopted as the main outcome. MR-Egger regression was also carried out in this study since the pleiotropy could be detected and adjusted by it and then obtaining a causal effect assessment to determine if directional horizontal pleiotropy is accountable to the results. Moreover, leave-one-out analysis was performed to evaluate the robustness of MR analysis results through any outlier SNP. All statistical analyses were carried out using the “TwoSampleMR” package in R version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria) and a two-tailed p value < 0.05 was regarded as statistical significance.

Results

Results of Mendelian randomization study
The overall design and summary of the results of this MR study are illustrated in Fig. 1. After matching the GU data, a total of 7 SNPs as the instruments (FA supplementary therapy) were included in the MR analysis. The final results were analyzed using seven MR analysis approaches, including simple mode, weighted mode, inverse-variance weighted multiplicative random effects, inverse-variance weighted fixed-effect, simple median, weighted median, and penalized weighted median (Fig. 3). The IVW models of both fixed and random effects showed that FA complementary therapy was related to a decreased risk of GU (OR, 0.870; 95% CI, 0.825–0.918, p < 0.001; OR, 0.870; 95% CI, 0.826–0.917, p < 0.001), as indicated in Fig. 4 (Table 2). This causality was also detected in simple median method (OR, 0.835; 95% CI, 0.773–0.901, p < 0.001), weighted median (OR, 0.854; 95% CI, 0.794–0.919, p < 0.001), penalized weighted median (OR, 0.849; 95% CI, 0.789–0.914, p < 0.001), simple mode (OR, 0.826; 95% CI, 0.724–0.943, p = 0.030) and weighted mode (OR, 0.828; 95% CI, 0.728–0.941, p = 0.028) as shown in Table 2. Heterogeneity may exist in the IVW analysis (Q = 5.645, p = 0.464) and MR-Egger analysis (Q = 5.172, p = 0.395). MR-Egger regression showed that there was a directed pleiotropy among the genetic variants (intercept, 0.0003; p = 0.529). The leave-one-out sensitivity investigation indicated that the relationship between FA adscititious therapy and GU was not essentially driven by any single SNP (Fig. 5).

### Table 2

<table>
<thead>
<tr>
<th>Method</th>
<th>Beta</th>
<th>SE</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVW (random effects)</td>
<td>-0.139</td>
<td>0.026</td>
<td>0.870</td>
<td>0.826–0.917</td>
<td>0.001</td>
</tr>
<tr>
<td>IVW (fixed effects)</td>
<td>-0.139</td>
<td>0.027</td>
<td>0.870</td>
<td>0.825–0.918</td>
<td>0.001</td>
</tr>
<tr>
<td>Simple mode</td>
<td>-0.191</td>
<td>0.068</td>
<td>0.826</td>
<td>0.724–0.943</td>
<td>0.030</td>
</tr>
<tr>
<td>Weighted mode</td>
<td>-0.189</td>
<td>0.065</td>
<td>0.828</td>
<td>0.728–0.941</td>
<td>0.028</td>
</tr>
<tr>
<td>Simple median</td>
<td>-0.181</td>
<td>0.039</td>
<td>0.835</td>
<td>0.773–0.901</td>
<td>0.001</td>
</tr>
<tr>
<td>Weighted median</td>
<td>-0.158</td>
<td>0.037</td>
<td>0.854</td>
<td>0.794–0.919</td>
<td>0.001</td>
</tr>
<tr>
<td>Penalised weighted median</td>
<td>-0.164</td>
<td>0.038</td>
<td>0.849</td>
<td>0.789–0.914</td>
<td>0.001</td>
</tr>
</tbody>
</table>

FA, Folic acid or folate; OR, odds ratio; CI, confidence interval; IVW, inverse variance-weighted; MR, Mendelian Randomization.

### Discussion

The occurrence of GU is widely recognized to be closely linked to the invasion of gastric mucosal injury factors and the reduction of mucosal self-defense ability [29, 30]. About 80%-90% of GU cases are caused by abuse of NSAID and the infection with H. pylori, a bacterium classified by the WHO as a class I (definite) carcinogen that promotes an inflammatory response in the host, stimulates gastric acid secretion, and eventually leads to ulcers. [31, 32]. The primary mechanism of NSAID-induced ulcers is inhibition of cyclooxygenase-1 (COX-1), which reduces the synthesis of prostaglandins and impairs mucosal regeneration. Reduced gastric blood flow and ischemia are also among the factors contributing...
to NSAID-induced ulcers [33, 34]. Folic acid, a water-soluble B vitamin that is essential for human health, is found in many foods and serves as a 1-carbon source that participates in various key cellular pathways, including DNA, RNA, and protein methylation, as well as DNA synthesis and maintenance [8, 35, 36]. Folate may be the regulating factor in all of these responses. Thus, folate deficiency is closely associated with the occurrence of various diseases.

Due to the widespread presence of FA in food and the high incidence of GU worldwide, it is crucial to elucidate the role of FA in the prevention and treatment of GU. Accumulating evidence suggests that FA complementary therapy can prevent the occurrence and progression of GU. The underlying mechanisms may be as follows: Firstly, folate may improve GU through antioxidant mechanisms and the inhibition of gastric acid secretion. For instance, a study on indomethacin-induced GU found that pretreatment with folate increased the concentration of superoxide dismutase and mucus, thus effectively preventing the formation of GU by clearing free radicals and protecting the mucosa from damage [17]. Secondly, folate can promote the proliferation of mucosal cells and angiogenesis, accelerating the healing of ulcers. A recent study demonstrated that folate supplementation enhanced the expression of angiogenesis-related factors such as EGF and VEGF, increased the expression of Ki-67 related to cell proliferation, and reduced the severity of ulcers in mice with GU [21]. In addition, folate can protect gastric mucosa from ethanol-induced acute damage through anti-inflammatory and anti-apoptotic mechanisms [20]. However, it should be noted that high-dose folate may damage the integrity of gastric mucosa, as suggested by some studies [19]. A rare case report of "Melanosis" of the duodenum showed that the patient had both GU and FA deficiency. After correcting the folate deficiency, the GU healed faster and the duodenal melanosis was relieved [37]. Furthermore, a study at the human genetic level found that ulcer-healing genes such as TFF2, PPARG, and RUNX3 can effectively recover from mucosal damage by coordinating methylation [38]. Folate is an essential substrate for the synthesis and maintenance of DNA methylation [39, 40], which further strengthens the link between FA folate supplementation and GU.

However, current research on the relationship between FA adjuvant therapy and GU still faces several limitations. First, clinical observational studies are rare. Most current studies have been conducted on animals and in vitro, which greatly reduces the reliability of the results. Second, the relationship between FA and GU in future clinical studies may be reverse causality and unmeasured confounding interference, which could affect the stability of experimental results. Finally, it is worth noting that in multiple clinical studies of FA supplementation have reported varying results due to differences in dose, study population, intervention time, and metabolic processes. Thus, subsequent clinical studies must carefully consider these factors to ensure the accuracy and generalizability of the outcomes.

The greatest strength of this paper lies in the use of Mendelian randomization (MR), a genetic epidemiological design that is similar to RCT [25, 41, 42]. MR studies are advantageous in that they avoid reverse causality and minimize confounding factors, thereby leading to more reliable causal inferences [43, 44]. Furthermore, unlike RCT that typically assess the effect of short-term treatment, MR Studies can reflect the situation of lifetime exposure as the genetic variation is already fixed at the time of conception [45]. Another important advantage of MR studies is their large sample size. In this study, we
used seven MR methods to estimate fully the association between FA supplementation and GU in a large sample of more than 360,000 GU cases. The results of MR all seven methods showed that FA was a protective factor for GU, and these findings were statistically significant. Therefore, our work provides more substantial evidence for the current research, indicating that FA supplementation can prevent the occurrence of GU.

This study was subject to certain limitations that should be taken into consideration. Firstly, the group involved in the MR analysis is of European origin. Therefore, whether the outcomes are representative of the population as a whole remains to be verified. Secondly, the potential overlapping participants in exposure and outcome studies are difficult to estimate.

**Conclusions**

In summary, this study has identified a causal association between FA supplementary therapy and a decreased risk of GU. This significant causal association sheds light on the potential benefits of FA supplementation in managing GU. While there is a dearth of observational evidence, our research contributes to a deeper understanding of the impact of FA supplementation on GU and may serve as a valuable guide for the dietary management of individuals with GU.

**Abbreviations**

Mendelian randomization: MR; gastric ulcer: GU; folic acid: FA; *Helicobacter pylori*. *Hp*; inverse variance-weighted: IVW; OR: odds ratio; CI: confidence interval; non-steroidal anti-inflammatory drugs: NSAID; Proton-Pump Inhibitors: PPIs; Histamine-2 Receptor Antagonists: H2Ras; genome-wide association studies database: GWAS; single nucleotide polymorphisms: SNPs; cyclooxygenase -1: COX-1; randomized controlled trials: RCT.

**Declarations**

**Ethics approval and consent to participate**

We confirmed that all protocols were carried out following relevant guidelines and regulations. In the present research, we only used the aggregated data of these studies; hence, there is no need for additional ethical approval.

**Consent for publication**

Not applicable

**Availability of data and materials**

The original data of Folic acid supplementary therapy can be acquired from https://gwas.mrcieu.ac.uk/datasets/ukb-b-3563/; ICD: “ukb-b-3563”; The original data of gastric ulcer can
be acquired from (https://gwas.mrcieu.ac.uk/datasets/ukb-d-k25/; ICD: “ukb-d-k25”. The analysis data involved in this study are included in the supplementary table1.

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**Author contributions**

Fuhao Li wrote the initial paper;

Fuhao Li, Fengming Huang and Yifan Lu analyzed and interpreted data;

Xi Wang, Meng Li prepared the figure1-5 and tables1-2;

Fan Zhang, Hao jiang revised and reviewed the manuscript for logical, grammatical and structural errors

Bin Lv and Jun Chen conceived idea of the study, supervised overall work and reviewed & revised final draft. Bin Lv had primary responsibility for final content.

All authors read and approved the final manuscript.

**Conflict of Interest**

The authors declare no conflicts of interest in the present study.

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**Figures**
Figure 1

Mendelian randomization model of association between folic acid supplementation and the risk of gastric ulcer. The overall design and summary of the results of this study.

Figure 2
Mendelian randomization model of FA complementary therapy and GU. The design is under the assumption that the genetic variants are associated with FA supplementary therapy, independent of other confounders, and the genetic variations affect gastric ulcer only by FA supplement therapy. Folic acid, FA; SNP, single nucleotide polymorphism.

Figure 3
Scatter plot to visualize the causal effect of FA complementary therapy on GU genetically. The slope of the straight line represents the degree of the causality.

Figure 4

IVW analysis of fixed effect of causality between FA complementary therapy with GU. The black dots and bars represented the causal estimation and 95% CI by means of each SNP. Through MR-Egger and fixed-
effect inverse variance weighted method, the red dot and bar represented the overall estimated value and 95% CI meta-analyzed.

Figure 5

Sensitivity analysis of MR leave-one-out for GU therapy with FA adjuvant. Circles indicate that if each SNP was omitted in turn, MR estimation of FA-assisted GU is performed by inverse-variance weighted fixed-effect method.

Supplementary Files

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

- Supplementarytable1.csv