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ORIGINAL ARTICLE

Plasma *L*-aspartic acid predicts the risk of gastric cancer and modifies the primary prevention effect: a multistage metabolomic profiling and Mendelian randomization study

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ABSTRACT

Objective: Based on multistage metabolomic profiling and Mendelian randomization analyses, the current study identified plasma metabolites that predicted the risk of developing gastric cancer (GC) and determined whether key metabolite levels modified the GC primary prevention effects.

Methods: Plasma metabolites associated with GC risk were identified through a case-control study. Bi-directional two-sample Mendelian randomization analyses were performed to determine potential causal relationships utilizing the Shandong Intervention Trial (SIT), a nested case-control study of the Mass Intervention Trial in Linqu, Shandong province (MITS), China, the UK Biobank, and the FinnGen project.

Results: A higher genetic risk score for plasma *L*-aspartic acid was significantly associated with an increased GC risk in the northern Chinese population (SIT: HR = 1.26 per 1 *SD* change, 95% CI: 1.07–1.49; MITS: HR = 1.07, 95% CI: 1.00–1.14) and an increased gastric adenocarcinoma risk in FinnGen (OR = 1.68, 95% CI: 1.16–2.45). Genetically predicted plasma *L*-aspartic acid levels also modified the GC primary prevention effects with the beneficial effect of *Helicobacter pylori* eradication notably observed among individuals within the top quartile of *L*-aspartic acid level (*P*-interaction = 0.098) and the beneficial effect of garlic supplementation only for those within the lowest quartile of *L*-aspartic acid level (*P*-interaction = 0.02).

Conclusions: Elevated plasma *L*-aspartic acid levels significantly increased the risk of developing GC and modified the effects of GC primary prevention. Further studies from other populations are warranted to validate the modification effect of plasma *L*-aspartic acid levels on GC prevention and to elucidate the underlying mechanisms.

KEYWORDS Gastric cancer; plasma metabolites; Mendelian randomization; *L*-aspartic acid

Introduction

Gastric cancer (GC) poses a significant threat to human health, ranking fifth globally in both cancer incidence and mortality[1]. Although the global incidence of GC has decreased[2], the disease burden is expected to continue to increase due to population growth[3]. Early detection of GC remains challenging with 5-year survival rates ranging between 20% and 40% in most regions[4,5]. *Helicobacter pylori* (*H. pylori*) infection is a well-established major risk factor for GC[6], the eradication of which significantly reduces GC incidence and mortality[7,8]. In addition, the occurrence of GC involves the interplay between multiple genetic, host, and environmental characteristics[7,9-11].

Metabolomic dysregulation is a major molecular event underlying gastric carcinogenesis, with previous studies on plasma, urine, and tissue samples highlighting metabolite changes in glucose, amino acids, lipids, and nucleotides in GC[12-18]. Although prior research has provided insight into metabolite alterations associated with GC risk, compelling

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evidence on key metabolite signatures and causal links to GC development is limited[15]. It is not clear if key metabolites signify the risk of developing GC and serve as indicators for the effect of *H. pylori* eradication and nutritional supplementation. Traditional observational metabolomics studies are often constrained by confounding factors and reverse causation[19]. In contrast, Mendelian randomization (MR) effectively minimizes these external influences by using genetic variants, which are randomly distributed as instrumental variables (IVs) to infer causality[20,21]. Although a past MR study that focused on the relationship between lipoproteins and GC[22], there is a conspicuous absence of comprehensive MR studies encompassing a diverse array of metabolites.

To address the gap in knowledge regarding metabolites that predict GC risk, a comprehensive examination of plasma metabolites that causally impact GC risk was undertaken. The current study combined a population-based case-control metabolomic study with bi-directional MR analyses, leveraging data from Linqu County and publicly available databases. Linqu County, located in Shandong Province, China, is a recognized high-risk area for GC. Several studies have been conducted in this geographic area on the etiology and GC epidemiologic risk factors since 1983, as well as the strategies to prevent GC[7,8,23-26]. How key plasma metabolites modify the long-term preventive effects of *H. pylori* eradication and nutrition supplementation on GC were determined to further elucidate the public health implications.

Materials and methods

Overall study design

The overall study design is illustrated in **Study Flow** and **Table S1**. The study began by identifying plasma metabolites associated with GC based on a case-control study involving 400 participants from Linqu County, Shandong Province of China who participated in the Upper Gastrointestinal Cancer Early Detection (UGCED) program[27].

Bi-directional two-sample MR analyses were then carried out to determine the causal relationships between plasma metabolites and GC. Forward MR analyses were performed to identify plasma metabolites that were causally associated with GC risk utilizing single-nucleotide polymorphisms (SNPs) associated with plasma metabolite levels as IVs. IVs for the Chinese population were identified based on our in-house metabolite genome-wide association study (GWAS) data for the UGCED population that was free of cancer, while SNPs associated with plasma metabolites in Europeans were obtained based on a literature research. Outcome cohorts relied on individual-level genetic data from the Shandong Intervention Trial (SIT, n = 2,816)[7,26,28-30], a nested case-control study of the Mass Intervention Trial in Linqu, Shandong province (MITS, n = 2,804)[8] and the UK Biobank (UKB, n = 251,716), as well as GC GWAS summary statistics from the FinnGen project (n = 315,616). There were no overlapping subjects between the exposure and outcome cohorts. Additionally, reverse MR analyses were used to determine whether GC risk would causally change the plasma metabolite levels utilizing the genetic susceptibility of GC as IVs. The current study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) guidelines[31,32].

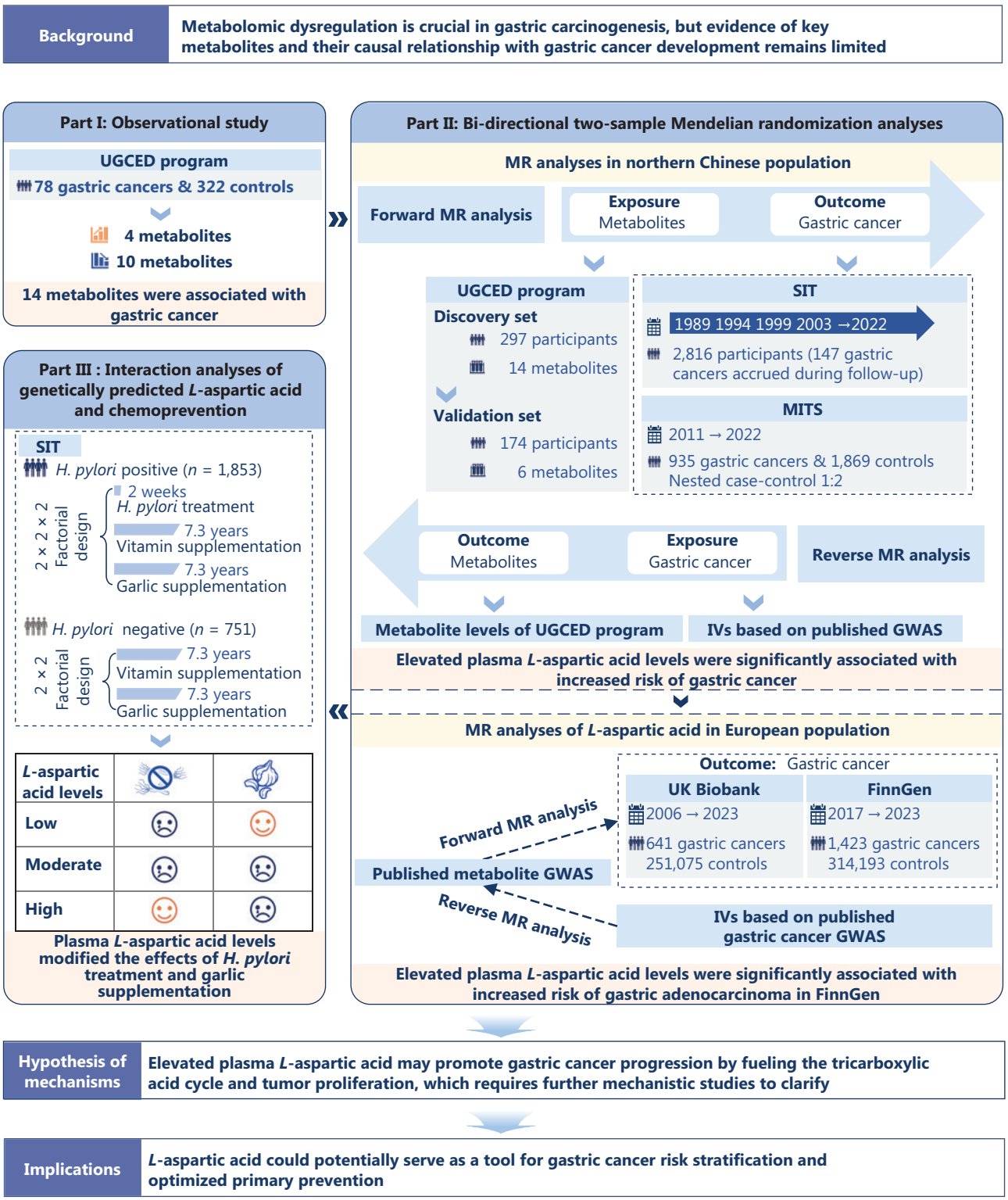
For metabolites with a potential causal relationship in the forward MR, whether metabolite levels interacted with *H. pylori* eradication, garlic supplementation, and vitamin supplementation on GC risk based on the SIT participants attending the trial (n = 2,604) was also determined.

The study was approved by the Institutional Review Board of Peking University Cancer Hospital (Approval No. 2022KT159).

Participants

Approximately 3,500 residents in Linqu County (40–69 years of age) undergo endoscopic examinations per year at no charge within the framework of the national UGCED program. Details of the UGCED program, including the inclusion and exclusion criteria, gastroendoscopic and pathologic diagnosis, questionnaire survey, and biospecimen collection, have been described previously[18,27,33]. The observational analyses examining plasma metabolites associated with GC included a total of 400 subjects (78 GC cases and 322 controls) from those attending the UGCED program. A GWAS was performed on 297 controls with eligible genetic data, which served as the discovery set to identify the IVs for plasma metabolites in the forward MR analyses and the outcome dataset in the reverse MR analyses of northern Chinese. In addition, 174 independent non-cancer subjects with metabolomics and genetic data were used as the validation set using our in-house database based on the UGCED program (recruited in 2016)[34].

The SIT commenced in 1989, enrolling 3,386 residents 35–64 years of age from 14 villages in Linqu County[23,35]. The



Study Flow Chart Part I involves an observational case-control study to identify plasma metabolites associated with gastric cancer risk using the UGCED program. Part II outlines bi-directional two-sample MR analyses utilizing genetic susceptibility of metabolites and gastric cancer risk as IVs to explore causal relationships with data from UGCED, SIT, MITS, the UK Biobank, and the FinnGen project. Part III examines the interaction of genetically predicted plasma *L*-aspartic acid levels with *H. pylori* eradication, garlic supplementation, and vitamin supplementation in

modifying gastric cancer risk. The study highlights *L*-aspartic acid as a potential biomarker for gastric cancer risk stratification and optimized primary prevention. GWAS, genome-wide association study; *Helicobacter pylori*, *H. pylori*; IV, instrumental variable; MITS, Mass Intervention Trial in Linqu, Shandong province; MR, Mendelian randomization; SIT, Shandong Intervention Trial; UGCED, Upper Gastrointestinal Cancer Early Detection.

participants underwent baseline endoscopic examinations. The exclusion criteria included serious illnesses, mental disorders, pregnancy, or prior *H. pylori* treatment. A total of 3,365 eligible individuals were randomized into a 2 × 2 × 2 factorial intervention trial in 1995[7,26,28]. Interventions included *H. pylori* treatment (amoxicillin and omeprazole for 2 weeks), garlic supplementation, and vitamin supplementation (both for 7.3 years) with matching placebos. Detailed treatment regimens have been reported previously[7]. The current study included 2,816 participants with genotyping data for the forward MR analyses in a northern Chinese population[30], of which 2,604 participating in the intervention trial were also used for the interaction analyses between genetically predicted metabolite level and primary prevention of GC.

The MITS is a community-based, cluster-randomized, controlled, superiority intervention trial to test the GC prevention effect of anti-*H. pylori* therapy[8,36]. A total of 180,284 eligible participants from 980 villages were enrolled and a total of 1,035 cases of incident GC were documented over 11.8 years of follow-up. There were no overlapping subjects between the SIT and MITS cohorts. The current study utilized a nested case-control design in which newly diagnosed GC patients during the follow-up period were selected as cases and controls were randomly chosen in a 1:2 ratio for genotyping. After quality control, a total of 935 GC cases and 1,869 healthy controls were included in the forward MR analyses of northern Chinese.

The UKB (https://www.ukbiobank.ac.uk/) is a large population-based cohort having recruited approximately 0.5 million participants aged between 40 and 69 years of age from 2006–2010 in the United Kingdom. Comprehensive medical data of participants were collected from the UK National Health Service, including records of disease events, prescriptions, and mortality. In the current study we focused on individuals of European ancestry, excluding individuals lacking genetic data, individuals with mismatches between self-reported and genotyped gender, or individuals with abnormal heterozygosity or high missingness rates. A total of 641 GC patients and 251,075 controls with no past history of other cancers were included for the forward MR analysis of the European (UKB application No. 90999).

The FinnGen study (https://www.finngen.fi/) collected the genomes and health data of approximately 0.5 million people[37]. FinnGen has been recruiting Finnish volunteers ≥ 18 years of age since 2017. Summary statistics of the FinnGen GWAS on GC and gastric adenocarcinoma (GAC) were downloaded (released on 18 December 2023) for the current study, including data from 1,423 GC patients and 314,193 individuals without a history of cancers were used for the forward MR analysis of the European.

Diagnosis of GC

Individuals underwent endoscopic examinations and pathologic diagnoses for the early detection of GC as part of the UGCED program. Patients with high-grade intraepithelial neoplasia (HGIN) and invasive GC were combined as the GC group because the program followed similar treatment principles for patients diagnosed with HGIN and early invasive GC. All attendees were actively followed until 31 August 2022 as well as attendees with advanced gastric lesions receiving repeated endoscopic examinations. All participants in the SIT underwent routine gastric endoscopic screening in 1989, 1994, 1999, and 2003 with continuous follow-up evaluations until 31 August 2022[30]. The MITS study conducted prospective follow-up evaluations until 31 December 2022. UGCED program, SIT, and MITS participants were also followed using passive and active approaches for GC incidence and mortality. The database was linked to cancer registry and death reporting system management by the Chinese Center for Disease Prevention and Control for passive follow-up care. Active clinical follow-up care was provided by village physicians, local program coordinators, and staff from Peking University Cancer Hospital.

Cancer registry data from the UKB cohort was used to identify GC and other cancer cases. The registry provides detailed information on histologic tumor types. All participant cancer registry data for FinnGen were collected and processed from the Finnish Cancer Registry and Cause of Death Registry.

The diagnoses of cancers were coded using the World Health Organization International Classification of Diseases

[10th revision (ICD-10)] or Finnish ICD-10. The pathologic types were referenced from the International Classification of Diseases for Oncology [3rd edition (ICD-O-3)].

Genotyping and imputation

Peripheral blood leukocyte DNA samples were genotyped using the Global Screening Array beadchip (Illumina Inc., San Diego, CA, USA) for UGCED and SIT participants and the Asian Screening Array beadchip (Illumina, Inc.) for MITS participants. The quality control and imputation of genotyped variants have been described previously[30] following standard procedures[38]. Approximately 488K participants genotyped using custom-designed Affymetrix UK BiLEVE Axiom or UK Biobank Axiom arrays (Affymetrix, Santa Clara, CA, USA). Detailed information on genotyping, quality control, and genotype imputation have been described in a previous study[39].

Metabolomic profiling and quality control

Non-targeted metabolomics assay was performed using ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS) for the observational study part utilizing UGCED participants following the methodology reported in the literature[18,34,40]. All detected ions were extracted using MarkerView 1.3 (AB Sciex, Concord, ON, Canada) into Excel in the format of dimensional matrix, including mass-to-charge ratio (m/z), retention time, and peak area; isotopic peaks were filtered. PeakView 2.2 (AB Sciex) was applied to extract MS/MS data. Metabolites were then annotated using standard references and established databases, including Metabolites (AB Sciex), HMDB (https://hmdb.ca/), and METLIN (https://metlin.scripps.edu/). Data from metabolomics assays were log-transformed (excluding values exceeding the mean ± 4 *SD*) and normalized with min-max scaling.

Statistical analysis

Observational study

Unconditional logistic regression models were used to calculate the odds ratio (OR) and 95% confidence interval (CI) for each plasma metabolite associated with GC cases *vs.* controls, adjusting for age, gender, and *H. pylori* infection. A false discovery rate (FDR)–adjusted $q < 0.05$ was considered statistically significant.

Selection of genetic IVs for forward MR analyses

An additive linear model was used to examine the associations of SNPs with each metabolite in the UGCED population adjusting for age, gender, and three principal components of genetic structure using PLINK (version 2.0). The GWAS significance threshold was set at a $P < 5 \times 10^{-8}$ for the discovery stage with a marginal significance threshold of $P < 1 \times 10^{-4}$. Selected metabolite-associated SNPs with a $P < 0.05$ and consistent directions in association across both stages were considered validated for metabolites with GWAS data in the validation stage. A meta-analysis of the results from both stages was performed using inverse variance weighting under a fixed effects model with METAL. The Q-Q plot and Manhattan plot for each metabolite GWAS were generated using R packages ('qqman' and 'CMplot'). LocusZoom was used to plot association regions between SNPs and plasma metabolite levels based on linkage disequilibrium (LD) information from the 1,000 Genomes Project for Asian populations with SNP annotation performed using ANNOVAR software.

IVs for 14 selected plasma metabolites in the Chinese population were defined utilizing our in-house metabolite GWAS data. Metabolite-associated SNPs after 2-stage validation ($P < 1 \times 10^{-4}$ in the discovery and $P < 0.05$ in the validation set) were used as the IVs for 6 metabolites with 2-stage GWAS data. A $P < 5 \times 10^{-5}$ and *F*-statistic > 10 were used for metabolite-related SNPs to avoid weak IV bias for the other 8 metabolites with GWAS data available in the discovery set[41]. The variance in plasma level explained by each IV (R^2) and the strength of each IV (*F*-statistics) were calculated using the following formulas for each metabolite:

$$R^2 = 2 \times MAF \times (1 - MAF) \times \left(\frac{\beta}{sd}\right)^2 \quad (1)$$

$$F = \frac{R^2/K}{(1 - R^2)/\left(N - K - 1\right)}, \quad (2)$$

where *MAF* denotes minor effect allele frequency, and β and *sd* represent the effect size and standard deviation of the SNP-metabolite association, respectively, *N* is the sample size of the metabolite GWAS, and *K* is the number of SNPs screened.

IVs for metabolites in the European population were obtained by searching PubMed and the GWAS Catalog. Information on genotyping, filling panels, and subject characteristics are available in the original article and its appendices[42]. LD clumping was performed with a window size of 1,000 kilobases to independently select SNPs (pairwise LD $r^2 < 0.01$) associated with plasma metabolites at a $P < 5 \times 10^{-6}$.

PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) was applied to identify and subsequently exclude any potential confounding SNPs associated with GC and its risk factors for the specified IVs of metabolites. Constrained by the scarcity of GWASs on *H. pylori* infection for the Asian population based on available databases, the associations between plasma metabolite IVs and *H. pylori* infection in the Chinese population were examined using an additive linear model with PLINK adjusting for age, gender, and three principal components for population stratification.

Forward MR analyses

The weighted genetic risk score (wGRS) for plasma metabolites was constructed utilizing the selected IVs. The hazard ratios (HRs) and corresponding 95% CIs were calculated for the associations between wGRSs of metabolites and GC risk using Cox proportional hazards regression models (SIT cohort) or inverse probability weighting Cox proportional hazards regression models (nested case-control study of the MITS) to determine the causal relationship between plasma metabolites and the risk of GC, adjusting for age, gender, *H. pylori* infection, and three principal components. A sensitivity analysis was performed by excluding IVs associated with *H. pylori* infection to eliminate potential pleiotropic effects. Unconditional logistic regression analysis was used to calculate the ORs (95% CIs) for the associations between wGRSs and GC risk for UKB, adjusting for age, gender, and three principal components. In a sensitivity analysis the synthetic minority oversampling technique (SMOTE)[43] was used to address the effect of class imbalance in UKB.

The inverse variance-weighted (IVW) method was employed using the "TwoSampleMR" package in R for FinnGen summary statistics. Pleiotropy was assessed using MR-Egger regression (P for intercept > 0.05, indicating no horizontal pleiotropy). Leave-one-out analysis, funnel plots, and forest plots were applied to evaluate stability and heterogeneity of effect estimates.

Reverse MR analyses

Reverse MR analyses were performed to determine whether GC causally influences plasma metabolite levels utilizing genetic susceptibility to GC as IVs. Genetic IVs were selected based on the studies by Yan et al.[44] and Hess et al.[45] for Chinese and European populations, respectively. We used our in-house GWAS data of metabolites based on the UGCED program (n = 297) as the outcome dataset for the reverse MR analyses in northern Chinese and the published GWAS of plasma metabolites (n = 8,253)[42] for the reverse MR analyses in Europeans. The IVW method was employed using the "TwoSampleMR" package in R.

Interaction between genetically predicted plasma metabolites and primary prevention of GC

Cox proportional hazards regression models were used to determine the associations of *H. pylori* treatment, vitamin supplementation, and garlic supplementation with GC risk during the follow-up period (1995–2022) by different genetically predicted plasma metabolite levels for the SIT participants. P values for interaction between the genetically predicted plasma metabolite levels and assessed intervention were calculated for each analysis. Absolute risk reduction (ARR) in the GC incidence and number of participants needed to treat (NNT) to prevent 1 case of GC in > 27.1 years of follow-up were calculated for each intervention[46,47]. Unless otherwise specified, all analyses were performed using R (version 4.1.1).

Results

Plasma metabolites associated with GC in the observational study

A total of 400 subjects were included from the UGCED program for the observational part of the study. The mean age of the subjects was 57.1 years (*SD*: 7.8 years) with 260 men (65.0%) and 140 women (35.0%). The patients with GC (n = 78) were older at the time of enrollment and had a higher percentage of men than the controls (n = 322; **Table S2**). The case-control study identified 14 metabolites that were significantly associated with the odds of having GC (FDR–adjusted q < 0.05; **Table 1**). Ten metabolites, including alpha-linolenic acid, sn-2 LysoPC (20:3), linoleic acid, sn-2 LysoPC (18:3), uracil, palmitic acid, sn-2 LysoPC (20:2), arachidonic acid, sn-2 LysoPC (18:1), and *L*-glutamine, were inversely associated with GC. Four metabolites, including *L*-alanine, *L*-aspartic acid, *N*-acetylglutamine, and alpha-*N*-phenylacetyl-*L*-glutamine, were positively associated with GC.

SNPs associated with plasma metabolites in the northern Chinese population

GWASs on plasma metabolites were performed to identify metabolite-associated SNPs as IVs for subsequent forward MR

Table 1 Plasma metabolites associated with gastric cancer in the observational study (n = 400)[a]

Metabolites	OR (95% CI)	P	FDR
Alpha-Linolenic acid	0.48 (0.32–0.71)	2.30×10^{-4}	0.004
sn-2 LysoPC (20:3)	0.51 (0.35–0.75)	5.65×10^{-4}	0.004
Linoleic acid	0.53 (0.37–0.76)	6.08×10^{-4}	0.004
sn-2 LysoPC (18:3)	0.49 (0.33–0.74)	6.09×10^{-4}	0.004
Uracil	0.63 (0.48–0.82)	8.01×10^{-4}	0.004
L-Alanine	1.57 (1.20–2.05)	0.001	0.005
Palmitic acid	0.61 (0.44–0.86)	0.004	0.02
sn-2 LysoPC (20:2)	0.58 (0.39–0.85)	0.005	0.02
Arachidonic acid	0.62 (0.44–0.87)	0.005	0.02
sn-2 LysoPC (18:1)	0.63 (0.44–0.90)	0.01	0.03
L-Aspartic acid	1.41 (1.08–1.83)	0.01	0.03
N-Acetylglutamine	1.45 (1.09–1.93)	0.01	0.03
Alpha-*N*-Phenylacetyl-*L*-glutamine	1.40 (1.07–1.84)	0.01	0.03
L-Glutamine	0.74 (0.57–0.95)	0.02	0.04

[a]A total of 14 plasma metabolites associated with gastric cancer (FDR–adjusted q < 0.05) are shown here. Logistic regression analyses were conducted adjusting for age, gender, and *H. pylori* infection. Metabolites were ranked by the P values. CI, confidence interval; FDR, false discovery rate; OR, odds ratio.

analyses based on discovery (n = 297) and validation sets (n = 174). No significant differences in age and gender were found between the two sets (**Table S3**).

The genetic inflation factor for the 14 GC-related metabolites ranged from 0.99–1.08 in the discovery set (**Figure S1**). The Q-Q and Manhattan plots for the genome-wide associations are shown in **Figures S1 and S2**. A total of 966 independent SNPs were marginally associated with the 14 plasma metabolites ($P < 1 \times 10^{-4}$) but only 1 SNP, rs3132557, associated with sn-2 LysoPC (18:1) met genome-wide significance ($P = 2.25 \times 10^{-8}$). Forty-five independent loci (**Table S4**) were significantly associated (P < 0.05) and showed consistent association directions in both the discovery and validation sets for the 6 plasma metabolites with available GWAS data in the validation set. Based on these results, six metabolites with five or more validated related-SNPs in the two-stage validation process were selected as instruments for the respective plasma metabolites. SNPs identified in the discovery cohort ($P < 5 \times 10^{-5}$, F > 10) were used as instruments (**Table S5**) for the remaining eight metabolites. A total of 328 SNPs were included as instruments for plasma metabolites in the Chinese population for subsequent forward MR analyses (**Table S6**).

Forward MR analyses in northern Chinese population

The forward MR analyses leveraged prospective follow-up of two population-based cohorts, including the SIT and MITS, from high-risk areas in China. The mean age at enrollment was 46.9 years (*SD*: 9.1 years) for the 2,816 participants in SIT with 147 GC cases diagnosed during 33.2 years of follow-up (1989–2022). A nested case-control study was designed within the framework of the MITS, including 935 newly diagnosed GCs (mean age, 48.2 years; *SD*, 5.8 years) during follow-up (2011–2022) and 1,869 controls (mean age, 42.9 years; *SD*, 7.5 years; **Table S7**).

The forward MR analyses revealed that plasma *L*-aspartic acid levels might be a significant risk factor for the development of GC (**Figure 1**). Higher genetically predicted levels of *L*-aspartic acid were significantly associated with an increased risk of GC in the Chinese population (SIT: HR = 1.26, 95% CI: 1.07–1.49, P = 0.006; MITS: HR = 1.07, 95% CI: 1.00–1.14, P = 0.04). Genetically predicted plasma linoleic acid was positively associated with GC risk in the SIT only. Genetically predicted plasma alpha-*N*-phenylacetyl-*L*-glutamine and *L*-glutamine showed potential positive associations with the risk of GC based on the MITS only. No other plasma metabolites were significantly associated with GC risk in the forward MR analyses of the Chinese population (P > 0.05). Among 328 plasma metabolite-related IVs, no statistically significant associations with *H. pylori* infection were observed after multiple comparison corrections [$P > 1.52 \times 10^{-4}$ (0.05/328)] and 13 were significant at a P < 0.05. Sensitivity analyses, excluding these 13 *H. pylori* infection-related SNPs (**Table S8**) for 6 involved metabolites, yielded similar results with the primary analyses (**Table S9**) and ensured the robustness of our findings.

GAC risk, the most common pathologic type of GC, was determined. Both genetically predicted plasma *L*-aspartic acid (HR = 1.08, 95% CI: 1.00–1.16, P = 0.046) and alpha-*N*-phenylacetyl-*L*-glutamine (HR = 1.09, 95% CI: 1.01–1.17, P = 0.02) were positively associated with GAC risk in the MITS (**Table S10**).

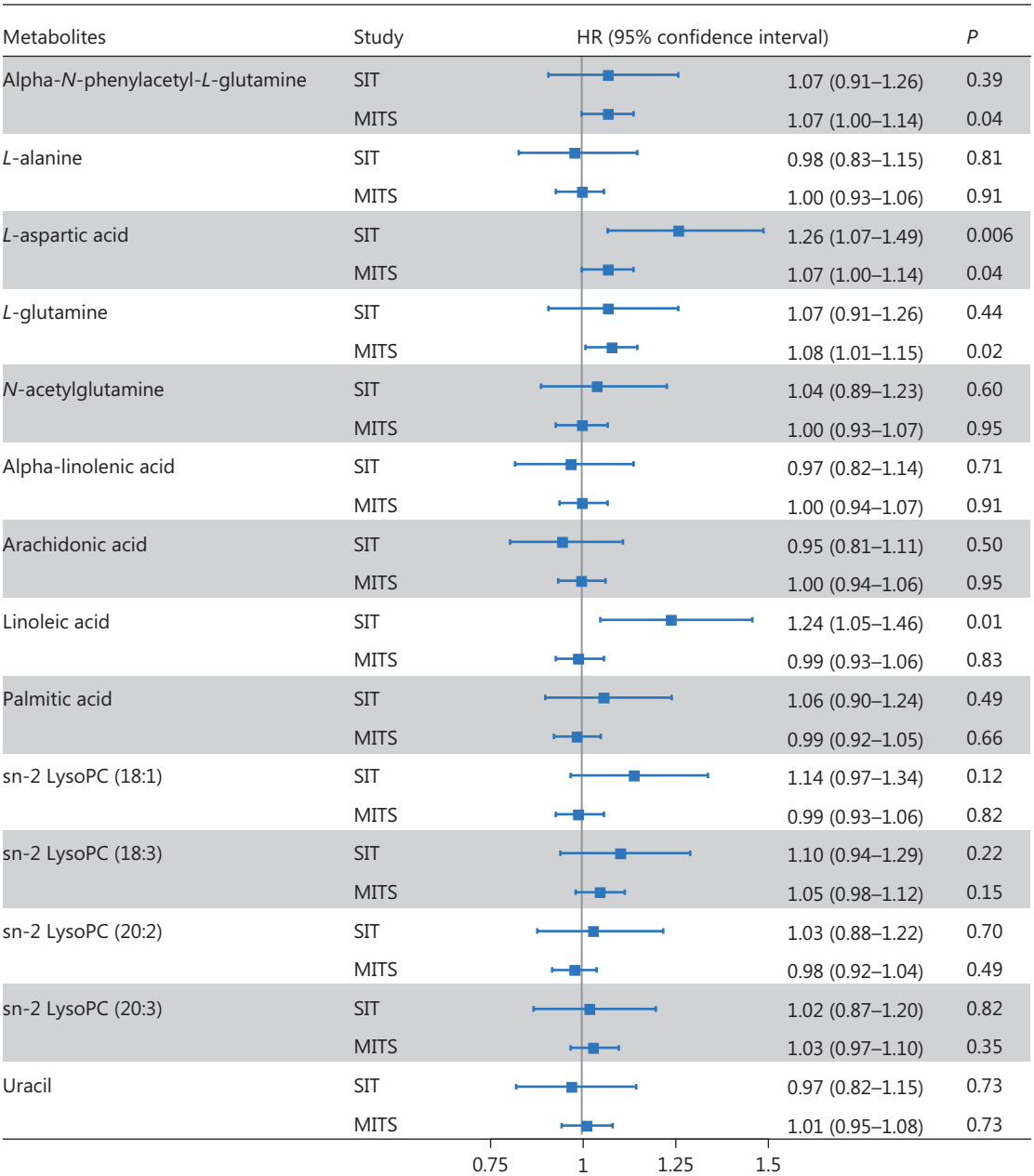


Figure 1 Association between genetically predicted plasma metabolites and risk of gastric cancer in northern Chinese population. Cox proportional hazards regression analyses were conducted adjusting for age, gender, baseline *Helicobacter pylori* infection, and three principal components in SIT (n = 2,816) and MITS (n = 2,804, including 935 gastric cancer cases and 1,869 controls). Metabolites are ranked by classes and names. HR, hazard ratio; MITS, Mass Intervention Trial in Linqu, Shandong province; SIT, Shandong Intervention Trial.

Reverse MR analyses in northern Chinese population

A literature review[44] identified 5 IVs of GC susceptibility in the Chinese population (**Table S11**) for reverse MR analyses. No significant associations between these IVs and the plasma metabolite levels were detected based on the UGCED program (all $P > 0.05$; **Figure 2**), indicating that the plasma metabolite levels were less likely to be causally altered by the occurrence of GC.

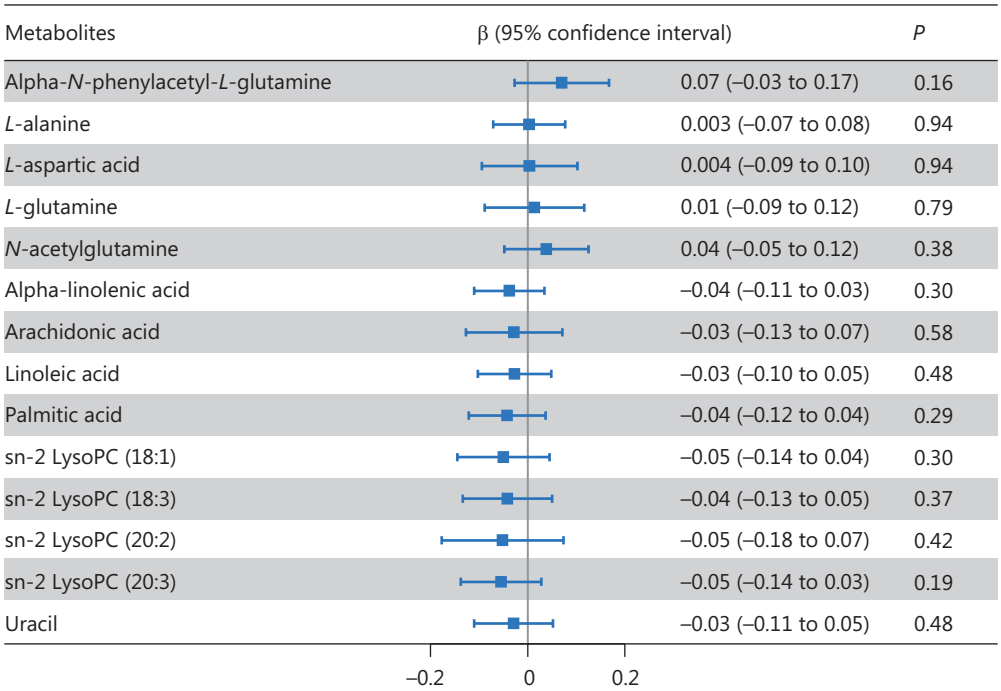


Figure 2 Reverse Mendelian randomization analyses between plasma metabolites and risk of gastric cancer in northern Chinese population. Inverse variance-weighted analysis was conducted based on the UGCED program (n = 297). Metabolites are ranked by class and metabolite name.

MR analyses of plasma *L*-aspartic acid and GC in the European population

Given the significant association of genetically predicted plasma *L*-aspartic acid and GC risk in two northern Chinese cohorts, the association in the European population was further validated using 13 independent loci as IVs (**Table S12**). The mean age was 61.5 years (*SD*: 6.4 years) for the 641 GC cases in UKB and 55.9 years (*SD*: 8.0 years) for the 251,075 controls (**Table S7**). Information on demographic characteristics is not available for the FinnGen project because only the GWAS summary statistics was utilized. The OR for the association between *L*-aspartic acid and GC was 1.01 (95% CI: 0.93–1.09, P = 0.85; **Table 2**) in the UKB. The sensitivity analysis yielded similar results with the OR values ranging between 1.01 and 1.02 across different resampling parameters with the SMOTE (**Figure S3**), which was consistent with the primary analysis. A potential causal link between *L*-aspartic acid and increased risk of GAC was suggested based on the FinnGen project (OR = 1.68, 95% CI: 1.16–2.45, P = 0.006; **Table 2**). The MR-Egger regression based on FinnGen summary statistics did not indicate violations of the independence or exclusion restriction assumptions for this association analysis (P-intercept = 0.17). Sensitivity analyses also confirmed the robustness of the results in FinnGen, showing no influence of pleiotropy (**Figure S4**).

Table 2 Association between genetically predicted plasma *L*-aspartic acid and risk of gastric cancer in the European population[a]

Study	Gastric cancer			Gastric adenocarcinoma		
	OR (95% CI)		*P*	OR (95% CI)		*P*
UK Biobank	1.01	(0.93–1.09)	0.85	0.99	(0.90–1.09)	0.81
FinnGen	1.23	(0.94–1.62)	0.14	1.68	(1.16–2.45)	0.006

[a]Unconditional logistic regression analyses were conducted adjusting for age, gender, and 3 principal components in UK Biobank (gastric cancers: 641, controls: 251,075). Inverse variance-weighted analysis was conducted in FinnGen (gastric cancers: 1,423, controls: 314,193). CI, confidence interval; OR, odds ratio.

IVs for GC susceptibility were selected based on literature review for reverse MR analysis in the European population[45] (**Table S11**). No significant association was demonstrated between genetic susceptibility to GC and *L*-aspartic acid levels (β = 0.003, 95% CI: –0.09–0.10, P = 0.95).

Effect of *H. pylori* eradication, garlic supplementation, and vitamin supplementation on GC prevention by genetically predicted plasma *L*-aspartic acid

A total of 139 GCs were documented during the follow-up period of 27.1 years (1995–2022; **Table S13**) based on 2,604 participants in the SIT (1,853 *H. pylori* positive and 751 *H. pylori* negative individuals). Participants were categorized as high (top quartile), moderate, and low (bottom quartile) levels of genetically predicted plasma *L*-aspartic acid. The beneficial effect of *H. pylori* eradication on reducing GC risk was observed for the high *L*-aspartic acid level group (HR = 0.44, 95% CI: 0.22–0.88, *P* = 0.02, ARR = 6.44%, NNT = 15.54), but not for the groups with low or moderate levels (*P*-interaction = 0.098). In contrast, individuals with the low *L*-aspartic acid level showed the greatest benefit from garlic supplementation (HR = 0.31, 95% CI: 0.12–0.78, *P* = 0.01, ARR = 4.38%, NNT = 22.84), while no such effect existed for individuals with high or moderate levels (*P*-interaction = 0.02). No interaction was demonstrated for the effect of vitamin supplementation (*P*-interaction = 0.91; **Figure 3**).

Discussion

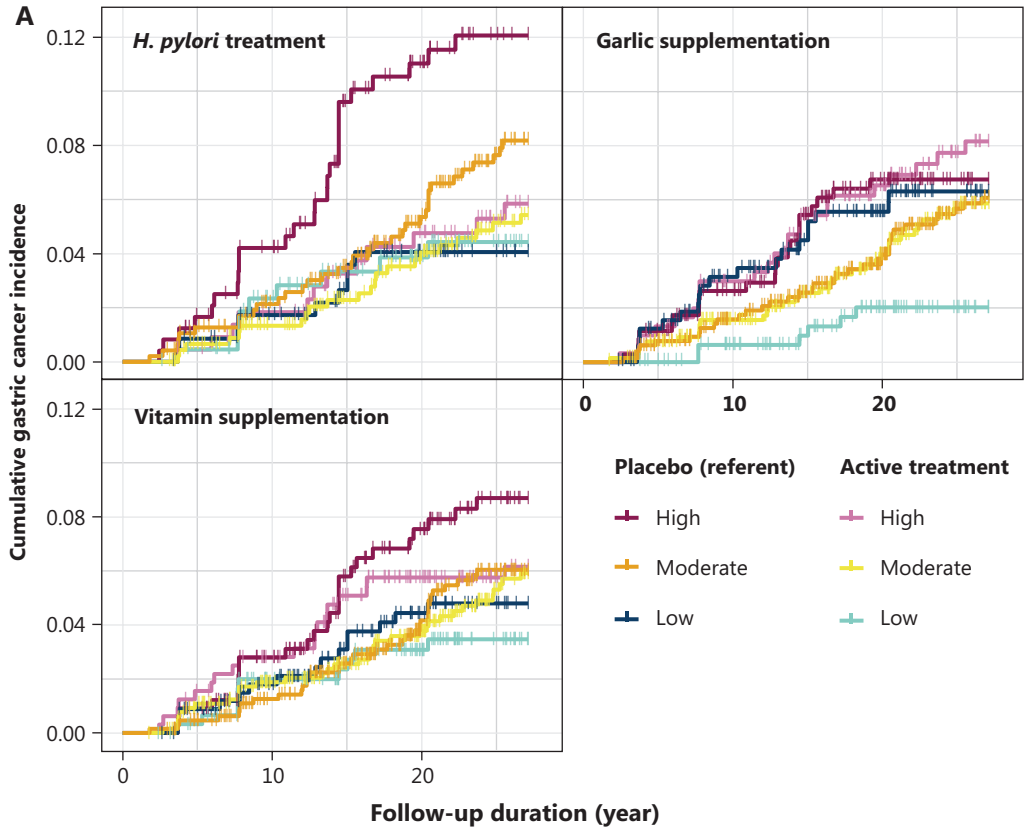
The relationships between plasma metabolites and GC were systematically examined based on multistage metabolomic profiling and MR analyses. Among plasma metabolites associated with GC risk in the observational study, individuals with higher genetically predicted plasma *L*-aspartic acid levels had a significantly increased GC risk in two northern Chinese cohorts and an increased GAC risk in the FinnGen project, indicating a possible role of elevated plasma *L*-aspartic acid in GC development. In addition, genetically predicted plasma *L*-aspartic acid levels also modified the effects of GC primary prevention. These findings underscore the potential public health significance of utilizing genetically influenced metabotypes for risk stratification and primary prevention of GC.

Metabolomics has emerged as a pivotal tool in discovering molecular signatures underlying carcinogenesis. Nevertheless, inconsistencies and concerns on robustness may arise in observational metabolomic studies due to variations in sample types, origins, and analytical platforms. Most studies involving the metabolite-GC nexus have yet to delve into a causal relationship, leaving a crucial gap in the identification of metabolite biomarkers for risk assessment. A bi-directional two-sample MR analysis was subsequently performed based on the observational findings, which helped mitigate the possibility of reverse causality that is often inherent in observational studies.

In the current study the plasma *L*-aspartic acid levels were significantly associated with GC risk and a potential causal relationship was further supported by forward MR analyses, especially in two northern Chinese cohorts. In addition, genetically predicted *L*-aspartic acid was significantly associated with GAC risk in the FinnGen, a well-known European cohort. *L*-aspartic acid is a non-essential amino acid interwoven with protein synthesis, the urea cycle, and the malate-aspartate shuttle[48]. A prior MR study has reported that elevated serum aspartate might be a risk factor for prostate and breast cancers[49]. Pivoting to cancer biology, prior research has underscored the crucial role of aspartate, the ionic form of aspartic acid, and glutamine in propelling the tricarboxylic acid cycle[50]. Aspartate also stands as a limiting metabolite for tumor proliferation[51] with its scarcity under hypoxic conditions posing a restriction on tumor growth[52,53]. Given the hypoxic niche of tumor cells, targeting aspartate metabolism emerges as a promising therapeutic avenue[54]. Prior small-scale molecular epidemiological studies have yielded inconsistent findings on the association between GC and circulating *L*-aspartic acid or aspartate[15]. The results herein highlight the potential of elevated *L*-aspartic acid as a key biomarker for GC progression. Notably, reverse MR analysis did not suggest a causal link between GC susceptibility and *L*-aspartic acid levels. Evidence was provided herein that testing plasma *L*-aspartic acid concentrations could serve as a tool for GC risk assessment and stratification.

Treatment of *H. pylori* infection prevents GC development. The SIT and MITS trials that our team conducted offer compelling evidence for the effectiveness of *H. pylori* eradication[7,8,29]. The SIT also supports the beneficial effect of vitamin and garlic supplementation on GC[7]. Even so, given the multifaceted etiology of GC, along with the global threat of antibiotic resistance and numerous factors affecting treatment success, a 'one-size-fits-all' prevention approach may not be biologically viable or practically efficient[55]. Therefore, it is crucial to concentrate prevention efforts on the specific populations that are most at risk. Indeed, our recent study showed that *H. pylori* treatment particularly benefited patients with a high polygenic risk score for GC[30]. An interaction between nutrition supplementation and lifestyle factors has also been reported[56]. The current research revealed that *H. pylori* eradication significantly reduces GC risk in individuals with high genetically

A



B

Categories	HR (95% CI)	ARR (%)	NNT	*P* for interaction
***H. pylori* treatment**				
Low	1.13 (0.45–2.87)	NA	NA	0.098
Moderate	0.65 (0.38–1.12)	2.75	36.43	
High	0.44 (0.22–0.88)	6.44	15.54	
Garlic supplementation				
Low	0.31 (0.12–0.78)	4.38	22.84	0.02
Moderate	0.92 (0.57–1.48)	0.18	551.87	
High	1.16 (0.64–2.10)	NA	NA	
Vitamin supplementation				
Low	0.67 (0.30–1.50)	1.28	78.17	0.91
Moderate	0.98 (0.61–1.57)	0.15	660.86	
High	0.80 (0.44–1.46)	2.29	43.69	

Figure 3 Effect of *H. pylori* treatment, garlic supplementation, and vitamin supplementation on the risk of gastric cancer by genetically predicted *L*-aspartic acid levels in the Shandong Intervention Trial. Participants were categorized as high (top quartile), moderate, and low (bottom quartile) levels of genetically predicted plasma *L*-aspartic acid. (**A**) Cumulative gastric cancer incidence for individuals receiving active treatment or placebo according to genetically predicted *L*-aspartic acid levels. (**B**) Association between *H. pylori* treatment and nutrition supplementation and the risk of incident gastric cancer by different genetically predicted *L*-aspartic acid levels. Cox proportional hazards regression analyses were conducted adjusting for age, gender, baseline *H. pylori* infection, and other interventions in the Shandong Intervention Trial (n = 2,604). ARR and NNT were calculated for the entire follow-up period (27.1 years). ARR, absolute risk reduction; CI, confidence interval; *H. pylori*, *Helicobacter pylori*; HR, hazard ratio; NA, not applicable; NNT, number needed to treat.

predicted *L*-aspartic acid levels. We also report a modification of genetically predicted *L*-aspartic acid levels on the beneficial effect of garlic supplementation. Thus, by integrating host characteristics with genetic susceptibility, a novel pathway may be paved for a tailored approach to GC prevention, leading to a more targeted and effective prevention strategy.

The strength of the current study lies in its integration of long-term prospective cohorts, public data resources, and a multi-tiered study design, providing evidence for GC risk assessment and tailored prevention, while accounting for population heterogeneity through MR studies in different populations. The current study had limitations. First, the GWAS of plasma metabolites based on the UGCED program had a relatively modest sample size and the validation set only covered part of the selected metabolites. While a larger sample size could more effectively detect rare quantitative trait loci, rigorous methods for IV selection and MR analyses were followed[57]. Second, the association analyses and MR study for the Chinese population were based solely on data from the high-risk region of GC in northern China. For the reverse MR analyses, IVs of GC susceptibility for Chinese population were utilized[44] but the analysis was then conducted based on the UGCED program alone. The sample representativeness and the generalizability of our findings to populations with a low GC risk and in southern China remain to be confirmed in future research. Third, although potential interactions were reported between genetically predicted *L*-aspartic acid levels and *H. pylori* eradication and garlic supplementation, the results need to be interpreted with caution. The findings were derived based on only one trial and the *P*-interaction was 0.098 for *H. pylori* eradication. Fourth, the associations between plasma metabolites and clinical features, such as stage, survival, or disease-free survival, were not examined due to the lack of detailed information on these clinical characteristics and a modest sample size of GC cases in the UGCED program. Finally, while MR studies can provide evidence on potential causal links between plasma metabolites and GC, MR studies do not elucidate the mechanisms through which these metabolites influence cancer risk. Further research is needed to explore the biological pathways and functional roles of these metabolites in GC development.

Conclusions

In conclusion, individuals with elevated plasma *L*-aspartic acid levels harbored an increased risk of developing GC, which may potentially serve as a molecular signature for risk stratification of GC. Genetically predicted plasma *L*-aspartic acid levels modified the effect of *H. pylori* eradication and garlic supplementation on GC prevention based on a large intervention trial in a high-risk area of China. Further studies are also warranted to evaluate the potential of utilizing genetically influenced metabotypes as biomarkers and to explore the biological mechanisms.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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Performed the analysis: Mengyuan Wang, Zhouyi Yin.
Wrote the paper: Mengyuan Wang, Zhouyi Yin, Wenqing Li.

Data availability statement

The data generated in this study are available upon request from the corresponding author.

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