Environmental factors, seven GWAS-identified susceptibility loci, and risk of gastric cancer and its precursors in a Chinese population

Meng Cai1,2, Shuyang Dai1,2, Wanqing Chen2, Changfa Xia2, Lingeng Lu3, Shuguang Dai4, Jun Qi5, Minjie Wang6, Meilin Wang6,7, Lanping Zhou1, Fuhua Lei8, Tingting Zuo2, Hongmei Zeng2 & Xiaohang Zhao1

1State Key Laboratory of Molecular Oncology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
2National Office for Cancer Prevention and Control, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
3Department of Chronic Disease Epidemiology, Yale School of Public Health, School of Medicine, Yale Cancer Center, Yale University, New Haven, Connecticut, USA
4Center for Disease Control and Prevention of Sheyang County, Sheyang, Jiangsu, China
5Department of Clinical Laboratory, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
6Department of Environmental Genomics, Jiangsu Key Laboratory of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Cancer Personalized Medicine, Nanjing Medical University, Nanjing, Jiangsu, China
7Department of Genetic Toxicology, the Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, Jiangsu, China
8Department of Pathology, Feicheng People Hospital, Feicheng, Shandong, China

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Abstract
Gene–environment interactions may increase gastric cancer (GC) risk. Seven susceptibility loci identified by genome-wide association studies (GWASs) suggest that genetic factors play a role in gastric carcinogenesis. Meanwhile, Helicobacter pylori (H. pylori) infection, smoking, and alcohol drinking are also important environmental factors for gastric cancer. However, studies to explore the role of gene–environment interactions in gastric carcinogenesis, and particularly the relationship between the seven susceptibility loci and their potential interactions with H. pylori infection, smoking, and alcohol drinking in risk of GC, and severe intestinal metaplasia (IM)/dysplasia, have been inconclusive. A total of 1273 subjects in a Chinese population were recruited, and genotyping was carried out using the competitive allele-specific PCR (KASP) method. Unconditional logistic regression was applied to model the associations between genetic polymorphisms and the disease risk. Effect modifications by H. pylori infection, smoking and alcohol drinking were evaluated. PSMA rs2294008/rs2976392 showed a significant, multiplicative interaction with H. pylori infection in risk of GC. Meanwhile, PRKAA1 rs13361707 had an additive interaction with H. pylori infection. SLC52A3 rs13042395 showed an interaction with alcohol drinking in risk of GC. Moreover, three SNPs, MUC1 rs4072037, ZBTB20 rs9841504 and PRKAA1 rs13361707, were associated with precancerous gastric lesions (severe IM/dysplasia). Our data suggest that genetic predisposition factors identified by GWAS may interact with environmental risk factors, Particularly for H. pylori infection and alcohol consumption, to increase the risk of GC.
Introduction

Gastric cancer (GC) is one of the most common cancers worldwide and more than 40% of GC cases occur in China. In 2015, approximately 500,000 patients in China died of GC [1]. GC can be divided into intestinal and diffuse types according to its histopathological features. The carcinogenesis of GC is a multifactorial process, resulting from the combined consequences of genetic predisposition and environmental risk factors including *Helicobacter pylori* (*H. pylori*) infection, smoking, alcohol drinking, and dietary factors [2].

Among the environmental risk factors for GC, it is widely recognized that *H. pylori* is a major etiological factor [3]. *Helicobacter pylori*, a Gram-negative bacterium, presents higher prevalence in developing countries than in developed countries [4]. Nevertheless, only a small proportion of *H. pylori*-infected individuals progress to GC [5], suggesting that environmental factors other than *H. pylori* infection, such as smoking and alcohol drinking, and genetic predisposition may also influence the outcome of gastric pathogenesis [6]. Several studies have found that *H. pylori* cytotoxin-associated gene A (CagA)*H. pylori* and/or vacuolating cytotoxin gene A (VacA)*H. pylori* strains can increase the risk of developing gastric diseases. In particular, Type I strains of *H. pylori* with CagA*H. pylori* and/or VacA*H. pylori* have higher pathogenicity than Type II strains of *H. pylori* without CagA*H. pylori* and VacA*H. pylori* [7]. Some in vitro studies have demonstrated that several genes within the *H. pylori* cag islands are involved in the release of proinflammatory cytokines [8, 9]. VacA is a secreted protein that stimulates epithelial cell apoptosis and induces vacuole formation in eukaryotic cells [10].

Four genome-wide association studies (GWASs) have identified seven GC genetic variants, including MUC1 rs4072037 at 1q22, ZBTB20 rs9841504 at 3q13, PRKAA1 rs13361707 at 5p13, PSCA rs2976392 and rs2294008 at 8q24, PLCE1 rs2274223 at 10q23, and SLCl23A rs13042395 at 20p13 [11–15]. These studies provide important clues to the etiology of the disease. Subjects carrying a certain genetic variant may be at greater risk for GC if a related environmental factor exists. Such gene–environment interactions have been identified in several cancers, including cancers of the bladder, esophagus and stomach [16–18]. Our previous study found that TLR5 gene polymorphism might be modified by *H. pylori* infection [19]. Li et al. [20] revealed that MUC1 rs4072037, PRKAA1 rs13361707, and PLCE1 rs2274223 may interact with *H. pylori* infection and increase the risk of GC. Severe intestinal metaplasia (IM) and dysplasia are precancerous lesions of GC. The risk of GC generally increases with the histological grade (from low to high grade) of IM and dysplasia [21]. Many polymorphisms associated with GC may also be related to gastric precancerous lesions [22, 23].

In view of the role of genetic effects and gene–environment interactions in gastric carcinogenesis, we were particularly interested in the seven GWAS-identified genetic variants and their potential roles in GC and severe IM/dysplasia. We explored the seven susceptibility loci and their potential interactions with *H. pylori* infection, smoking and alcohol drinking in risk of GC and its precursors in a total of 1273 subjects in a Chinese population.

Materials and Methods

Study subjects

In 2014, we launched an endoscopic screening program for GC in a cohort from Sheyang County, Jiangsu Province, China. Overall, 2189 subjects were recruited for endoscopic screening of GC. All screened subjects received an endoscopic survey and provided blood samples for DNA isolation and *H. pylori* serological detection. Biopsy samples were taken from the standard locations of the stomach in each subject, and the histopathological diagnosis of each sample was made by senior pathologists. Each biopsy was classified into either normal, nonatrophic gastritis, atrophic gastritis, mild/severe IM, low/high grade dysplasia, or GC based on the Chinese System and the criteria for the Updated Sydney System [24, 25]. The TNM stages were evaluated according to the American Joint Committee on Cancer Staging Manual, Sixth Edition. Demographic and epidemiologic information, including age, sex, smoking, alcohol drinking and family history of cancer, was recorded on a questionnaire through face-to-face interviews by a trained doctor.

The present study included 501 GC cases from the baseline gastroscopic screening program in Sheyang County of China and from the Cancer Hospital of Chinese Academy of Medical Sciences diagnosed during the same period. Additionally, 489 controls with either normal or nonatrophic gastritis were randomly selected from the cohort and frequency-matched to the GC cases by gender and age. Moreover, 283 cases with severe IM (31) or dysplasia (252) identified in the gastroscopic screening cohort were included. Ethics approval was obtained from the Institutional Review Board of the Cancer Hospital of the Chinese Academy of Medical Sciences, and all participants provided written informed consent.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood using a whole-blood DNA kit (Biomed Corporation, China) following the manufacturer’s instructions. Isolated genomic DNA was analyzed by 0.8% agarose gel electrophoresis.
to evaluate DNA quality, and DNA quantity was assessed using a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The DNA samples were stored at −20°C until use. Genotyping of the seven candidate SNPs was performed by competitive allele-specific PCR (KASP) assay (Compass Biotechnology, Beijing, China). Detailed information on the genotyping method has been described previously [26, 27]. The sequences of the primers used in this study are available upon request. All the primers were synthesized by LGC Genomics (Herts, UK). The call rate for each SNP was >95%. Three percent of the samples were randomly selected for validation via DNA sequencing, and the concordance was 100%.

**Helicobacter pylori serology**

Helicobacter pylori infection status was determined using an immunoblotting assay according to the manufacturer’s instructions (Shenzhen Blot Biotech Co., Ltd., Shenzhen, China). The sensitivity and specificity of the assay were 96.8% and 97.6%, which were higher than another similar immunoblotting kit Helicoblot 2.1 (with 95.6% and 92.6% of the sensitivity and specificity) [28–30]. The assay allows the identification of specific antibody responses against the distinct *H. pylori* antigens CagA, VacA, UreA and UreB. Briefly, the lysate from *H. pylori* was separated by SDS-PAGE and then electrotransferred onto polyvinylidene fluoride membranes and incubated with 1:100 diluted serum for 30 min. After washing, the membrane strips were incubated with horse radish peroxidase-conjugated anti-human immune globulin antibodies (IgG, dilution 1:50) for 30 min at room temperature. After washing three times, the bound antibodies were visualized through a staining reaction, and the corresponding antigens formed dark bands on the strip. If such a band formed, the subject was considered seropositive for the specific *H. pylori* antigen. *Helicobacter pylori* infection status was defined as seropositive if any of the four antigens tested, CagA, VacA, UreA and UreB, was positive. Subjects positive for CagA and/or VacA were classified as Type I *H. pylori* seropositive, and those positive for only UreA and/or UreB were classified as Type II *H. pylori* seropositive.

**Statistical analysis**

The chi-square test was used to compare the basic characteristics and the genotypes between cases and controls. Goodness-of-fit chi-square test was used to test the Hardy–Weinberg equilibrium in the controls. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated for the association of genetic variants with risk of GC and severe IM/dysplasia, as well as for gene–gene or gene–environmental factor joint effect analysis using unconditional logistic regression models. All analyses were adjusted for potential confounders including age, sex, *H. pylori* infection, smoking and alcohol drinking. For gene–gene or gene–environment (*H. pylori, smoking and alcohol drinking*) joint effect analysis, different gene–gene or gene–environment combination categories were transformed into dummy variables, with the “except” category used as reference; all the other categories were entered into the unconditional logistic regression model to calculate the adjusted ORs and 95% CIs for each category compared with the reference. Multiplicative gene–gene or gene–environment interactions were measured by including main effect variables and their product terms in the logistic regression model with likelihood ratio tests.

For the additive interaction analysis, we used a bootstrapping test of goodness of fit of the null hypothesis of no departure from an additive model versus an alternative hypothesis of a departure from an additive model using Stata (version 12.0; StataCorp LP, College Station, TX). A synergy index (SI) and 95% CI were reported. Linkage disequilibrium (LD) coefficients were calculated with Haplovie 4.0 software. All P values reported were unadjusted, two-sided, and considered statistically significant at $P \leq 0.05$. The Benjamini–Hochberg procedure was performed for multiple test comparison correction, and the false discovery rate (FDR)-adjusted $P$ value was considered as well [31]. All the statistical analyses were performed using SPSS (version 16.0).

**Results**

**Characteristics of study subjects**

A total of 1273 subjects, including 870 males (68.3%) and 403 females (31.7%) were included in our study, and the mean age of the participants was 58.0 years. The baseline characteristics of the study population are shown in Table 1. Of 501 GC cases, 384 (83.8%) occurred at a noncardia site and 74 (16.2%) were at a cardia site. There were no significant differences in the distributions of age and sex between GC cases and controls. However, the percentages of smoking, alcohol drinking, and *H. pylori* infection were significantly higher in the GC cases than in the controls ($P = 0.037$ for smoking status, $P < 0.001$ for alcohol drinking and $P < 0.001$ for *H. pylori* seropositivity). For IM/dysplasia and controls, the distribution of age, smoking and alcohol drinking was similar between groups. However, the percentage of female and *H. pylori* seropositivity were significantly higher in the IM/dysplasia cases than in the controls ($P < 0.001$ for sex and $P < 0.001$ for *H. pylori* infection).

For type of *H. pylori* infection, there was a higher degree of *H. pylori* CagA positivity and VacA positivity in GC
patients than in controls (65.7% vs. 32.4% and 46.5% vs. 28.1%). Subjects with Type I *H. pylori* infection had a significantly higher risk of GC compared with those without *H. pylori* infection (OR: 7.12, 95% CI: 5.04–10.04). For subjects with Type II *H. pylori* infection, they had a two-fold increased risk of GC compared to those without *H. pylori* infection.

**Main effects of the seven SNPs with risk of GC**

The genotype distribution of the seven SNPs in all subjects is shown in Table 2. The *PSCA* rs2294008 was in 100% LD with rs2976392 ($r^2 = 1.00$). Under the dominant models, we found that individuals with at least one variant allele of the six polymorphisms had an altered risk of GC (OR: 1.48, 95% CI: 1.09–1.99 for *PLCE1* rs2274223; OR: 1.41, 95% CI: 1.05–1.89 for *PSCA* rs2294008; OR: 1.56, 95% CI: 1.05–2.30 for *PSCA* rs2976392; OR: 0.59, 95% CI: 0.42–0.81 for *MUC1* rs4072037; OR: 0.70, 95% CI: 0.52–0.93 for *SLC52A3* rs13042395; and OR: 0.43, 95% CI: 0.31–0.61 for *PRKAA1* rs13361707). For *ZBTB20* rs9841504, although subjects carrying the CG genotype had a decreased risk of GC (OR: 0.65, 95% CI: 0.45–0.92), we did not observe a significant association between *ZBTB20* rs9841504 and risk of GC under the dominant model.

**Table 1. Basic characteristics of the study subjects.**

|                     | GC cases | Controls | Severe IM/dysplasia |
|---------------------|----------|----------|---------------------|
| n                   | 501      | 489      | 283                 |
| Age, n (%)          |          |          |                     |
| <58                 | 246 (49.1)| 233 (47.6)| 133 (47.0)          |
| ≥58                 | 255 (50.9)| 256 (52.4)| 150 (53.0)          |
| p                  | 0.647    |          | 0.861               |
| Sex, n (%)          |          |          |                     |
| Male                | 365 (72.9)| 352 (72.0)| 153 (54.1)          |
| Female              | 136 (27.1)| 137 (28.0)| 130 (45.9)          |
| p                  | 0.759    |          | <0.001              |
| Smoking, n (%)      |          |          |                     |
| Yes                 | 199 (46.9)| 196 (40.1)| 112 (39.6)          |
| No                  | 225 (53.1)| 293 (59.9)| 171 (60.4)          |
| p                  | 0.037    |          | 0.890               |
| Alcohol drinking, n (%) |     |          |                     |
| Yes                 | 186 (44.3)| 119 (24.3)| 65 (23.0)           |
| No                  | 234 (55.7)| 370 (75.7)| 217 (77.0)          |
| p                  | <0.001   |          | 0.687               |
| *H. pylori* infection, n (%) |     |          |                     |
| Positive            | 431 (88.0)| 280 (57.4)| 217 (78.3)          |
| Negative            | 59 (12.0) | 208 (42.6)| 60 (21.7)           |
| p                  | <0.001   |          | <0.001              |
| Type of *H. pylori* infection, n (%) |     |          |                     |
| CagA+               | 322 (65.7)| 158 (32.4)| 115 (41.5)          |
| CagA−               | 168 (34.3)| 330 (67.6)| 162 (58.5)          |
| p                  | <0.001   |          | 0.011               |
| Type of *H. pylori* infection, n (%) |     |          |                     |
| VacA+               | 228 (46.5)| 137 (28.1)| 85 (30.7)           |
| VacA−               | 262 (53.5)| 351 (71.9)| 192 (69.3)          |
| p                  | <0.001   |          | 0.444               |
| Type of *H. pylori* infection, n (%) |     |          |                     |
| Type I              | 329 (67.1)| 163 (33.4)| 133 (48.0)          |
| Type II             | 102 (20.8)| 117 (24.0)| 84 (30.3)           |
| Not infected        | 59 (12.1) | 208 (42.6)| 60 (21.7)           |
| p                  | <0.001   |          | <0.001              |
| Cancer site, n (%)  |          |          |                     |
| Cardia              | 74 (16.2)|          |                     |
| Noncardia           | 384 (83.8)|          |                     |
| Stage, n (%)        |          |          |                     |
| Stage I             | 98 (25.8)|          |                     |
| Stage II           | 282 (74.2)|          |                     |

1P values for chi-square test.
Given that six polymorphisms showed significant associations with GC risk, we further investigated their joint effects on risk of GC. Because PSCA rs2294008 was in 100% LD with PSCA rs2976392, we excluded PSCA rs2976392 in the following analysis. Compared with subjects carrying 0–1 risk genotypes, individuals carrying 2–3 risk genotypes had an increased risk for GC (OR: 2.13, 95% CI: 1.55–2.93), and those carrying 4–5 risk genotypes had a 4.76-fold increased risk (OR: 5.76, 95% CI: 3.61–9.18).

The risks of GC related to the polymorphisms were further examined in relation to subtype of GC. We found that the associations of SNPs between controls and non-cardia GC cases were similar to those between controls and all GC cases (Table S1). Because there were relatively few cases of cardia GC (74) in our study, the results linking SNPs and risk of cardia GC should be interpreted with caution (Table S2).

Associations between genetic variants and risk of GC by status of H. pylori infection

The main effects of the genetic polymorphisms stratified by type of H. pylori infection are shown in Table 3. For PLCE1 rs2274223 and PSCA rs2294008, the effects were mainly present in subjects with Type I H. pylori infection. Their adjusted ORs were 1.59 (95% CI: 1.04–2.43) and 1.93 (95% CI: 1.28–2.90), respectively. For MUC1 rs4072037, similar significant associations between PRKAA1 rs13361707 and decreased risk of GC were found in subjects with both Types I and II infection. The ORs of MUC1 rs4072037 in subjects with Types I and II H. pylori infection were 0.64 (95% CI: 0.42–0.99) and 0.47 (95% CI: 0.23–0.96), respectively. For PRKAA1 rs13361707, we found significant associations between PRKAA1 rs13361707 and decreased risk of GC in subjects with Types I and II infection, and the ORs were 0.46 (95% CI: 0.29–0.73) and 0.39 (95% CI: 0.20–0.78), respectively. Analysis of ZBTB20 rs9841504 and SLC52A3 rs13042395 separately did not reveal any effect in subgroups.

Effect modification by H. pylori infection status

We investigated the potential interactions between genetic variants and H. pylori infection in the risk of GC (Table 4). Significant multiplicative and additive interactions between PSCA rs2294008 and H. pylori infection on risk of GC were found. The OR due to multiplicative interaction was 3.05 (95% CI: 1.43–6.53) and the SI on an additive interaction scale was 2.45 (95% CI: 1.55–5.52). In contrast, we observed only an additive interaction between PRKAA1 rs13361707 and H. pylori infection on risk of GC (SI on an additive interaction scale: 2.21, 95% CI: 1.39–3.69).

Associations between the genetic variants and risk of GC by age, gender, smoking, and alcohol drinking status

The main effects of the genetic polymorphisms stratified by age, gender, smoking and alcohol drinking status are shown in Tables S3–S6. PSCA rs2294008, PSCA rs2976392, MUC1 rs4072037, and SLC52A3 rs13042395 were associated with GC risk in subjects who were at young age, male, or smoking. In contrast, for PRKAA1 rs13361707, significant associations were shown in the subjects irrespective of the age groups, sex, smoking, or drinking status.

Effect modification by smoking and alcohol drinking status

We did not find any significant interactions between the genetic variants and smoking on risk of GC either in a multiplicative model or on an additive scale (data not shown). However, in the gene–alcohol drinking interaction analysis, there was a significant additive interaction between alcohol drinking and SLC52A3 rs13042395 in risk of GC, and the SI was 2.13 (95% CI: 1.04–5.73) (Table 5).

Association of the seven SNPs with risk of severe IM/dysplasia

We further performed associations between seven genetic variants and risk of severe IM/dysplasia (Table 6). The MUC1 rs4072037 AG/GG genotype was associated with a decreased risk of severe IM/dysplasia, with an OR of 0.65 (95% CI: 0.46–0.92). We found that the ZBTB20 rs9841504, CG/GG genotype was associated with a protective effect against risk of severe IM/dysplasia, with an adjusted OR of 0.69 (95% CI: 0.48–0.99). In addition, subjects with a PRKAA1 rs13361707 CT/TT genotype had a decreased risk of severe IM/dysplasia, with an adjusted OR of 0.68 (95% CI: 0.47–0.98).

Discussion

In this study, we covered all seven GWAS-identified GC susceptibility loci and explored their potential interactions with environmental factors (H. pylori infection, smoking and alcohol drinking) on risk of GC. We further examined their potential effects on risk of precancerous lesions of the stomach. We confirmed that PLCE1 rs2274223, PSCA rs2294008, PSCA rs2976392, MUC1 rs4072037, SLC52A3 rs13042395 and PRKAA1 rs13361707 were associated with GC risk in a Chinese population. The risk of GC may be modulated by PSCA rs2294008 and PRKAA1 rs13361707, possibly in combination with H. pylori infection. Meanwhile, SLC52A3 rs13042395 had a potential
Table 2. Distribution of genotype frequencies of the susceptibility loci and their associations with GC risk.

| Locus   | Gene    | SNP   | Genetic model | Controls n (%) | GC cases n (%) | OR (95% CI)\(^1\) | P   |
|---------|---------|-------|---------------|---------------|---------------|-----------------|-----|
| **PLCE1 rs2274223** |        |       |               |               |               |                 |     |
| AA      | Codominant | 317 (65.0) | 291 (59.3) | 1             |               |                 |     |
| AG      |            | 153 (31.3) | 174 (35.4) | 1.40 (1.03–1.91) | 0.033 |               |     |
| GG      |            | 18 (3.7) | 26 (5.3) | 2.18 (1.07–4.45) | 0.032 |               |     |
| AA      | Dominant   | 317 (65.0) | 291 (59.3) | 1             |               |                 |     |
| AG+GG   |            | 171 (35.0) | 200 (40.7) | 1.48 (1.09–1.99) | 0.011 |               |     |
| G allele| Additive   | 266 (54.9) | 215 (44.3) | 1             |               |                 |     |
| **PSCA rs2294008** |        |       |               |               |               |                 |     |
| CC      | Codominant | 268 (54.9) | 215 (44.3) | 1             |               |                 |     |
| CT      |            | 173 (35.5) | 225 (46.4) | 1.50 (1.10–2.04) | 0.01 |               |     |
| TT      |            | 47 (9.6) | 45 (9.3) | 1.10 (0.66–1.83) | 0.725 |               |     |
| CC      | Dominant   | 268 (54.9) | 215 (44.3) | 1             |               |                 |     |
| CT+TT   |            | 220 (45.1) | 270 (55.7) | 1.41 (1.05–1.89) | 0.021 |               |     |
| T allele| Additive   | 266 (54.9) | 215 (44.3) | 1             |               |                 |     |
| **PSCA rs2976392** |        |       |               |               |               |                 |     |
| GG      | Codominant | 268 (54.9) | 215 (44.3) | 1             |               |                 |     |
| AG      |            | 173 (35.5) | 225 (46.4) | 1.49 (1.10–2.04) | 0.011 |               |     |
| AA      |            | 47 (9.6) | 45 (9.3) | 1.11 (0.66–1.85) | 0.699 |               |     |
| GG      | Dominant   | 268 (54.9) | 215 (44.3) | 1             |               |                 |     |
| AA+AG   |            | 220 (45.1) | 268 (55.7) | 1.41 (1.05–1.89) | 0.021 |               |     |
| A allele| Additive   | 266 (54.9) | 215 (44.3) | 1             |               |                 |     |
| **MUC1 rs4072037** |        |       |               |               |               |                 |     |
| AA      | Codominant | 318 (65.2) | 371 (77.3) | 1             |               |                 |     |
| AG      |            | 152 (31.1) | 92 (19.2) | 0.54 (0.38–0.76) | <0.001 |               |     |
| GG      |            | 18 (3.7) | 17 (3.5) | 1.01 (0.48–2.15) | 0.974 |               |     |
| AA      | Dominant   | 318 (65.2) | 371 (77.3) | 1             |               |                 |     |
| AG+GG   |            | 170 (34.8) | 109 (22.7) | 0.59 (0.42–0.81) | 0.001 |               |     |
| G allele| Additive   | 318 (65.2) | 371 (77.3) | 1             |               |                 |     |
| **ZBTB20 rs9841504** |        |       |               |               |               |                 |     |
| CC      | Codominant | 349 (71.5) | 368 (75.6) | 1             |               |                 |     |
| CG      |            | 124 (25.4) | 95 (19.5) | 0.65 (0.45–0.92) | 0.015 |               |     |
| GG      |            | 15 (3.1) | 24 (4.9) | 1.85 (0.86–3.97) | 0.114 |               |     |
| CC      | Dominant   | 349 (71.5) | 368 (75.6) | 1             |               |                 |     |
| CG+GG   |            | 139 (28.5) | 119 (24.4) | 0.76 (0.54–1.05) | 0.094 |               |     |
| G allele| Additive   | 349 (71.5) | 368 (75.6) | 1             |               |                 |     |
| **SLC52A3 rs13042395** |       |       |               |               |               |                 |     |
| CC      | Codominant | 180 (36.8) | 238 (48.7) | 1             |               |                 |     |
| CT      |            | 247 (50.5) | 211 (43.1) | 0.77 (0.56–1.04) | 0.088 |               |     |
| TT      |            | 62 (12.7) | 40 (8.2) | 0.46 (0.27–0.76) | 0.003 |               |     |
| CC      | Dominant   | 180 (36.8) | 238 (48.7) | 1             |               |                 |     |
| CT+TT   |            | 309 (63.2) | 251 (51.3) | 0.70 (0.52–0.93) | 0.016 |               |     |
| T allele| Additive   | 180 (36.8) | 238 (48.7) | 1             |               |                 |     |
| **PRKAA1 rs13361707** |        |       |               |               |               |                 |     |
| CC      | Codominant | 98 (20.1) | 172 (36.4) | 1             |               |                 |     |
| CT      |            | 246 (50.5) | 213 (45.0) | 0.48 (0.34–0.68) | <0.001 |               |     |
| TT      |            | 143 (29.4) | 88 (18.6) | 0.35 (0.23–0.54) | <0.001 |               |     |
| CC      | Dominant   | 98 (20.1) | 172 (36.4) | 1             |               |                 |     |
| CT+TT   |            | 389 (79.9) | 301 (63.6) | 0.43 (0.31–0.61) | <0.001 |               |     |
| T allele| Additive   | 98 (20.1) | 172 (36.4) | 1             |               |                 |     |
| **Risk genotype** |        |       |               |               |               |                 |     |
| 0–1     |            | 156 (32.0) | 74 (15.7) | 1             |               |                 |     |
| 2–3     |            | 294 (60.4) | 297 (62.9) | 2.13 (1.55–2.93) | <0.001 |               |     |
| 4–5     |            | 37 (7.6) | 101 (21.4) | 5.76 (3.61–9.18) | <0.001 |               |     |

\(^1\)Adjusted for age, sex, smoking, drinking status, and Helicobacter pylori infection status in logistic models.
interaction with alcohol drinking and had an effect on risk of GC. Moreover, three SNPs, MUC1 rs4072037, ZBTB20 rs9841504, and PRKAA1 rs13361707, were also associated with gastric severe IM/dysplasia.

Studies on interactions between these seven genetic polymorphisms and *H. pylori* infection on risk of GC are limited and yielded inconsistent results [20, 32]. This inconsistency may be due to variations in study conditions.

### Table 3. Association between each of the susceptibility loci and risk of GC, stratified by Helicobacter pylori infection.

| H. pylori infection Genotype | Controls n (%) | Cases n (%) | OR (95% CI)\(^1\) | P |
|-----------------------------|---------------|-------------|-------------------|---|
| **PLCE1 rs2274223**         |               |             |                   |   |
| – AA                        | 134 (64.7)    | 34 (60.7)   | 1.00              |   |
| – AG+GG                     | 73 (35.3)     | 22 (39.3)   | 1.59 (0.82–3.09)  | 0.174 |
| I AA                        | 111 (68.1)    | 186 (57.6)  | 1.00              |   |
| I AG+GG                     | 52 (31.9)     | 137 (42.4)  | 1.59 (1.04–2.43)  | 0.032 |
| II AA                       | 72 (61.5)     | 61 (60.4)   | 1.00              |   |
| II AG+GG                    | 45 (38.5)     | 40 (39.6)   | 1.34 (0.74–2.43)  | 0.328 |
| **PSCA rs2294008**          |               |             |                   |   |
| – CC                        | 109 (52.7)    | 33 (60.0)   | 1.00              |   |
| – CT+TT                     | 98 (47.3)     | 22 (40.0)   | 0.57 (0.28–1.15)  | 0.116 |
| I CC                        | 93 (57.1)     | 133 (41.7)  | 1.00              |   |
| I CT+TT                     | 70 (42.9)     | 186 (58.3)  | 1.93 (1.28–2.90)  | 0.002 |
| II CC                       | 65 (55.6)     | 43 (43.0)   | 1.00              |   |
| II CT+TT                    | 52 (44.4)     | 57 (57.0)   | 1.45 (0.81–2.60)  | 0.206 |
| **PSCA rs2976392**          |               |             |                   |   |
| – GG                        | 109 (52.7)    | 33 (60.0)   | 1.00              |   |
| – AA+AG                     | 98 (47.3)     | 22 (40.0)   | 0.57 (0.28–1.15)  | 0.116 |
| I GG                        | 93 (57.1)     | 132 (41.8)  | 1.00              |   |
| I AA+AG                     | 70 (42.9)     | 184 (58.2)  | 1.92 (1.27–2.89)  | 0.002 |
| II GG                       | 65 (55.6)     | 42 (42.4)   | 1.00              |   |
| II AA+AG                    | 52 (44.4)     | 57 (57.6)   | 1.50 (0.84–2.69)  | 0.173 |
| **MUC1 rs4072037**          |               |             |                   |   |
| – AA                        | 136 (65.7)    | 45 (81.8)   | 1.00              |   |
| – AG+GG                     | 71 (34.3)     | 10 (18.2)   | 0.44 (0.19–1.01)  | 0.053 |
| I AA                        | 101 (62.0)    | 237 (75.0)  | 1.00              |   |
| I AG+GG                     | 62 (38.0)     | 79 (25.0)   | 0.64 (0.42–0.99)  | 0.046 |
| II AA                       | 80 (68.4)     | 80 (81.6)   | 1.00              |   |
| II AG+GG                    | 52 (44.4)     | 57 (57.6)   | 1.50 (0.83–2.96)  | 0.037 |
| **ZBTB20 rs9841504**        |               |             |                   |   |
| – CC                        | 151 (72.9)    | 45 (80.4)   | 1.00              |   |
| – CG+GG                     | 56 (27.1)     | 11 (19.6)   | 0.57 (0.25–1.33)  | 0.192 |
| I CC                        | 115 (70.6)    | 238 (74.4)  | 1.00              |   |
| I CG+GG                     | 48 (29.4)     | 82 (25.6)   | 0.83 (0.53–1.30)  | 0.419 |
| II CC                       | 83 (70.9)     | 76 (76.0)   | 1.00              |   |
| II CG+GG                    | 34 (29.1)     | 24 (24.0)   | 0.74 (0.38–1.43)  | 0.371 |
| **SLC52A3 rs13042395**      |               |             |                   |   |
| – CC                        | 72 (34.6)     | 25 (44.6)   | 1.00              |   |
| – CT+TT                     | 136 (65.4)    | 31 (55.4)   | 0.76 (0.38–1.49)  | 0.417 |
| I CC                        | 67 (41.1)     | 158 (49.2)  | 1.00              |   |
| I CT+TT                     | 96 (58.9)     | 163 (50.8)  | 0.74 (0.49–1.11)  | 0.148 |
| II CC                       | 40 (34.2)     | 46 (45.5)   | 1.00              |   |
| II CT+TT                    | 77 (65.8)     | 55 (54.5)   | 0.64 (0.35–1.15)  | 0.137 |
| **PRKAA1 rs13361707**       |               |             |                   |   |
| – CC                        | 44 (21.4)     | 21 (38.2)   | 1.00              |   |
| – CT+TT                     | 162 (78.6)    | 34 (61.8)   | 0.50 (0.24–1.04)  | 0.064 |
| I CC                        | 34 (20.9)     | 116 (37.3)  | 1.00              |   |
| I CT+TT                     | 129 (79.1)    | 195 (62.7)  | 0.46 (0.29–0.73)  | 0.001 |
| II CC                       | 20 (17.1)     | 32 (33.3)   | 1.00              |   |
| II CT+TT                    | 97 (82.9)     | 64 (66.7)   | 0.39 (0.20–0.78)  | 0.007 |

\(^1\)Adjusted for age, sex, smoking, and alcohol drinking status in logistic models.
### Table 4. Joint effects of Helicobacter pylori seropositivity and genetic variants on risk of GC.

| Genotype     | H. pylori infection | Controls n (%) | Cases n (%) | OR (95% CI) | P     |
|--------------|---------------------|----------------|-------------|-------------|-------|
| **PLCE1 rs2274223** |                     |                |             |             |       |
| AA           | –                   | 134 (27.5)     | 34 (7.1)    | 1.00        |       |
| AA           | +                   | 183 (37.6)     | 247 (51.4)  | 6.07 (3.72–9.88) | <0.002 |
| AG/GG        | –                   | 73 (15.0)      | 22 (4.6)    | 1.56 (0.80–3.02) | 0.192 |
| AG/GG        | +                   | 97 (19.9)      | 177 (36.9)  | 8.82 (5.28–14.74) | <0.001 |
| Multiplicative interaction |                  |                |             | 0.94 (0.45–1.97) | 0.861 |
| Additive interaction: SI |                  |                |             | 1.39 (0.93–2.12) |       |
| **PSCA rs2294008** |                     |                |             |             |       |
| CC           | –                   | 109 (22.4)     | 33 (7.0)    | 1.00        |       |
| CC           | +                   | 158 (32.4)     | 176 (37.1)  | 3.56 (2.20–5.75) | <0.001 |
| CT/TT        | –                   | 98 (20.1)      | 22 (4.6)    | 0.57 (0.29–1.14) | 0.110 |
| CT/TT        | +                   | 122 (25.1)     | 243 (51.3)  | 6.20 (3.83–10.05) | <0.001 |
| Multiplicative interaction |                  |                |             | 3.05 (1.43–6.53) | 0.004 |
| Additive interaction: SI |                  |                |             | 2.45 (1.55–5.52) |       |
| **PSCA rs2976392** |                     |                |             |             |       |
| GG           | –                   | 109 (22.4)     | 33 (7.0)    | 1.00        |       |
| GG           | +                   | 158 (32.4)     | 174 (37.0)  | 3.51 (2.17–5.69) | <0.001 |
| AG/AA        | –                   | 98 (20.1)      | 22 (4.7)    | 0.57 (0.29–1.13) | 0.110 |
| AG/AA        | +                   | 122 (25.1)     | 241 (51.3)  | 6.14 (3.79–9.95) | <0.001 |
| Multiplicative interaction |                  |                |             | 3.06 (1.43–6.54) | 0.004 |
| Additive interaction: SI |                  |                |             | 2.47 (1.43–5.61) |       |
| **PUC1 rs4072037** |                     |                |             |             |       |
| AG/GG        | –                   | 71 (14.6)      | 10 (2.1)    | 1.00        |       |
| AG/GG        | +                   | 99 (20.3)      | 97 (20.7)   | 7.49 (3.38–16.63) | <0.001 |
| AA           | –                   | 136 (27.9)     | 45 (9.6)    | 2.19 (0.95–5.02) | 0.065 |
| AA           | +                   | 181 (37.2)     | 317 (67.6)  | 12.19 (5.67–26.21) | <0.001 |
| Multiplicative interaction |                  |                |             | 0.74 (0.30–1.83) | 0.520 |
| Additive interaction: SI |                  |                |             | 1.46 (0.97–2.33) |       |
| **ZBTB20 rs9841504** |                     |                |             |             |       |
| CG/GG        | –                   | 56 (11.5)      | 11 (2.3)    | 1.00        |       |
| CG/GG        | +                   | 82 (16.8)      | 106 (22.3)  | 7.97 (3.54–17.93) | <0.001 |
| CC           | –                   | 151 (31.0)     | 45 (9.4)    | 1.84 (0.80–4.23) | 0.154 |
| CC           | +                   | 198 (40.7)     | 314 (66.0)  | 9.87 (4.55–21.43) | <0.001 |
| Multiplicative interaction |                  |                |             | 0.68 (0.27–1.68) | 0.397 |
| Additive interaction: SI |                  |                |             | 1.14 (0.79–1.76) |       |
| **SLC52A3 rs13042395** |                     |                |             |             |       |
| CT/TT        | –                   | 136 (27.9)     | 31 (6.5)    | 1.00        |       |
| CT/TT        | +                   | 173 (35.4)     | 218 (45.6)  | 5.67 (3.51–9.15) | <0.001 |
| CC           | –                   | 72 (14.8)      | 25 (5.2)    | 1.32 (0.67–2.60) | 0.423 |
| CC           | +                   | 107 (21.9)     | 204 (42.7)  | 8.32 (5.08–13.65) | <0.001 |
| Multiplicative interaction |                  |                |             | 1.11 (0.52–2.36) | 0.781 |
| Additive interaction: SI |                  |                |             | 1.47 (0.99–2.24) |       |
| **PRKAA1 rs13361707** |                     |                |             |             |       |
| CT/TT        | –                   | 162 (33.3)     | 34 (7.4)    | 1.00        |       |
| CT/TT        | +                   | 226 (46.5)     | 259 (56.1)  | 5.47 (3.51–8.55) | <0.001 |
| CC           | –                   | 44 (9.1)       | 21 (4.5)    | 2.00 (0.97–4.12) | 0.061 |
| CC           | +                   | 54 (11.1)      | 148 (32.0)  | 13.11 (7.80–22.04) | <0.001 |
| Multiplicative interaction |                  |                |             | 1.20 (0.53–2.71) | 0.663 |
| Additive interaction: SI |                  |                |             | 2.21 (1.39–3.69) |       |

1 Adjusted for age, sex, smoking, and drinking status in logistic models.
Table 5. Joint effects of alcohol drinking status and genetic variants on risk of GC.

| Genotype     | Alcohol drinking | Controls n (%) | Cases n (%) | OR (95% CI) \(^1\) | \(P\)  |
|--------------|------------------|----------------|-------------|---------------------|-------|
| **PLCE1** rs2274223 |                  |                |             |                     |       |
| AA           | –                | 240 (49.2)     | 134 (32.5)  | 1.00                |       |
| AA           | +                | 77 (15.8)      | 101 (24.5)  | 2.83 (1.81–4.42)    | <0.001|
| AG/GG        | –                | 130 (26.6)     | 95 (23.1)   | 1.35 (0.94–1.95)    | 0.106 |
| AG/GG        | +                | 41 (8.4)       | 82 (19.9)   | 4.96 (2.98–8.26)    | <0.001|
| Multiplicative interaction |       |                |             | 1.30 (0.69–2.45)    | 0.421 |
| Additive interaction: SI |       |                |             | 1.82 (0.89–3.87)    |       |
| **PSCA** rs2294008 |                  |                |             |                     |       |
| CC           | –                | 207 (42.4)     | 107 (26.3)  | 1.00                |       |
| CC           | +                | 61 (12.5)      | 77 (18.9)   | 2.86 (1.74–4.70)    | <0.001|
| CT/TT        | –                | 163 (33.4)     | 119 (29.2)  | 1.33 (0.93–1.89)    | 0.122 |
| CT/TT        | +                | 57 (11.7)      | 104 (25.6)  | 4.57 (2.82–7.41)    | <0.001|
| Multiplicative interaction |       |                |             | 1.21 (0.65–2.24)    | 0.555 |
| Additive interaction: SI |       |                |             | 1.63 (0.83–3.70)    |       |
| **PSCA** rs2976392 |                  |                |             |                     |       |
| GG           | –                | 207 (42.4)     | 106 (26.3)  | 1.00                |       |
| GG           | +                | 61 (12.5)      | 76 (18.8)   | 2.85 (1.74–4.69)    | <0.001|
| AG/AA        | –                | 163 (33.4)     | 118 (29.3)  | 1.32 (0.93–1.89)    | 0.125 |
| AG/AA        | +                | 57 (11.7)      | 103 (25.6)  | 4.57 (2.82–7.41)    | <0.001|
| Multiplicative interaction |       |                |             | 1.21 (0.65–2.26)    | 0.545 |
| Additive interaction: SI |       |                |             | 1.64 (0.83–3.83)    |       |
| **MUC1** rs4072037 |                  |                |             |                     |       |
| AG/GG        | –                | 130 (26.6)     | 55 (13.7)   | 1.00                |       |
| AG/GG        | +                | 40 (8.2)       | 40 (10.0)   | 2.95 (1.59–5.49)    | 0.001 |
| AA           | –                | 240 (49.2)     | 169 (42.0)  | 1.67 (1.12–2.49)    | 0.011 |
| AA           | +                | 78 (16.0)      | 138 (34.3)  | 5.26 (3.21–8.63)    | <0.001|
| Multiplicative interaction |       |                |             | 1.07 (0.54–2.12)    | 0.857 |
| Additive interaction: SI |       |                |             | 1.62 (0.88–3.66)    |       |
| **ZBTB20** rs9841504 |                  |                |             |                     |       |
| CG/GG        | –                | 101 (20.7)     | 51 (12.5)   | 1.00                |       |
| CG/GG        | +                | 38 (7.8)       | 51 (12.5)   | 3.16 (1.72–5.79)    | <0.001|
| CC           | –                | 269 (55.1)     | 177 (43.4)  | 3.13 (0.87–1.98)    | 0.194 |
| CC           | +                | 80 (16.4)      | 129 (31.6)  | 4.23 (2.52–7.09)    | <0.001|
| Multiplicative interaction |       |                |             | 1.02 (0.52–2.02)    | 0.955 |
| Additive interaction: SI |       |                |             | 1.62 (0.88–3.66)    |       |
| **SLC52A3** rs13042395 |                  |                |             |                     |       |
| CT/TT        | –                | 231 (47.2)     | 124 (30.3)  | 1.00                |       |
| CT/TT        | +                | 78 (16.0)      | 86 (21.0)   | 2.63 (1.67–4.14)    | <0.001|
| CC           | –                | 139 (28.4)     | 104 (25.4)  | 1.25 (0.87–1.80)    | 0.221 |
| CC           | +                | 41 (8.4)       | 95 (23.3)   | 5.01 (3.02–8.31)    | <0.001|
| Multiplicative interaction |       |                |             | 1.52 (0.81–2.86)    | 0.192d|
| Additive interaction: SI |       |                |             | 2.13 (1.04–5.73)    |       |
| **PRKAA1** rs13361707 |                  |                |             |                     |       |
| CT/TT        | –                | 296 (60.8)     | 141 (35.5)  | 1.00                |       |
| CT/TT        | +                | 93 (19.1)      | 113 (28.5)  | 3.33 (2.19–5.10)    | <0.001|
| CC           | –                | 74 (15.2)      | 81 (20.4)   | 2.46 (1.64–3.68)    | <0.001|
| CC           | +                | 24 (4.9)       | 62 (15.6)   | 6.67 (3.70–12.03)   | <0.001|
| Multiplicative interaction |       |                |             | 0.82 (0.40–1.67)    | 0.577 |
| Additive interaction: SI |       |                |             | 1.50 (0.73–3.00)    |       |

\(^1\) Adjusted for age, sex, smoking status, and *Helicobacter pylori* infection status in logistic regression models.
populations with sample sizes. Previous reports from Japanese and Caucasians [32, 33] found that individuals who had \textit{H. pylori} infection and a \textit{PSCA} risk genotype might have a higher risk of GC than subjects without \textit{H. pylori} infection, consistent with our results. However, these authors did not find significant interactions in their study, perhaps due to the small sample sizes in their studies. In our study, we found significant interactions...
between the PSCA polymorphism and _H. pylori_ infection on risk of GC. An individual who carries the PSCA risk genotype and Type I _H. pylori_ infection may have a higher risk of GC than an individual with Type II infection. PSCA, a tumor suppressor, is thought to have an inhibitory effect on the proliferation of differentiating gastric epithelial cells, and the downregulation of PSCA expression can be found in GC tissues [34]. The T allele of PSCA rs2294008 may reduce the transcriptional activity of the PSCA promoter in gastric cell lines [13]. Type I _H. pylori_ strain, expressing CagA and/or VacA, release proinflammatory cytokines and induce vacuole formation, which results in higher toxicity than Type II strain [8, 10]. Studies indicate that the PSCA genotype may be related in some way to parietal cell mass or to the regulation of gastric acid secretion, which then may influence the effects of _H. pylori_ -associated inflammation [35]. The different pathogenicity of the two types of _H. pylori_ and their joint effect with PSCA genotype may play a part of process of inflammatory-related gastric carcinogenesis, which makes the individual who carries PSCA risk genotype and Type I _H. pylori_ infection have a higher risk of GC. We found significant interactions between PRKAA1 rs13361707 and _H. pylori_ infection in risk of GC. Eom et al. [36] found an additive interaction between _H. pylori_ CagA infection and PRKAA1 polymorphism, and our results are also consistent with their findings (data not shown). We further identified that in subjects with Type I and II _H. pylori_ infection, there were significant associations between PRKAA1 polymorphisms and GC risk.

SLC52A3 rs13042395 is located on chromosome 20p13. This gene encodes riboflavin transporter 2 protein (RFT2), which plays a role in gastric carcinogenesis [37]. A previous study conducted by Dong et al. [38] found that subjects carrying SLC52A3 rs13042395 T carriers showed a significantly increased risk of noncardia GC when they were alcohol drinkers, similar to the results of our study. In addition, we identified an additive interaction between SLC52A3 rs13042395 and alcohol drinking status, suggesting that SLC52A3 may interact with certain metabolic changes induced by alcohol drinking and influence the susceptibility to GC.

GC is an end result of the transformation of multistage precancerous gastric lesions, including chronic atrophic gastritis, IM, and dysplasia. Previous studies have found that certain genetic variants associated with GC, such as PSCA rs2294008 may also have an effect on risk of precancerous gastric lesions, though the results were inconsistent [23, 39], potentially due to different sample sizes and population heterogeneity. In our study, we identified that MUC1 rs4072037 and PRKAA1 rs13361707, which were significantly associated with GC, and ZBTB20 rs9841504, which was not, were also related to severe IM/dysplasia, indicating that these polymorphisms may affect an early stage of gastric carcinogenesis.

One of the strengths of our study is that we collected detailed personal information regarding environmental exposures from the study subjects, which enabled us to estimate the effect modification of environmental factors. Moreover, the severe IM/dysplasia was diagnosed from the same screening population as the control subjects, allowing us to explore the effects of seven susceptibility loci on the risk of precancerous lesions as well as GC. However, there are some limitations in our study. First, although we had a total of 1273 subjects in our study, the sample size was not sufficiently large for stratification analysis. Due to the multiple comparisons performed, false-positive results might have occurred. However, after multiple test comparison correction using the Benjamini–Hochberg procedure, we were still able to observe significant independent and interactive associations of _H. pylori_ infection and genetic polymorphisms on GC risk. Future large-scale studies and functional studies will be needed to further validate our results.

In summary, we systematically explored the seven GWAS-identified susceptibility loci and their potential interactions with environmental factors in risk of GC. We found that PSCA and PRKAA1 polymorphisms may interact with _H. pylori_ infection and that they had a synergistic effect on risk of GC. Meanwhile, SLC52A3 rs13042395 had an additive interaction with alcohol drinking that may contribute to GC risk. Three genetic variants that were associated with risk of GC may also be related to risk of severe IM/dysplasia. Knowledge of gene–environment interactions is important for risk prediction and the identification of certain high-risk populations, which in turn informs public health strategies for targeted prevention. Our study may also be helpful for gaining insight into the biological mechanisms underlying the associations between specific risk factors and gastric carcinogenesis. Additional studies with larger cohorts, as well as mechanistic studies of these findings are warranted.

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**Conflict of Interest**

We have no conflict of interest to declare.
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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Association of the seven SNPs with risk of noncardia cases and controls.
Table S2. Association of the seven SNPs with risk of cardia GC cases and controls.
Table S3. Association between each of the susceptibility loci and risk of GC, stratified by age.
Table S4. Association between each of the susceptibility loci and risk of GC, stratified by sex.
Table S5. Association between each of the susceptibility loci and risk of GC, stratified by smoking status.
Table S6. Association between each of the susceptibility loci and risk of GC, stratified by alcohol drinking status.