GSTT1 and *GSTM1* Null Genotypes and the Risk of Gastric Cancer: A Case-Control Study in a Chinese Population[1]

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Abstract

Glutathione *S*-tranferase (GST) enzymes are involved in detoxification of many potentially carcinogenic compounds. The homozygous deletions or null genotypes of *GSTT1* (θ class) and *GSTM1* (μ class) genes may be associated with an increased risk of cancer. Few studies have evaluated the relationship between *GSTT1, GSTM1* and the risk of gastric cancer, as well as the potential interactions between these genetic markers and other risk factors of gastric cancer in the Chinese population. We conducted a case-control study with 143 cases with gastric cancer, 166 chronic gastritis (CG) cases and 433 cancer-free population controls from Yangzhong County, China. The epidemiological data were collected by a standard questionnaire for all of the subjects, and blood samples were obtained from 91 gastric cancer cases, 146 CG cases, and 429 controls. *GSTT1* and *GSTM1* genotypes were assayed by the PCR method, and *Helicobacter pylori* infection was measured by the ELISA method. Using logistic regression model in SAS, we assessed the independent effects of *GSTT1* and *GSTM1* null genotypes on the risk of gastric cancer and their potential interactions with other factors. The prevalence of *GSTM1* null genotype was 48% in gastric cancer cases, 60% in CG patients, and 51% in controls. The prevalence of *GSTT1* null genotype was 54% in gastric cancer cases, 48% in CG patients, and 46% in controls. After controlling for age, gender, education, pack-years of smoking, alcohol drinking, body mass index, *H. pylori* infection, and fruit and salt intake, the adjusted odds ratio (OR) for *GSTT1* and gastric cancer was 2.50 (95% confidence interval (CI), 1.01–6.22). When gastric cancer cases were compared with CG patients, the adjusted OR for *GSTT1* was 2.33 (95% CI, 0.75–7.25). However, *GSTT1* null genotype was not associated with the risk of CG when using population controls. No obvious association was found between *GSTM1* and the risk of both gastric cancer and CG. Our results suggest that *GSTT1* null genotype may be associated with an increased risk of gastric cancer in a Chinese population.

Introduction

Despite the downward trend in incidence and mortality in most countries, gastric cancer was recently estimated to be the second most common cancer in the world after lung cancer. Thirty-five percent of worldwide cases occur in China, where it remains the most common cancer in both sexes, as it is elsewhere in Eastern Asia. Gastric cancer remains the most frequent cancer in men (ahead of lung cancer) in tropical South America, and high rates are also present in both sexes in the former Union of Soviet Socialist Republics (1). The etiology of gastric cancer is not well established, although nutritional (excessive salt intake and deficient vegetable/fruit intake), microbial (*H. pylori* infection), and genetic factors have been suggested in a multi-step and multifactorial process (2).

The glutathione *S*-tranferase supergene family consists of four gene subfamilies (*GSTA, GSTM, GSTT*, and *GSTP*) that play a central role in the inactivation of toxic and carcinogenic electrophiles (3, 4). Certain genes within the *GSTM* and *GSTT* (*GSTM1* and *GSTT1*) subfamilies exhibit homozygous deletion (null genotype) polymorphisms that have been considered as potentially important modifiers of individual risk of environmentally induced cancers (5). The prevalence of the null genotype of *GSTM1* and *GSTT1* has been found to vary among ethnic groups (6). The *GSTM1* is absent in 35–60% of individuals (7–9), and *GSTT1* is absent in 10–65% of the human population (6, 9). Research on the relationship between homozygous deletion polymorphisms and the risk of cancer is important for a better understanding of interindividual variation in response to carcinogen exposures and cancer susceptibility.

Previous studies have shown that the *GSTM1* null genotype has been associated with an increased susceptibility to lung cancer (10–14), bladder cancers (7, 15), and cutaneous cancers (16). Studies with regard to an association between *GSTM1* and gastric cancer have been limited, and the results have been inconsistent (17, 18).

GSTT1 null genotype may be a risk modifier in the occurrence of colorectal cancer, and it is suggested that this

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enzyme is important in the detoxification of unidentified xenobiotics in the large intestine (19). Individuals with *GSTT1* null genotype may be at increased risk for genotoxic damage from environmental or occupational 1,3-butadiene exposures (20). A study of the *GSTT1* gene may provide insights into the nature of common environmental or dietary exposures that produce chromosomal damage. Persons with the *GSTT1* null genotype show reduced ability to detoxify metabolites of ethylene oxide (5). There is a strong association between the *GSTT1* normal genotype and the level of isothiocyanates (degradation products of glucosinolates, which occur naturally in a variety of cruciferous vegetables and have been shown to exhibit chemopreventive activity) in urine when compared with the null *GSTT1* genotype depending on the level of cruciferous vegetable intake (21).

The increased consumption of vegetables and fresh fruit has been shown to reduce the risk of gastric cancer (22–25), whereas high consumption of salt tends to increase the risk of gastric cancer (26, 27). Tobacco smoking has been considered a potential risk factor for gastric cancer. From the previous epidemiological studies, a risk of gastric cancer among smokers was increased compared with nonsmokers (22, 28–30). *Helicobacter pylori* has been implicated as an etiological factor for gastric cancer (31, 32). Correa and Stemmermann in separate studies (2, 33) have suggested the general hypothesis of precancerous sequences for gastric carcinogenesis, especially for the intestinal types of gastric cancer, as follows: superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and cancer. Epidemiological studies have shown the positive association between seropositivity of *H. pylori* IgG antibody, tissue positivity of *H. pylori* infection, and the risk of gastric cancer in several studies (31, 32, 34, 35), and *H. pylori* infection may be a risk factor of chronic atrophic gastritis (35).

Few studies have correlated environmental factors and genetic susceptibility with the risk of gastric cancer, especially in the Chinese population, which has one of the highest incidences of gastric cancer in the world. In this study, we evaluated the association between *GSTM1* and *GSTT1* and the risk of gastric cancer and chronic gastritis. We also explored the potential interactions between *GSTM1, GSTT1*, and other risk factors such as *H. pylori* infection, smoking, and salt and fruit intake in a Chinese population.

Materials and Methods

Background. Yangzhong City (formerly Yangzhong County, before 1995) is an island situated on the Yangtze River in the southeast part of Jiangsu province, China. It has one of the highest rates of alimentary cancer in the world. The average annual death rate from gastric cancer, adjusted for world standard-population, was 127/100,000 and as high as 1,862/100,000 among the men ages 70–74 years old. The phenomenon of high frequency of gastric cancer is very rare in the world and may be associated with the genetic or environmental background of the population. (23, 36)

Study Population. A case-control study of gastric cancer was conducted in Yangzhong City, Jiangsu province. Data included questionnaire data, medical record review, and blood samples for assaying *H. pylori* infection and other molecular markers. The two-case groups included patients with gastric cancer and chronic gastritis. The population healthy control group was a random sample from the local population from which the cases came.

Gastric Cancer Cases. Eligible cases were all of the patients examined at Yangzhong Central Hospital-Endoscopy Unit from January 1, 1995, to June 30, 1995, with pathologically confirmed diagnoses of gastric adenocarcinoma. We interviewed all of the incident patients with gastric cancer during the study period who consented to be interviewed with the following restrictions: patients must be newly diagnosed, not restricted by age, in stable medical condition as determined by their physician, and willing to participate. The study was restricted to people living in Yangzhong for 1 year or more. In the 6-month study period, we recruited a total of 200 patients with esophageal or gastric cancer from the Endoscopic Unit. Of these, 57 patients with esophageal cancer were excluded. We had a total of 143 incident cases with gastric cancer in this study, which represented 86% of all of the new cases diagnosed in the same study period in Yangzhong (the estimated total incident cases with gastric cancer in Yangzhong was 166 in the study period). Blood specimens were collected in 64% (91 of 143) of the gastric cancer patients who had an interview.

Chronic Gastritis. Eligible cases were randomly selected patients at Yangzhong Central Hospital Endoscopy Unit between January 1, 1995 and June 30, 1995, with pathologically confirmed diagnoses of chronic (superficial or atrophic) gastritis. We interviewed all of the randomly selected incident patients with chronic gastritis, with consent to be interviewed, with the following restrictions: patients must be newly diagnosed, not restricted by age, in stable medical condition as determined by their physician and willing to participate. The study was restricted to people living in Yangzhong for 1 year or more. In the 6-month study period, we approached 205 patients and interviewed 166 patients with chronic gastritis (81%) from the Endoscopic Unit. Blood specimens were collected in 88% (146 of 166) of patients with chronic gastritis who had an interview.

Controls. Eligible controls were healthy and cancer-free individuals. We interviewed all of the randomly selected eligible controls during the study period with the following criteria: not restricted by age, in a stable medical condition, and willing to participate. The study was restricted to people living in Yangzhong for 1 year or more. They were randomly selected from the name list of each village in Yangzhong. Following the selected list, the interviewer located the controls, explained the study, interviewed them at their home, and collected 5 ml or more of blood sample. A total of 477 potential healthy controls were approached, and 433 controls had completed interviews (91%). Blood specimens were collected in 99% (429 of 433) of population controls who had an interview.

Histological Diagnosis. The subjects received an upper gastrointestinal endoscopic examination and biopsies taken from seven standard sites in the stomach: four from the antrum, one from the angulus, and one each from the lesser and greater curvature of the body. Pathological diagnoses were made according to criteria proposed by the Chinese Association of Gastric Cancer. The details of the classification criteria, along with photographs of superficial gastritis, chronic atrophic gastritis, intestinal metaplasia and dysplasia, can be found in an earlier publication. (37)

Epidemiological Data Collection. We interviewed cases and controls using a standard epidemiological questionnaire. The collected information included (*a*) demographic factors; (*b*) occupational history; (*c*) detailed information on gastric or duodenal ulcers; (*d*) family history of gastric cancer; (*e*) detailed data on consumption of salt and oil (current and 5 years ago); (*f*) data of green tea consumption; (*g*) smoking and drinking history; (*h*) dietary habits; (*i*) body heights and

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weights; and (*j*) BMI.[3] The medical records for patients were abstracted for relevant clinical data including endoscopy and pathology examinations. Blood specimens were obtained for *GSTT1* and *GSTM1* assays and to test serology for *H. pylori* infection from 91 gastric cancer cases, 146 chronic gastritis cases, and 429 population controls.

Blood Samples Collection and Storage. After cases and controls were identified, study nurses collected 5 ml or more of whole blood into tubes. All blood samples were transported to the laboratory at 4°C as soon as possible. The blood then was centrifuged at 400 × *g* for 10 min to collect the serum. The serum was then divided into three 1-ml aliquots, and the coagulated blood was put into one 5-ml tube. Both the serum and the coagulated blood were stored at −20°C until shipment. At the end of the study, blood specimens were hand-carried on dry ice to Shanghai Medical University from Yangzhong and stored at −20°C for further shipment. Blood samples were then packed in styrofoam containers with large amounts of dry ice and shipped from Shanghai to New York in November 1995. All of the blood specimens were finally stored at −70°C at the Molecular Epidemiology Laboratory of the University of California-Los Angeles Jonsson Comprehensive Cancer Center.

DNA Extraction. Genomic DNA was isolated from 200 μl of whole blood using the QIAmp Blood Kit from Qiagen (Valencia, CA). The 200 μl of sample were mixed with the protease and lysis buffer provided in the kit and were incubated at 70°C for 10 min. After ethanol was added, the mixture was transferred to the QIAmp spin column. The spin column was washed three times using the provided buffer to purify the DNA that bound to the column. The DNA was then eluted from the column and collected. The DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260/280 nm.

PCR Analysis of *GSTM1* and *GSTT1* Polymorphisms. Genotyping for *GSTM1* was modified from the method described previously (7). All of the reagents were obtained from Life Technologies, Inc. (Gaithersburg, MD). Reactions were carried out in a total volume of 50 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 μM each *GSTM1* primers, 0.25 μM each β-globin primers (internal control), 2.5 U Taq DNA polymerase, 200 μM each dNTPs, and 100 ng of DNA. The *GSTM1* primers were 5′-GAACTCCCTGAAAAGCTAAGC and 5′-GTTGGGCTCAAATATACGGTGG. PCR conditions were 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 1.5 min with a final extension at 72°C for 10 min. The *GSTT1* genotyping was performed using a modified procedure described previously (20) with β-globin as an internal control. Reactions were carried out as *GSTM1* except the *GSTT1* primers were used. The *GSTT1* primers were 5′-TTCCTTACTGGTCCTCACATCTC and 5′-TCACCGGATCATGGCCAGCA. PCR conditions were 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 10 min. PCR products were analyzed on a premade 4% Nusieve 1% agarose gel (FMC Bioproducts, Rockland, MN) stained with ethidium bromide and photographed under UV light.

Measurement of Serum Antibody IgG to *H. pylori*. The presence of serum IgG antibodies to *H. pylori* were measured by ELISA using HM-CAP kit from Enteric Product, Inc. (Stony Brook, NY). By using urea breath test as the reference procedure, the sensitivity and specificity of this assay were 97.6% and 93.6%, respectively. In brief, the serum samples were diluted 101 times with wash buffer and were pipetted into a microwell plate coated with *H. pylori* antigen. The plate was incubated at room temperature for 20 min, which was followed by three washings. After the conjugate was added, the plate was incubated for another 20 min. Next, the plate was washed three times followed by dispensing the substrate solution. Finally, stop solution was added, and the plate was read under 450/630 nm dual wavelengths. The EVs were calculated by the standard curve generated from calibrator sera provided by the manufacturer. The EV above 2.2 was considered positive, and the value below 1.8 was negative. Samples with EVs between 1.8–2.2 were retested at least three times. If the value of these samples still fell within this range, an EV of 2.0 was used as a cutoff point.

Statistical Analysis. The relationships between gastric cancer and putative risk factors were measured using the OR and their 95% CIs derived from logistic regression analysis using SAS software. The per capita consumption of salt and oil was calculated by dividing the total of monthly intake in kg per family by the number of family members. The categorizations of smoking variables were based on the most recently published literature. The categorization of the intake of fruit, vegetables, and salt, as well as the BMI, were based on their distributions in the population controls. We used the quartile distribution for four categories and the median for binary variables as cutoff points. Crude ORs were calculated for all of the independent variables. Dummy variables were used to estimate the OR for each category of exposure in logistic regression analysis. On the basis of these distributions and prior knowledge of the risk factors for gastric cancer, gender (M/F) and the continuous variables of age and education were adjusted for in the logistic regression model when the adjusted ORs were estimated. For the "further adjusted" ORs, the continuous variables of BMI, pack-years of smoking, fruit intake, and salt intake, and the categorical variables of alcohol drinking and *H. pylori* infection (no/yes) were included in the logistic regression model when appropriate, in addition to the variables included in the "adjusted" OR model. We focused on the effect of *GSTM1* and *GSTT1* null genotype in gastric cancer, and logistic regression was used to assess the interaction effects between *GSTM1* and *GSTT1* null genotype and other possible risk factors of gastric cancer. We evaluated departures from additive and multiplicative interaction effects between *GSTM1, GSTT1*, and other potential risk or protective factors for gastric cancer. The null hypotheses of additivity and multiplicativity were tested. A more than additive interaction was indicated when:

$$OR_{11} > OR_{10} + OR_{01} - 1$$

where OR_{11} = OR when both factors are present, OR_{10} = OR when only factor 1 is present, and OR_{01} = OR when only factor 2 is present. A more than multiplicative interaction was suggested when:

$$OR_{11} > OR_{10} * OR_{01}$$

The departures from additive and multiplicative effects were assessed by including main effect variables and their product terms in the logistic regression model.

Results

Table 1 shows the distribution of age, gender, education, alcohol drinking, salt intake, pack-years of smoking, fruit intake,

[3] The abbreviations used are: BMI, body mass index; EV, ELISA value; OR, odds ratio; CI, confidence interval.

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Table 1 Distribution of selected variables in gastric cancer cases, chronic gastritis, and controls by blood sample availability

	Gastric cancer *n* (%)		Chronic gastritis *n* (%)		Control *n* (%)	
	With	Without	With	Without	With	Without
Age groups						
≤40 yr	0 (0)	2 (3.9)	63 (43.2)	5 (25.0)	132 (30.9)	2 (50.0)
41–50 yr	15 (16.5)	6 (11.5)	30 (20.5)	6 (30.0)	142 (33.3)	1 (25.0)
51–60 yr	36 (39.6)	13 (25.0)	35 (24.0)	7 (35.0)	97 (22.7)	1 (25.0)
>60 yr	40 (44.0)	31 (59.6)	18 (12.3)	2 (10.0)	56 (13.1)	0 (0)
Total	91	52	146	20	427	4
χ^2 *P*	0.051		0.37		0.91	
Gender						
Male	65 (71.4)	34 (65.4)	97 (66.4)	17 (85.0)	213 (49.7)	1 (25.0)
Female	26 (28.6)	18 (34.6)	49 (33.6)	3 (15.0)	216 (50.3)	3 (75.0)
Total	91	52	146	20	429	4
χ^2 *P*	0.46		0.12		0.62	
Education						
Illiterate	22 (25.3)	27 (52.9)	23 (16.8)	3 (15.8)	103 (24.5)	0 (0)
Primary	49 (56.3)	18 (35.3)	49 (35.8)	7 (36.8)	168 (40.0)	2 (50.0)
Middle	11 (12.6)	5 (9.8)	40 (29.2)	3 (15.8)	110 (26.2)	1 (25.0)
High school	2 (2.3)	1 (2.0)	25 (18.2)	6 (31.6)	39 (9.3)	1 (25.0)
College	3 (3.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	87	51	137	19	420	4
χ^2 *P*	0.012		0.46		0.41	
Alcohol drinking						
No	60 (66.0)	25 (48.1)	100 (68.5)	12 (60.0)	290 (67.6)	3 (75.0)
Yes	31 (34.1)	27 (51.9)	46 (31.5)	8 (40.0)	139 (32.4)	1 (25.0)
Total	91	52	146	20	429	4
χ^2 *P*	0.051		0.45		1.00	
Salt intake (kg)						
<0.5	12 (19.0)	5 (10.6)	33 (30.6)	3 (21.4)	92 (22.8)	0 (0)
0.5–0.69	18 (28.6)	18 (38.3)	38 (35.2)	3 (21.4)	120 (29.8)	0 (0)
0.7–0.89	9 (14.3)	7 (14.9)	17 (15.7)	5 (35.7)	68 (16.9)	1 (25.0)
≥0.9	24 (38.1)	17 (36.2)	20 (18.5)	3 (21.4)	123 (30.5)	3 (75.0)
Total	63	47	108	14	403	4
χ^2 *P*	0.57		0.31		0.15	
Pack-year						
0	46 (51.1)	27 (51.9)	83 (56.8)	7 (35.0)	279 (66.3)	3 (75.0)
1–20	20 (22.2)	17 (32.7)	41 (28.1)	6 (30.0)	92 (21.9)	1 (25.0)
21–40	15 (16.7)	7 (13.5)	14 (9.6)	6 (30.0)	35 (8.3)	0 (0)
>40	9 (10.0)	1 (1.9)	8 (5.5)	1 (5.0)	15 (3.6)	0 (0)
Total	90	52	146	20	421	4
χ^2 *P*	0.20		0.058		1.00	
Fruit intake						
≤1–3/week	50 (66.7)	17 (34.7)	88 (68.8)	15 (83.3)	183 (47.7)	1 (33.3)
>1–3/week	25 (33.3)	32 (65.3)	40 (31.3)	3 (16.7)	201 (52.3)	2 (66.7)
Total	75	49	128	18	384	3
χ^2 *P*	<0.001		0.27		1.00	
BMI (kg/m^2)						
<19.5	37 (40.7)	17 (41.5)	39 (27.3)	6 (31.6)	38 (15.3)	1 (25.0)
19.5–21.09	19 (20.9)	10 (24.4)	30 (21.0)	7 (36.8)	59 (23.8)	2 (50.0)
21.1–23.49	21 (23.1)	8 (19.5)	37 (25.9)	3 (15.8)	72 (29.0)	0 (0)
≥23.5	14 (15.4)	6 (14.6)	37 (25.9)	3 (15.8)	79 (31.9)	1 (25.0)
Total	91	41	143	19	248	4
χ^2 *P*	0.96		0.37		0.39	

and BMI in subjects with and without blood samples. These selected variables are potential confounding factors based on the literature. The presentation of these distributions was to evaluate possible confounding factors and to justify the procedure for controlling these variables in the multivariate analysis. We also presented the selected variables for those who had blood samples and those who did not, to assess whether the individuals with blood samples were representative of the original study population. This attempt was to show whether there was possible selection bias due to missing samples that may threaten the validity of the study. For cases with gastric cancer, patients with blood samples were generally younger, more often male, more educated, drank less alcohol, and ate less fruit than those without blood samples. For patients with chronic gastritis and for controls, because the majority of them had blood samples, no obvious differences were observed between those with and without blood samples for variables listed in Table 1.

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Table 2 *GSTM1* and *GSTT1* genotype and the risk of gastric cancer and chronic gastritis (CG): ORs and 95% CIs

	Cases	Controls	Crude OR (95% CI)	Adjusted OR[a] (95% CI)	Further adjusted OR[b] (95% CI)
Gastric cancer *vs.* Control					
GSTM1					
Normal	45	207	1.00	1.00	1.00
Null	42	212	0.91 (0.57–1.45)	1.00 (0.59–1.70)	0.60 (0.26–1.38)
GSTT1					
Normal	37	228	1.00	1.00	1.00
Null	44	190	1.43 (0.89–2.30)	1.44 (0.83–2.49)	2.50 (1.01–6.22)
CG *vs.* Controls					
GSTM1					
Normal	58	207	1.00	1.00	1.00
Null	83	212	1.40 (0.95–2.06)	1.42 (0.95–2.13)	1.03 (0.56–1.90)
GSTT1					
Normal	74	228	1.00	1.00	1.00
Null	67	190	1.09 (0.74–1.59)	1.05 (0.70–1.56)	1.07 (0.58–1.96)
Gastric cancer *vs.* CG					
GSTM1					
Normal	45	58	1.00	1.00	1.00
Null	42	83	0.65 (0.38–1.12)	0.85 (0.43–1.68)	0.74 (0.26–2.13)
GSTT1					
Normal	37	74	1.00	1.00	1.00
Null	44	67	1.31 (0.76–2.27)	1.68 (0.84–3.35)	2.33 (0.75–7.25)

[a] OR adjusted for age, education (continuous), and sex (M/F).
[b] OR adjusted for sex (M/F), age, education, BMI, pack-years of smoking, fruit intake, salt intake (all continuous), *H. pylori* infection (yes/no), and alcohol drinking (yes/no).

The gastric cancer cases were more concentrated among the older group (ages >60 years) compared with chronic gastritis patients and population controls. In gastric cancer cases, chronic gastritis patients, and controls, the male proportions were higher than female. The highest proportion was found in the primary-school level of education for all of the subjects.

The *GSTM1* and *GSTT1* genotype prevalence in gastric cancer cases, chronic gastritis patients, and population controls are shown in Table 2. The prevalence of *GSTM1* null genotype was 48% in gastric cancer cases, 60% in chronic gastritis patients and 51% controls. The prevalence of *GSTT1* null genotype was 54% in gastric cancer cases, 48% in chronic gastritis patients, and 46% in controls.

In Table 2, the ORs and 95% CIs of *GSTM1* and *GSTT1* genotypes comparing gastric cancer cases to population controls were shown. Using normal *GSTM1* and *GSTT1* genotype as referent, we found that the crude ORs for *GSTM1* and *GSTT1* were 0.91 (95% CI, 0.57–1.45) and 1.43 (95% CI, 0.89–2.30), respectively. After controlling for age, gender, and education, the adjusted ORs for *GSTM1* and *GSTT1* were 1.00 (95% CI, 0.59–1.70) and 1.44 (95% CI, 0.83–2.49), respectively. Further adjustments for BMI, pack-years of smoking, alcohol drinking, *H. pylori* infection, and fruit and salt intake yield ORs for *GSTM1* and *GSTT1* of 0.60 (95% CI, 0.26–1.38) and 2.50 (95% CI, 1.01–6.22), respectively.

Table 2 also shows the ORs and 95% CIs of *GSTM1* and *GSTT1* genotypes comparing chronic gastritis patients to population controls. Normal *GSTM1* and *GSTT1* genotypes were used as referent. The crude ORs for *GSTM1* and *GSTT1* were 1.40 (95% CI, 0.95–2.06) and 1.09 (95% CI, 0.74–1.59), respectively. After controlling for age, gender, and education, the adjusted ORs for *GSTM1* and *GSTT1* were 1.42 (95% CI, 0.95–2.13) and 1.05 (95% CI, 0.70–1.56), respectively. Further adjustments for BMI, pack-years of smoking, alcohol drinking, *H. pylori* infection, and fruit and salt intake, yield ORs for *GSTM1* and *GSTT1* of 1.03 (95% CI, 0.56–1.90) and 1.07 (95% CI, 0.58–1.96), respectively.

The ORs and 95% CI of *GSTM1* and *GSTT1* genotypes comparing gastric cancer cases to chronic gastritis patients are shown in Table 2. Using normal *GSTM1* and *GSTT1* genotype as referent, the crude ORs for *GSTM1* and *GSTT1* were 0.65 (95% CI, 0.38–1.12) and 1.31 (95% CI, 0.76–2.27), respectively. After controlling for age, gender, and education, the adjusted ORs for *GSTM1* and *GSTT1* were 0.85 (95% CI, 0.43–1.68) and 1.68 (95% CI, 0.84–3.35), respectively. Further adjustments for BMI, pack-years of smoking, alcohol drinking, *H. pylori* infection, and fruit and salt intake yield ORs for *GSTM1* and *GSTT1* of 0.74 (95% CI, 0.26–2.13) and 2.33 (95% CI, 0.75–7.25), respectively.

The univariate analysis of the possible interactions between *GSTM1* and salt intake, fruit intake, smoking, alcohol drinking, BMI, and *H. pylori* infection are presented in Table 3. We observed possible interactions that were more than multiplicative between *GSTM1* and fruit intake, salt intake, pack-years of smoking, and smoking status. Normal *GSTM1* and high-fruit-intake group, normal *GSTM1* and <0.7-kg-salt-intake group, normal *GSTM1* and <20-pack-year group, and normal *GSTM1* and no-smoking group were used as referent.

In Table 4, we observed possibly more than multiplicative interactions between *GSTT1*, pack-years of smoking, and smoking status. Normal *GSTT1* genotype and <20-pack-year group and normal *GSTT1* and no-smoking group were used as referent.

In addition to assessing the gene-environmental interactions, we also assessed possible gene-gene interaction between *GSTM1* and *GSTT1* (data not shown). No obvious interaction was observed between these two genes on the risk of gastric cancer.

Discussion

Before interpretations of our results, methodological issues should be addressed. A population-based case-control study has an advantage. It is less susceptible to certain forms of selection

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Table 3 Interactions between *GSTM1* and selected variables and the risk of gastric cancer

Variables	*GSTM1*	Cases	Controls	Crude OR (95% CI)
Fruit intake				
High	Normal	15	95	1.00
Low	Normal	24	94	1.62 (0.80–3.27)
High	Null	8	102	0.50 (0.20–1.23)
Low	Null	24	88	1.73 (0.85–3.50)
Alcohol drinking				
No	Normal	29	136	1.00
Yes	Normal	16	71	1.06 (0.54–2.08)
No	Null	29	148	0.92 (0.52–1.62)
Yes	Null	13	64	0.95 (0.46–1.95)
BMI				
<21.1	Normal	27	42	1.00
≥21.1	Normal	18	72	0.39 (0.19–0.79)
<21.1	Null	26	50	0.81 (0.41–1.59)
≥21.1	Null	16	76	0.33 (0.16–0.68)
Pack-year				
≤20	Normal	36	177	1.00
>20	Normal	9	28	1.02 (0.46–2.27)
≤20	Null	26	189	0.87 (0.50–1.52)
>20	Null	15	20	5.00 (1.50–16.72)
Smoking				
No	Normal	26	131	1.00
Yes	Normal	19	76	1.26 (0.65–2.43)
No	Null	18	143	0.63 (0.33–1.21)
Yes	Null	24	69	1.75 (0.94–3.28)
Salt intake				
≤0.7	Normal	16	101	1.00
>0.7	Normal	14	94	0.94 (0.44–2.03)
≤0.7	Null	12	106	0.72 (0.32–1.59)
>0.7	Null	18	92	1.24 (0.60–2.56)
Family with gastric cancer				
No	Normal	40	203	1.00
Yes	Normal	5	4	6.34 (1.63–24.66)
No	Null	39	198	1.00 (0.62–1.62)
Yes	Null	3	14	1.09 (0.30–3.96)
H. pylori infection				
No	Normal	15	77	1.00
Yes	Normal	23	118	1.00 (0.49–2.04)
No	Null	9	68	0.68 (0.28–1.65)
Yes	Null	25	131	0.98 (0.49–1.97)

Table 4 Interactions between *GSTT1* and selected variables and the risk of gastric cancer

Variables	*GSTT1*	Cases	Controls	Crude OR (95% CI)
Fruit intake				
High	Normal	8	107	1.00
Low	Normal	21	102	2.75 (1.17–6.50)
High	Null	15	88	2.28 (0.92–5.63)
Low	Null	21	80	3.51 (1.48–8.33)
Alcohol drinking				
No	Normal	24	158	1.00
Yes	Normal	13	70	1.22 (0.59–2.54)
No	Null	30	125	1.58 (0.88–2.84)
Yes	Null	14	65	1.42 (0.69–2.91)
BMI				
<21.1	Normal	26	42	1.00
≥21.1	Normal	11	96	0.19 (0.08–0.41)
<21.1	Null	24	50	0.78 (0.39–1.55)
≥21.1	Null	20	53	0.61 (0.30–1.24)
Pack-year				
≤20	Normal	26	194	1.00
>20	Normal	10	29	1.38 (0.58–3.25)
≤20	Null	30	168	1.71 (0.94–3.11)
>20	Null	14	19	8.11 (2.14–30.75)
Smoking				
No	Normal	18	146	1.00
Yes	Normal	19	82	1.88 (0.93–3.78)
No	Null	21	127	1.34 (0.68–2.63)
Yes	Null	23	63	2.96 (1.49–5.87)
Salt intake				
≤0.7	Normal	9	114	1.00
>0.7	Normal	15	100	1.90 (0.78–4.53)
≤0.7	Null	14	93	1.91 (0.79–4.60)
>0.7	Null	16	85	2.38 (1.01–5.66)
Family with gastric cancer				
No	Normal	33	219	1.00
Yes	Normal	4	9	2.95 (0.86–10.13)
No	Null	40	181	1.47 (0.89–2.42)
Yes	Null	4	9	2.95 (0.86–10.13)
H. pylori infection				
No	Normal	9	85	1.00
Yes	Normal	21	128	1.55 (0.68–3.55)
No	Null	14	59	2.24 (0.91–5.52)
Yes	Null	25	121	1.95 (0.87–4.39)

bias in estimating exposure effect because the cancer-free controls were selected from the same population from which the gastric cancer and chronic gastritis cases arose. However, because not all of the subjects in our study had blood samples, the selection bias may exist when we study subjects with blood samples only. Because the gastric cancer patients with blood samples were younger, more educated, and drank less alcohol than those without blood samples, the selection bias may distort the association toward the null. On the other hand, the gastric cancer cases with blood samples had more males and had fewer fruit eaters than those without blood samples, which may lead to positive bias away from the null. In this study, the gastric cancer cases were older than the population controls. To minimize the possible confounding effects caused by difference in age for cases and controls, we controlled for age in all of our analyses, except in the assessment of interactions or stratified analysis by age because of the limited sample size. Considering that genetic polymorphisms under study (*GSTM1* and *GSTT1*) are inherited, age may not be associated with the *GSTM1* and *GSTT1* genotypes. However, because age is an important risk factor for gastric cancer, and some young individuals in the control group may eventually develop cancer at an older age, the possible confounding or misclassification bias by age may not be excluded.

Similar to the limitations of general case-control study, the information bias may exist. First, disease status may be misclassified. However, because all of our gastric cancer and chronic gastritis cases were pathologically confirmed, the possible disease misclassification is minimized. Second, exposure misclassification bias may be present. There is the possibility that exposure was misclassified by the subjects during the interview, possibly by recall bias and reporting bias. Because gastric cancer and chronic gastritis cases are aware of their disease status, it is possible for them to think about the possible causes of their illnesses. Thus, they may recall their exposure differently from the controls. Due to recall bias, an overestimation of OR is possible. On the other hand, reporting bias may also cause underestimation of OR. Especially if certain habits such as smoking and alcohol drinking are not socially acceptable for Chinese women.

Sample size is also a concern in our study in that we have a limited number of cases with blood samples (gastric cancer, $n = 91$ and chronic gastritis, $n = 146$). For the interactions between *GSTM1, GSTT1*, and some selected variables that have

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been associated with gastric cancer, many cells had a small number of cases ($n \leq 9$). The small number of cases may limit our ability to estimate OR precisely. With the large sample size of the control group ($n = 429$), the power will be increased slightly to detect the association. However, we still cannot totally exclude the potential effect of chance in the genotype distribution in the cases with the relatively small sample size.

The prevalence of *GSTM1* and *GSTT1* null genotypes varies among ethnic groups. In our study we found that the prevalence of *GSTM1* and *GSTT1* null in controls to be 51% and 46%, respectively. Our results were similar to a previous study that found the prevalence of *GSTM1* null genotype among healthy Chinese to be 49% in Hong Kong and 45% in Taiwan (38). Another study (39) also showed that the prevalence of *GSTM1* and *GSTT1* null among healthy Chinese in Shanghai to be both 49% which was fairly consistent with our results.

Very few studies in the past have studied the associations between *GSTM1* and *GSTT1* null genotype and the risk of gastric cancer. One study showed a weak association between *GSTM1*, but not *GSTT1*, genotype and gastric cancer in a Japanese population (17). Another study also showed an association between *GSTM1* and gastric cancer with 19 cases (40). A previous study with an English Caucasian population found that the prevalence of *GSTM1* null genotype was 52.9% among gastric cancer cases and 54.8% among controls (19), which is consistent with our results. In our study, the prevalence of *GSTM1* null genotype was 48.3% among gastric cancer cases and 50% among controls. We observed no association between *GSTM1* genotype and either gastric cancer or chronic gastritis. This result is also consistent with a previous study which found no association between *GSTM1* null genotype and gastric cancer in a Japanese population (18).

From two previous studies (17, 19), no association was observed between *GSTT1* and gastric cancer in a Japanese and a English Caucasian population. However, we found that the prevalence of *GSTT1* null was higher in gastric cancer cases (54%) than in chronic gastritis patients (48%) and controls (45%). The OR was 2.50 (95% CI, 1.01–6.22) for gastric cancer cases with *GSTT1* null genotype after controlling for age, gender, education, pack-years of smoking, alcohol drinking, *H. pylori* infection, and salt and fruit intake. No association was found between *GSTT1* genotype and chronic gastritis.

Logistic regression was used to assess the interactions (or effect modifications) between *GSTT1* and *GSTM1* null genotype and other possible risk factors of gastric cancer. Because of the limited number of cases, only univariate analysis was used to explore the possible interactions with smoking, fruit and salt intake, alcohol drinking, *H. pylori* infection, BMI, and family history of gastric cancer. Both GSTM1 and GSTT1 enzymes can catalyze the detoxification of compounds in cigarette smoke (3, 4). We observed possible interactions that were more than multiplicative between *GSTM1, GSTT1*, and smoking in gastric cancer. The ORs of 5.00 (95% CI, 1.50–16.72) and 8.11 (95% CI, 2.14–30.75) were observed in the heavier smokers (pack-year > 20) and *GSTM1* or *GSTT1* null type, respectively. This result of possible interaction between the *GSTM1* null and the heavy smoker category was consistent with one study (17). No previous studies have shown the possible interaction in gastric cancer between the *GSTT1* and smoking. The results of interactions need to be interpreted with caution because of the limited sample size.

For family history of gastric cancer, we observed the risk in individuals with family history of gastric cancer only with normal *GSTM1* and regardless of their *GSTT1* status. However, there were very few numbers of subjects for the strata (five cases and four controls); thus, the observed association may be due to chance.

A previous study found that *GSTM1* null genotype was more prevalent in gastric cancer cases with *H. pylori* infection than without the infection (41). We did not find this interaction in our study, and this is consistent with one other study (18). Interestingly, an increase in *GSTT1* activity has been associated with high cruciferous vegetable intake (21). Because vegetable and fruit intakes have been shown consistently as protective factors in gastric cancer, there may be an association between *GSTT1* activity and fruit intake. Unfortunately, the number of our gastric cancer cases was limited and, therefore, limited us in the assessment of additional possible interactions when controlling for potential confounding factors.

In conclusion, our results suggest that the *GSTT1* genotype may be associated with gastric cancer in a Chinese population. Studies with large sample size and detailed data on fruit and vegetable intake or other possible risk factors of gastric cancer are needed to replicate our results.

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