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

Fuhao Li[1,2#], Fengming Huang[1,2#], Yulong Tang[3#], Fan Zhang[2], Hao Jiang[2], Jun Chen[2*], Bin Lv[1,2*]

[1]Department of Gastroenterology, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou 310003, Zhejiang, China

[2]Key Laboratory of Digestive Pathophysiology of Zhejiang Province, The First Affiliated Hospital of Zhejiang Chinese Medical University, Zhejiang Chinese Medical University, Hubin Campus, Hangzhou 310006, China

[3]The Second School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang, China

***Corresponding Author:** Jun chen, Email address: 21818354@zju.edu.cn; 548 Binwen Road, Binwen District, Hangzhou City, Zhejiang Province, China; Bin Lv, 54 Post and Telecommunication Road in Shangcheng District, Hangzhou, Zhejiang, 310006, China Email address: lvbin@medmail.com.cn;

#co-author: Fuhao Li, Email address: li13903822400@163.com; Fengming Huang, Email address: fengminghuang0711@163.com; Yulong Tang, Email address: tyl-forward@foxmail.com

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI
10.1017/S0007114524002368
The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

Running title: Folic acid and gastric ulcers: an MR Study

Abbreviations

Mendelian randomization: MR; gastric ulcer: GU; folic acid: FA; *Helicobacter pylori*: *Hp*; inverse variance-weighted: IVW; OR: odds ratio; Cl: 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.

Abstract

Previous research has suggested a potential link between folic acid supplementary therapy and gastric ulcers. To investigate this relationship further, we conducted a Mendelian randomization (MR) analysis using data from the UK Biobank. Our analysis primarily employed inverse variance-weighted (IVW) methods, including both fixed-effect and random-effect models. To ensure the robustness of our findings, additional methods such as the simple median, the weighted median, and the penalized weighted median were also applied. The MR analysis aimed to explore the causal effect of FA supplementary therapy on gastric ulcers. Seven single nucleotide polymorphisms (SNPs) at genetic loci associated with FA supplementary therapy were identified. Both the random-effect and fixed-effect IVW models indicated that genetically predicted FA supplementary therapy significantly reduced the risk of gastric ulcers (OR, 0.870; 95% CI, 0.826-0.917, p<0.001). This result was consistent across other methods, with similar outcomes observed using the simple median (OR, 0.835; 95% CI, 0.773-0.901, p<0.001), the weighted median (OR, 0.854; 95% CI, 0.794-0.919, p<0.001), and the penalized weighted median (OR, 0.849; 95% CI, 0.789-0.914, p<0.001). Leave-one-out sensitivity analysis confirmed that no individual SNP significantly drove the association between FA supplementary therapy and gastric ulcers. This MR study provides genetic evidence that FA supplementary therapy may decrease the risk of gastric ulcers.

Keywords: mendelian randomization, folic acid, gastric ulcers, genetics, folate.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

CLINICAL PERSPECTIVE

What Is New?

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

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

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]. Folic acid is used in the treatment of gastric ulcer in the following two aspects: On the one hand, it reduces TNF-α and IL-1β by reducing the infiltration of neutrophils and inflammatory cells, but increases IL-4 and IL-10 to play an anti-secretion, anti-oxidation and anti-inflammatory role, reducing the damage to gastric mucosa[22]; On the other hand, it promotes the healing of gastric mucosa by regulating EGF, EGFR and Ki-67 to promote epithelial proliferation and increasing VEGF, CD31 and factor VIII expression to promote angiogenesis[23]. 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, based on the theory that the random allocation of genes is similar to randomized controlled trials.[24-26]. MR can avoid the interference of confounding factors, as genetic variation is randomly distributed among individuals and is not influenced by potential social or environmental factors. Compared to observational studies, MR provides more reliable causal inference because observational studies are more susceptible to confounding and selection bias, while MR reduces these biases by utilizing genetic variation. Therefore, 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 [27, 28].

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 supplementary 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 [29]. 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

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

only related to the results of investigated exposure [30]. The brief process of this work is displayed in Figure 2.

Data Source and Methods

Summary-level data on the correlations of FA supplementary therapy 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 ($p < 5 \times 10-8$). Finally, a total of 7 independent SNPs were genome-wide significant with FA supplementary therapy 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 is the primary method of analysis because it provides efficient and accurate causal effect estimates when there are many instrumental variables and the assumptions are met. 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. Cochrane's Q value was applied to assess the heterogeneity.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

IVW method with the random-effects model was adopted as the main outcome when the p value of Cochrane'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 Figure 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 (Figure 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 Figure 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 supplementary therapy and GU was not essentially driven by any single SNP (Figure 5).

Discussion

The occurrence Despite significant advances in the treatment of gastric ulcers in recent years, numerous challenges persist in clinical practice. Current therapeutic strategies primarily focus on eradicating *Hp*, inhibiting gastric acid secretion through proton pump inhibitors

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

(PPIs) or H2 receptor antagonists, and utilizing mucosal protective agents[31]. However, issues such as low *Hp* eradication rates, increased antibiotic resistance, poor patient adherence, the inability to discontinue NSAIDs, and high recurrence rates frequently lead to treatment failure. Furthermore, the long-term use of PPIs and H2RAs is associated with a range of side effects, including nutrient malabsorption[32], increased fracture risk[33], heightened infection susceptibility[34], development of drug tolerance[35], and potential renal, hepatic, and central nervous system damage[36]. Therefore, exploring more effective and personalized therapeutic strategies is of paramount importance.

This study investigates the role of folic acid as a potential adjunctive therapy in the prevention and treatment of gastric ulcers. Previous clinical studies have indicated that patients with gastric ulcers generally exhibit lower levels of folate[37], and another case report has suggested that folic acid supplementation may accelerate ulcer healing and alleviate related symptoms[38]. Building on this, our study employs MR to assess the impact of folic acid supplementation on the risk of gastric ulcer development from a genetic perspective. The results demonstrate that folic acid supplementation significantly reduces the risk of developing gastric ulcers, offering a novel perspective on the potential use of folic acid in the prevention and treatment of gastric ulcers.

Current research, primarily conducted in animal models and in vitro studies, supports the potential of folic acid in gastric ulcer prevention and treatment. Firstly, folic acid may improve gastric ulcers through antioxidant mechanisms and inhibition of gastric acid secretion. For example, in a study of indomethacin-induced gastric ulcers, folic acid pretreatment significantly increased the levels of superoxide dismutase and mucus, effectively preventing the formation of gastric ulcers by scavenging free radicals and protecting the mucosa[17]. Secondly, folic acid may promote mucosal cell proliferation and angiogenesis, thereby accelerating ulcer healing. One study showed that folic acid supplementation enhanced the expression of angiogenic factors (e.g., EGF and VEGF) and the cell proliferation marker Ki-67, thereby reducing the severity of gastric ulcers. Additionally, folic acid has shown potential in protecting the gastric mucosa from ethanol-induced acute damage through anti-inflammatory and anti-apoptotic mechanisms[39]. As an essential substrate for DNA methylation, folic acid may contribute to mucosal repair by promoting the coordinated methylation of ulcer-healing genes such as *TFF2*, *PPARG*, and *RUNX3*[40]. However, the potential of folic acid supplementation to reduce the risk of gastric ulcers still necessitates further basic research for validation.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

The greatest strength of this paper lies in the use of Mendelian randomization (MR), a genetic epidemiological design that is similar to RCT[27, 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 supplementary therapy 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 supplementary therapy can prevent the occurrence of GU.

Despite the encouraging findings from this study and other related research, it is important to acknowledge the limitations. This study is primarily based on European populations, and its findings may not be generalizable to other racial or ethnic groups, necessitating further validation. Given that our data are derived from European populations, it is important to consider the relationship between diet and folic acid deficiency within this demographic. Additionally, it is important to note that in MR studies, unmeasured confounders can still impact the stability of the findings. We also cannot entirely rule out the possibility that another condition influenced by folic acid could act as a confounder. Moreover, the long-term effects and safety of folic acid at different doses remain uncertain. Some studies have suggested that high doses of folic acid might cause gastric mucosal damage, leading to gastrointestinal discomfort and other adverse effects[46]. Therefore, future research should focus on optimizing folic acid dosing, evaluating its long-term effects and safety, and assessing its efficacy in diverse populations. Additionally, the potential synergistic effects of folic acid in combination with existing therapies should be explored to develop more personalized treatment regimens.

In conclusion, folic acid has shown significant potential in the prevention and treatment of gastric ulcers, but its precise mechanisms and clinical effects require further validation through high-quality clinical trials and basic research. Future studies should aim to optimize folic acid treatment protocols to enhance the quality of life and clinical outcomes for patients with gastric ulcers.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

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 supplementary therapy in managing GU. While there is a dearth of observational evidence, our research contributes to a deeper understanding of the impact of FA supplementary therapy on GU and may serve as a valuable guide for the dietary management of individuals with GU.

Acknowledgements

We thank all of participants for their great support for UK-Biobank (https://gwas.mrcieu.ac.uk/).

This research was supported by the National Natural Science Foundation of China (81970470). the National Natural Science Foundation of China (82174131) and the National Natural Science Foundation of China (81600426).

F. L. wrote the initial paper; F. L., F. H. and Y. T. analyzed and interpreted data; X. W., M. L. prepared the figure 1-5 and tables; F. Z., H. J. revised and reviewed the manuscript for logical, grammatical and structural errors. B. L. and J. C. conceived idea of the study, supervised overall work and reviewed & revised final draft. B. L. had primary responsibility for final content. All authors read and approved the final manuscript.

The authors declare no conflicts of interest.

The original contributions presented in the study are included in the article/Supplementary table 1, further inquiries can be directed to the corresponding authors.

Uncategorized References

[1] Sverden E, Agreus L, Dunn J M, et al. (2019) Peptic ulcer disease. *BMJ* **367**: l5495.

[2] Lanas A, Chan F K L. (2017) Peptic ulcer disease. *Lancet* **390**: 613-624.

[3] Seo J H, Hong S J, Kim J H, et al. (2016) Long-Term Recurrence Rates of Peptic Ulcers without Helicobacter pylori. *Gut Liver* **10**: 719-725.

[4] Allen A, Garner A. (1980) Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. *Gut* **21**: 249-262.

[5] Malfertheiner P, Megraud F, O'morain C A, et al. (2017) Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut* **66**: 6-30.

[6] Scally B, Emberson J R, Spata E, et al. (2018) Effects of gastroprotectant drugs for the prevention and treatment of peptic ulcer disease and its complications: a meta-analysis

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

of randomised trials. *Lancet Gastroenterol Hepatol* **3**: 231-241.

[7] Chan Y M, Bailey R, O'connor D L. (2013) Folate. *Adv Nutr* **4**: 123-125.

[8] Crider K S, Yang T P, Berry R J, et al. (2012) Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. *Adv Nutr* **3**: 21-38.

[9] Mathers J C. (2005) Reversal of DNA hypomethylation by folic acid supplements: possible role in colorectal cancer prevention. *Gut* **54**: 579-581.

[10] Choi J H, Yates Z, Veysey M, et al. (2014) Contemporary issues surrounding folic Acid fortification initiatives. *Prev Nutr Food Sci* **19**: 247-260.

[11] Dameshek W. (1948) Folic acid, pernicious anemia and pendulums. *Blood* **3**: 699-702.

[12] Czeizel A E, Dudas I, Vereczkey A, et al. (2013) Folate deficiency and folic acid supplementation: the prevention of neural-tube defects and congenital heart defects. *Nutrients* **5**: 4760-4775.

[13] Antoniades C, Antonopoulos A S, Tousoulis D, et al. (2009) Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. *Eur Heart J* **30**: 6-15.

[14] Mcnulty H, Rollins M, Cassidy T, et al. (2019) Effect of continued folic acid supplementation beyond the first trimester of pregnancy on cognitive performance in the child: a follow-up study from a randomized controlled trial (FASSTT Offspring Trial). *BMC Med* **17**: 196.

[15] Oaks B M, Dodd K W, Meinhold C L, et al. (2010) Folate intake, post-folic acid grain fortification, and pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr* **91**: 449-455.

[16] Pufulete M, Al-Ghnaniem R, Khushal A, et al. (2005) Effect of folic acid supplementation on genomic DNA methylation in patients with colorectal adenoma. *Gut* **54**: 648-653.

[17] Ajeigbe K O, Olaleye S B, Oladejo E O, et al. (2011) Effect of folic acid supplementation on oxidative gastric mucosa damage and acid secretory response in the rat. *Indian J Pharmacol* **43**: 578-581.

[18] Markle H V. (1997) Coronary artery disease associated with Helicobacter pylori infection is at least partially due to inadequate folate status. *Med Hypotheses* **49**: 289-292.

[19] Ajeigbe K, Oladejo E, Emikpe B, et al. (2012) The dual modulatory effect of folic acid supplementation on indomethacin-induced gastropathy in the rat. *Turk J Gastroenterol* **23**: 639-645.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

[20] Ajeigbe K O, Jaja L E, Onifade A A, et al. (2017) Folic Acid Supplementation Ameliorates Inflammation and Apoptosis in Ethanol-Induced Gastric Ulceration in Rats. *生物科学与医学(英文)*.

[21] Ajeigbe K, Aibangbee K, Saeed S, et al. (2022) Folic acid protects and heals gastric mucosa: role of acid output, inflammatory cytokines, angiogenic and growth factors. *The Journal of Basic and Applied Zoology* **83**: 15.

[22] Kakhki S, Goodarzi M, Abbaszade-Cheragheali A, et al. (2023) Folic acid supplementation improved cognitive deficits associated with lithium administration during pregnancy in rat offspring. *Int J Dev Neurosci* **83**: 615-630.

[23] Han S W, Oh D Y, Im S A, et al. (2009) Phase II study and biomarker analysis of cetuximab combined with modified FOLFOX6 in advanced gastric cancer. *Br J Cancer* **100**: 298-304.

[24] Liu G, Jin S, Jiang Q. (2019) Interleukin-6 Receptor and Inflammatory Bowel Disease: A Mendelian Randomization Study. *Gastroenterology* **156**: 823-824.

[25] Luo J, Le Cessie S, Van Heemst D, et al. (2021) Diet-Derived Circulating Antioxidants and Risk of Coronary Heart Disease: A Mendelian Randomization Study. *J Am Coll Cardiol* **77**: 45-54.

[26] Huang D, Lin S, He J, et al. (2022) Association between COVID-19 and telomere length: A bidirectional Mendelian randomization study. *J Med Virol* **94**: 5345-5353.

[27] Ference B A, Holmes M V, Smith G D. (2021) Using Mendelian Randomization to Improve the Design of Randomized Trials. *Cold Spring Harb Perspect Med* **11**.

[28] Ference B A, Kastelein J J P, Ginsberg H N, et al. (2017) Association of Genetic Variants Related to CETP Inhibitors and Statins With Lipoprotein Levels and Cardiovascular Risk. *JAMA* **318**: 947-956.

[29] Lawlor D A, Harbord R M, Sterne J A, et al. (2008) Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* **27**: 1133-1163.

[30] Emdin C A, Khera A V, Kathiresan S. (2017) Mendelian Randomization. *JAMA* **318**: 1925-1926.

[31] Gong H, Zhao N, Zhu C, et al. (2024) Treatment of gastric ulcer, traditional Chinese medicine may be a better choice. *J Ethnopharmacol* **324**: 117793.

[32] Teresa Selvin S, Thomas S, Bikeyeva V, et al. (2022) Establishing the Association Between Osteoporosis and Peptic Ulcer Disease: A Systematic Review. *Cureus* **14**: e27188.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

[33] Lespessailles E, Toumi H. (2022) Proton Pump Inhibitors and Bone Health: An Update Narrative Review. *Int J Mol Sci* **23**.

[34] Cunningham R, Dial S. (2008) Is over-use of proton pump inhibitors fuelling the current epidemic of Clostridium difficile-associated diarrhoea? *J Hosp Infect* **70**: 1-6.

[35] Sharma P. (2023) Over 30 Years of Omeprazole. *J Assoc Physicians India* **71**: 11-12.

[36] Fossmark R, Martinsen T C, Waldum H L. (2019) Adverse Effects of Proton Pump Inhibitors-Evidence and Plausibility. *Int J Mol Sci* **20**.

[37] Tamura A, Fujioka T, Nasu M. (2002) Relation of Helicobacter pylori infection to plasma vitamin B12, folic acid, and homocysteine levels in patients who underwent diagnostic coronary arteriography. *Am J Gastroenterol* **97**: 861-866.

[38] Sharp J R, Insalaco S J, Johnson L F. (1980) "Melanosis" of the duodenum associated with a gastric ulcer and folic acid deficiency. *Gastroenterology* **78**: 366-369.

[39] Zou Y, Cui X, Xiang Q, et al. (2021) Protective effect of against ethanol-induced gastric ulcer and its mechanism. *Zhejiang Da Xue Xue Bao Yi Xue Ban* **50**: 561-567.

[40] Hong S J, Oh J H, Jung Y C, et al. (2010) DNA methylation patterns of ulcer-healing genes associated with the normal gastric mucosa of gastric cancers. *J Korean Med Sci* **25**: 405-417.

[41] Gill D, Walker V M, Martin R M, et al. (2020) Comparison with randomized controlled trials as a strategy for evaluating instruments in Mendelian randomization. *Int J Epidemiol* **49**: 1404-1406.

[42] Thanassoulis G, O'donnell C J. (2009) Mendelian randomization: nature's randomized trial in the post-genome era. *JAMA* **301**: 2386-2388.

[43] Yuan S, Larsson S C. (2022) Coffee and Caffeine Consumption and Risk of Kidney Stones: A Mendelian Randomization Study. *Am J Kidney Dis* **79**: 9-14.e11.

[44] Bouras E, Karhunen V, Gill D, et al. (2022) Circulating inflammatory cytokines and risk of five cancers: a Mendelian randomization analysis. *BMC Med* **20**: 3.

[45] Labrecque J A, Swanson S A. (2019) Interpretation and Potential Biases of Mendelian Randomization Estimates With Time-Varying Exposures. *Am J Epidemiol* **188**: 231-238.

[46] Selhub J, Rosenberg I H. (2016) Excessive folic acid intake and relation to adverse health outcome. *Biochimie* **126**: 71-78.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

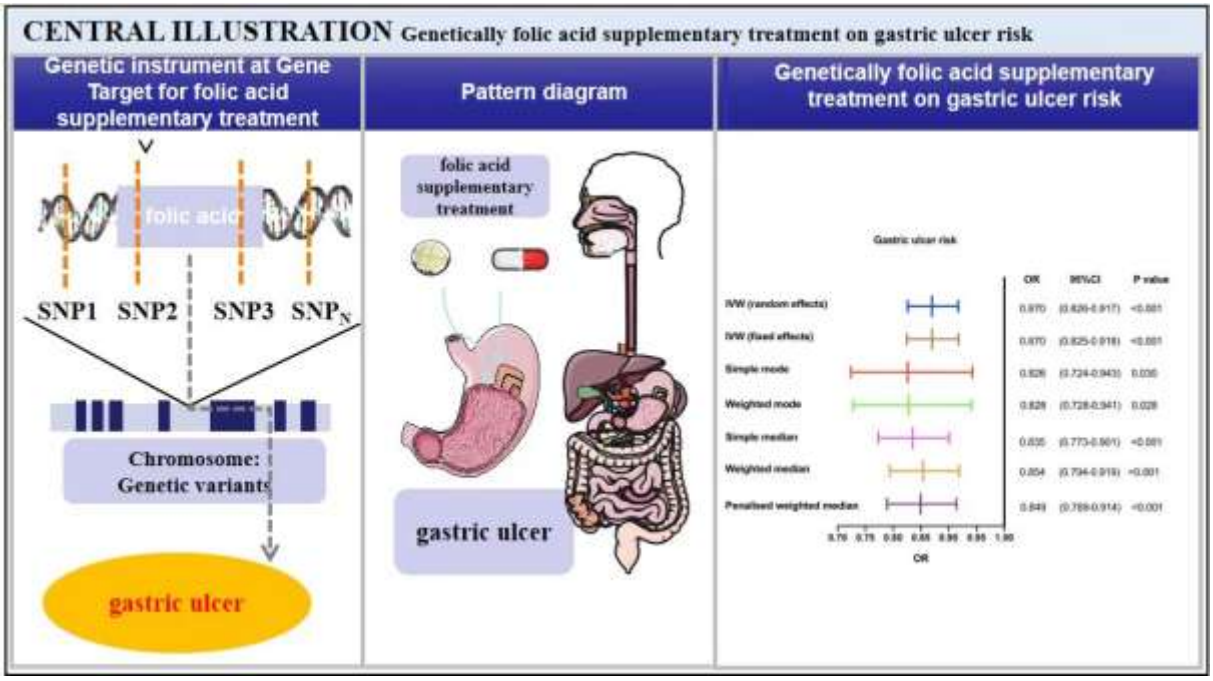

Figure 1. Mendelian randomization model of association between FA supplementary therapy and the risk of gastric ulcer. The overall design and summary of the results of this study**.**

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

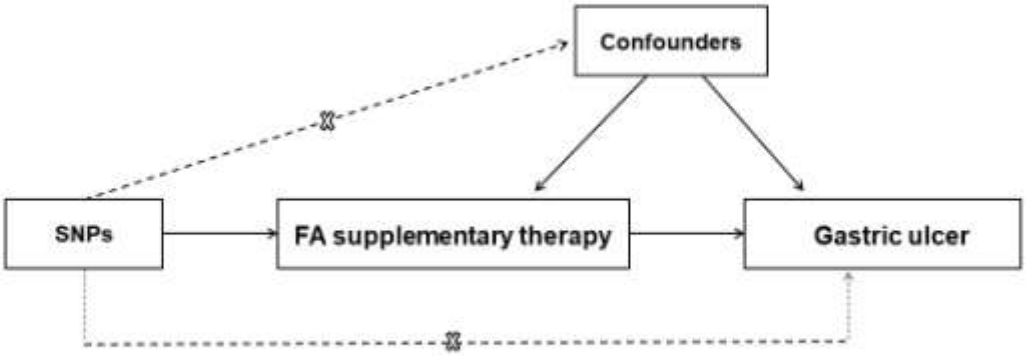

Figure 2. Mendelian randomization model of FA supplementary therapy and GU. The design is under the assumption that the genetic variants are associated with FA supplementary therapy, independent of other confounders, and the genetic variations affect gastric ulcer only by FA supplement therapy. Folic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

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

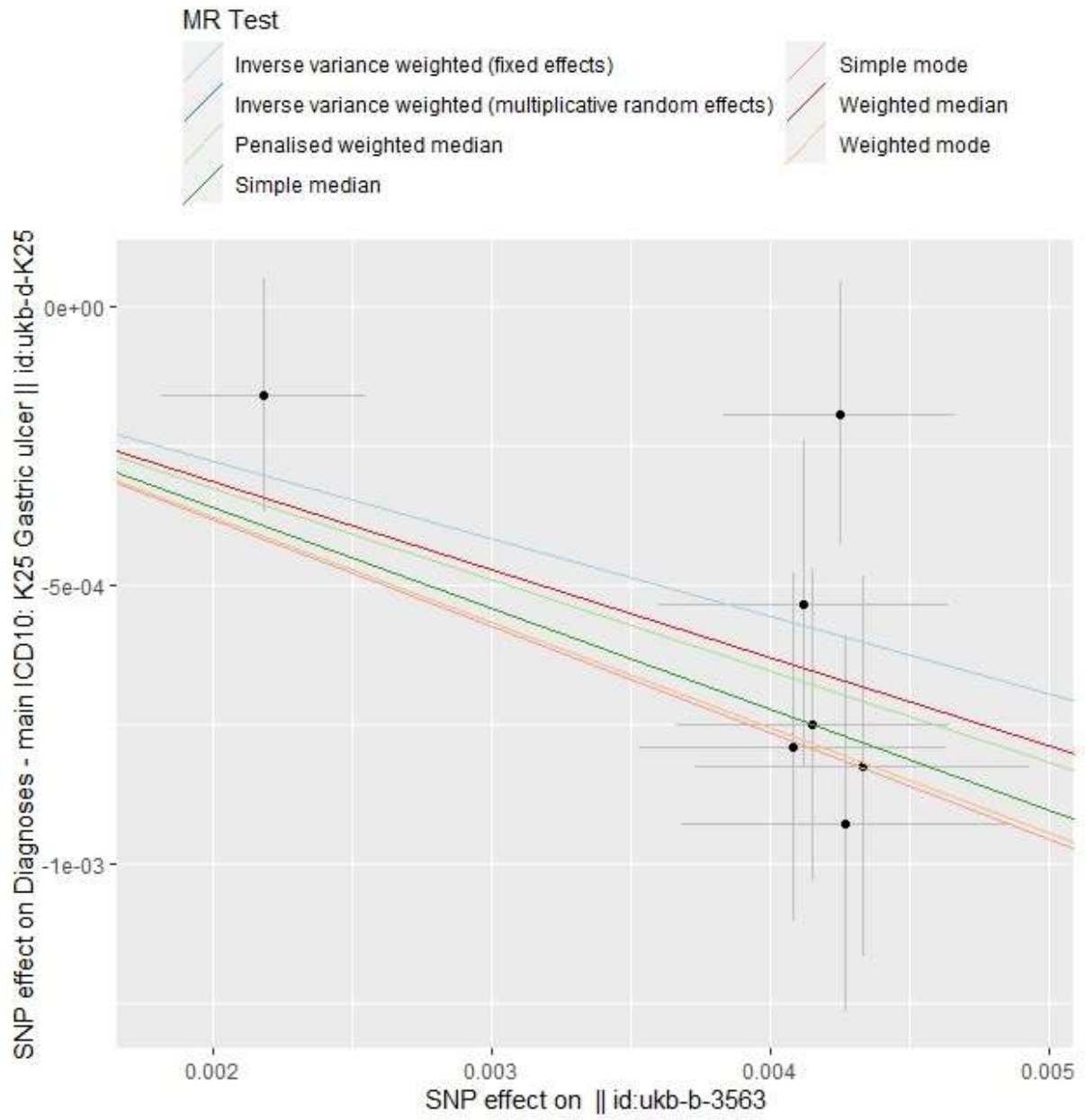

Figure 3. Scatter plot to visualize the causal effect of FA supplementary therapy on GU genetically. The black dots and bars represented the causal estimation and 95% CI by means of each SNP and slope of the straight line represents the degree of the causality.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

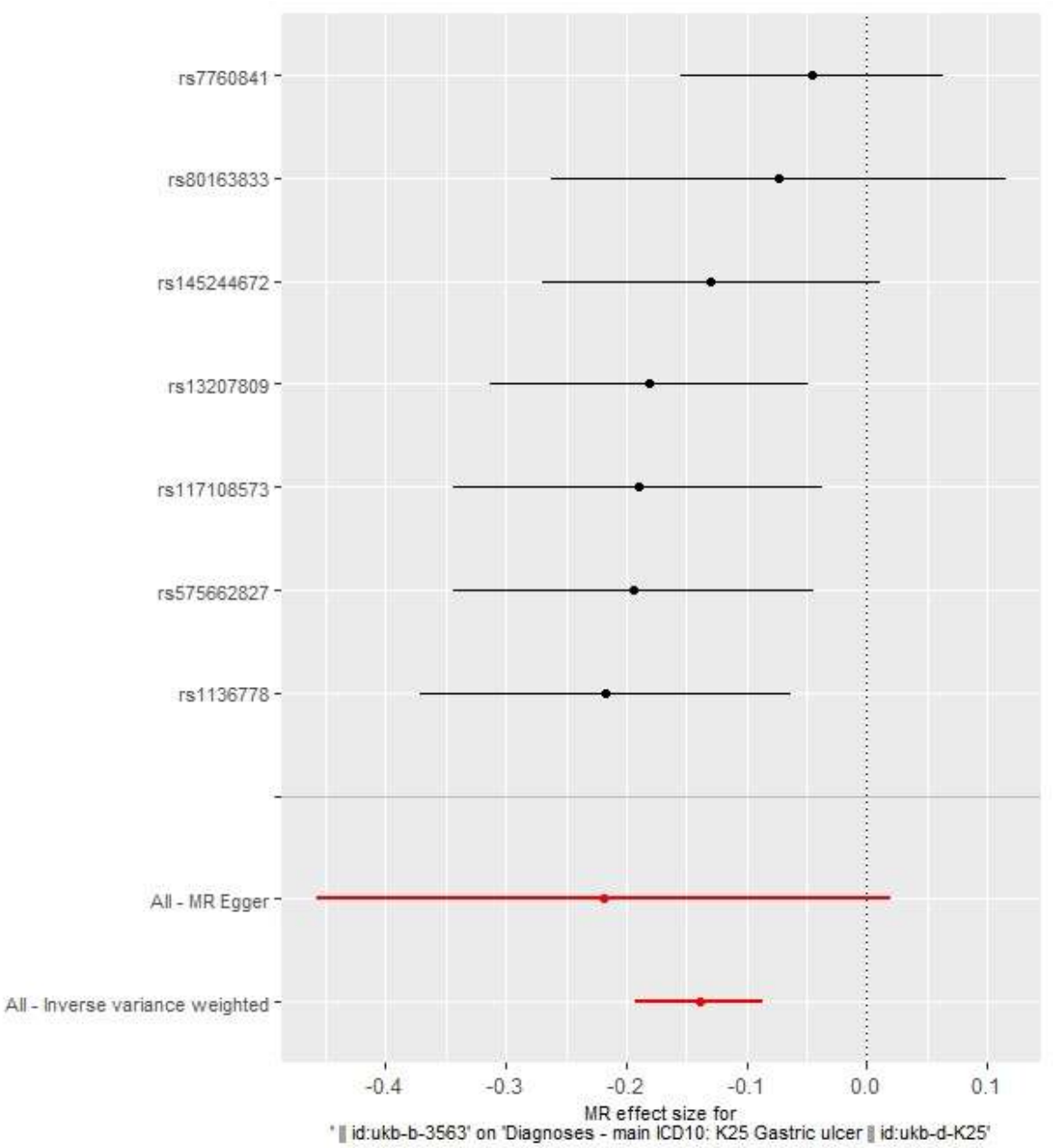

Figure 4. IVW analysis of fixed effect of causality between FA supplementary therapy with GU. The black dots and bars represented the causal estimation and 95% CI by means of each SNP. Through MR-Egger and fixed-effect inverse variance weighted method, the red dot and bar represented the overall estimated value and 95% CI meta-analyzed.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

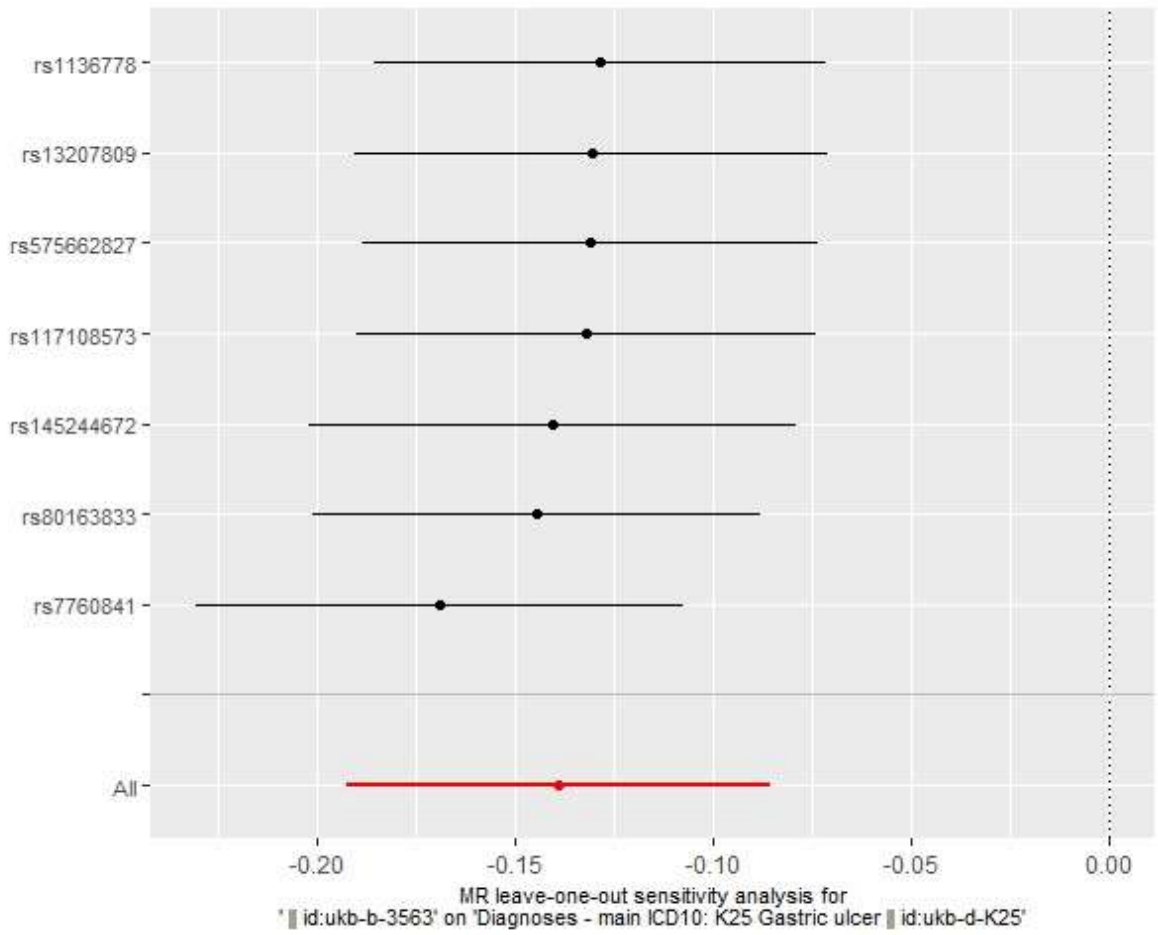

Figure 5. Sensitivity analysis of MR leave-one-out for GU therapy with FA supplementary therapy. Circles indicate that if each SNP was omitted in turn, MR estimation of FA-assisted GU is performed by inverse-variance weighted fixed-effect method.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

Table 1. The characteristics of 7 SNPs and their genetic connections with FA supplementary therapy and GU.

SNP	EAF	EA	OA	FA supplementary therapy			gastric ulcer		
				Beta	SE	P	Beta	SE	P
rs1136778	0.080863	G	A	0.004273	0.000592	5.50E-13	-0.000928	0.000338	0.005953
rs117108573	0.079232	T	C	0.004334	0.000601	5.50E-13	-0.000824	0.000341	0.015567
rs13207809	0.128791	C	G	0.004154	0.000489	2.10E-17	-0.000751	0.000279	0.007150
rs145244672	0.120871	G	T	0.004120	0.00052	2.00E-15	-0.000534	0.000295	0.070562
rs575662827	0.101384	C	T	0.004083	0.000549	1.00E-13	-0.00079	0.000312	0.011172
rs7760841	0.173206	T	C	0.004249	0.000413	8.80E-25	-0.000192	0.000236	0.414570
rs80163833	0.273883	T	C	0.002180	0.000369	3.40E-09	-0.000159	0.000211	0.451577

SNP, single nucleotide polymorphism; FA, Folic acid or folate; Chr, chromosome; EA, effect allele; OA, other allele; EAF, frequency of effect allele; SE, standard error.

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press

Table 2: The Association of FA supplementary therapy with GU using various methods.

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

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

https://doi.org/10.1017/S0007114524002368 Published online by Cambridge University Press