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Isothiocyanates, *glutathione S-transferase M1* and *T1* polymorphisms and gastric cancer risk: a prospective study of men in Shanghai, China

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Abstract

Isothiocyanates (ITC) in cruciferous vegetables may be chemopreventive against gastric cancer development. Glutathione *S*-transferases (GSTs) may modify the chemopreventive effect of ITC. The relationship between urinary total ITC and risk of gastric cancer was prospectively examined. Between 1986 and 1989, 18,244 middle-aged men in Shanghai, China were enrolled in a prospective study of diet and cancer and donated baseline urine and blood samples. Urinary ITC was quantified for 307 incident cases of gastric cancer that occurred during the first 16 years of follow-up, and 911 matched control subjects. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression methods. Seropositivity for antibodies to *Helicobacter pylori* and homozygous deletions of *GSTM1* and *GSTT1* were determined. Compared with the first tertile, ORs (95% CIs) of gastric cancer for the second and third tertiles of urinary total ITC were 0.83 (0.61–1.15) and 0.66 (0.47–0.94) (P_{trend}=0.02). A stronger protective effect of ITC against gastric cancer development was seen among men with homozygous deletion of *GSTM1* (third tertile versus first tertile, OR = 0.50, 95% CI = 0.27–0.93) or *GSTT1* (third tertile versus first tertile, OR = 0.47, 95% CI = 0.25–0.88), and particularly with deletions of both *GSTM1* and *GSTT1* (second and third tertiles versus first tertile, OR = 0.44, 95% CI = 0.21–0.93). In this cohort of Chinese men at high risk for gastric cancer, isothiocyanates may protect against the development of gastric cancer. The protection may be stronger for individuals genetically deficient in enzymes that metabolize these chemopreventive compounds.

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Novelty: The present study demonstrated an inverse association between the levels of total isothiocyanates in urine collected prior to cancer diagnosis and the risk of developing gastric cancer in humans. The inverse association was stronger in individuals lacking glutathione S-transferase M1 and T1.

Impact: Dietary isothiocyanates may protect against the development of gastric cancer. This protective effect may be modulated by *glutathione S-transferase* genotypes. The findings of the present study support that increased dietary intake of isothiocyanates may be a viable, cost-effective chemoprevention strategy for primary prevention of gastric cancer.

Keywords

cruciferous vegetables; ITC; *GST* polymorphisms; urinary biomarkers; gastric cancer

Introduction

Although the incidence and mortality rates of gastric cancer have declined over the past several decades, it remains the fourth most commonly diagnosed cancer and second most common cause of death from cancer worldwide.[1, 2] Incidence rates vary considerably across different regions. The highest rates are recorded in Asian countries such Japan and China where the age-standardized (world standard) incidence rates per 100,000 are as high as 40–60 in men and 20–25 in women, compared to 7.2 in men and 3.3 in women in the United States.[1, 3] The prognosis of gastric cancer is poor due to lack of effective therapeutic choices. Approximately only one in four patients with gastric cancer in the U.S. would survive for five years after cancer diagnosis.[4] Identification of viable chemopreventive agents for primary prevention against the development of gastric cancer would have great impact on both morbidity and mortality of this malignancy in humans.

Gastric cancer is a multi-factorial disease in which both environmental and genetic factors may contribute to its carcinogenesis process. Infection with the bacterium *Helicobacter pylori*, a gram-negative bacillus that colonizes the stomach, has been shown in epidemiologic studies to be a primary risk factor for gastric cancer.[5–7] The prevalence of infection with *H. pylori* highly correlates with the incidence of gastric cancer worldwide.[5] Prospective cohort studies including ours, have established an unequivocal link between infection with *H. pylori* and risk of gastric cancer.[6–8] Experimental studies demonstrated that infection with *H. pylori* resulted in the development of gastric tumors in infected animals.[9, 10] A randomized clinical trial demonstrated that eradication of the *H. pylori* bacterium with antibiotics reduced progression of intestinal metaplasia and enhanced the regression of multifocal non-metaplastic atrophy and intestinal metaplasia, precancerous lesions, in the stomach of high-risk individuals.[11] In 1994, The International Agency for Research on Cancer (IARC) concluded that *H. pylori* is a group I carcinogenic agent in humans.[12]

Dietary factors are believed to play an important role in the development of gastric cancer. Nitrosamines formed from the reaction of nitrites with other nitrogen-containing compounds present in preserved food are potential carcinogens for the stomach in humans.[13, 14] Dietary antioxidants can inhibit the process of nitrosation and are believed to exert protective effects on gastric carcinogenesis. There is a large body of literature on dietary antioxidants in relation to risk of gastric cancer in humans.[14] Individuals with low serum levels of vitamin C, carotenes, and lycopene at baseline experienced increased risk of developing gastric cancer.[15] A randomized clinical trial demonstrated that supplementation with vitamin C and beta-carotene for 72 months increased the rate of regression of precancerous lesions in the stomach in humans.[11] These findings implicate that oxidative stress due to nitrosamines is one possible mechanism of gastric carcinogenesis and dietary antioxidants may protect against the development of gastric cancer.

Besides antioxidants, data on the role of dietary bioactive compounds, such as isothiocyanates (ITC), in the development of gastric cancer are sparse. ITC, derived from glucosinolates in cruciferous vegetables such as broccoli, bok choi, cabbage, and watercress, has antioxidative properties and chemopreventive effects on the development of cancer of the lung and colon.[16, 17] In addition, in vitro and in vivo experimental studies have demonstrated that ITC possesses bactericidal properties against the *H. pylori* bacterium.[18–

20 Sulforaphane (a form of ITC) is bactericidal to both extracellular and intracellular forms of *H. pylori* and can inhibit tumor development in the forestomach of infected mice.18 Sulforaphane can also eradicate *H. pylori* in human gastric xenografts on nude mice.19 These data suggest that ITC is a potential chemopreventive agent against gastric cancer in humans.

Glutathione *S*-transferases (GSTs) belong to a super family of Phase II detoxifying enzymes which catalyze the conjugation of glutathione with activated carcinogens such as benzo[*a*]pyrene as well as other xenobiotics including ITC, for rapid urinary excretion.17, 21 Seven cytosolic isoenzymes of GSTs are expressed in human tissue: alpha, mu, pi, theta, zeta sigma and omega.22, 23 *GSTM*, *GSTT*, *GSTP* and *GSTA* are known to be polymorphic in humans24, 25 with homozygous deletions of *GSTM1* and *GSTT1* (null genotype) resulting in the abolishment of respective enzyme activity.26 As GSTs are involved in the detoxification of activated carcinogens as well as ITC, it is plausible that observed inter-individual variation of cancer susceptibility may be partially due to the polymorphic nature of *GSTM1* and *GSTT1*. We and others have found that the *GSTM1* and *GSTT1* genotypes modify the protective effect of ITC or cruciferous vegetables on risk of developing lung and colon cancers.27–31 We hypothesized that the individuals with *GSTM1 null* and/or *GSTT1 null* genotypes would derive a greater beneficial effect from ITC in protecting against the development of gastric cancer.

The present study used a specific, validated urinary biomarker for total ITC32, 33 to examine the relation between levels of total ITC in urine collected before cancer diagnosis and the risk of subsequently developing gastric cancer in the Shanghai Cohort Study, a prospective cohort of 18,244 middle-aged and older men in Shanghai, China, with up to 16 years of follow-up for incident cancer. We updated the results on the association between seropositivity of antibodies to *H. pylori* with 4 additional years of follow-up since our last report.7 The present study also examined the possible modifying effects of *GSTM1* and *GSTT1* genotypes on the ITC-gastric cancer risk association.

Materials and Methods

Study Population

The design of the Shanghai Cohort Study has been previously described in detail elsewhere.34, 35 Briefly, all male residents between the ages of 45 and 64 years of four geographically defined communities in Shanghai, with no prior history of cancer were invited to participate in a prospective study of diet and cancer. Between January 1, 1986 and September 30, 1989, 18,244 men (representing approximately 80% of the eligible subjects) were enrolled in the study. At enrollment, each participant was interviewed in person using a structured questionnaire to obtain demographic information, history of tobacco and alcohol use, usual adult diet and medical history. This study had been approved by the Institutional Review Boards at the University of Minnesota and the Shanghai Cancer Institute.

During the baseline interview, each participant was asked if he had ever smoked at least one cigarette per day for 6 months or more. If he answered yes, he was classified as a smoker. For smokers, information concerning current smoking status (yes, no), number of cigarettes smoked per day and number of years of smoking over lifetime was also obtained. For former smokers, the number of years since quitting was also recorded. Each participant was also asked if he had ever drunk alcoholic beverages at least once a week for 6 or more months. If he answered yes, information concerning the typical amount of beer, wine or spirits consumed per week, separately, was obtained. One drink was defined as 360 g of beer (12.6 g of ethanol), 103 g of wine (12.3 g of ethanol) or 30 g of spirits (12.9 g of ethanol).36 Current diet was assessed through a food frequency questionnaire that included 45 food

items that represented commonly consumed local foods. The baseline interview did not include any specific cruciferous vegetables.37

Following completion of the interview, a 10 ml nonfasting blood sample and a single-void (i.e. spot) urine sample were collected from each participant. Biospecimens were usually collected between 5 p.m. and 9 p.m., on average approximately 3 hours after the last meal. Urine samples were immediately placed in an ice box and transported on the same day to the processing laboratory where they were stored at 4°C. Urine samples were aliquoted into two vials with 10 ml each and an additional vial containing 25 ml the following morning. All aliquots were stored at −20 °C until 2001 when they were transferred to −70 °C until analysis. Beginning September 1, 2001, all surviving cohort members (N=14,531) were asked to donate buccal cell samples for DNA analysis. At the end of 2002, buccal cell samples had been obtained from 13,816 original cohort members (95% of surviving subjects).

Annual follow-up of the cohort for incident cancers and deaths has been carried out since 1986. We contacted all surviving cohort members in-person annually and performed record linkage analysis with databases of the Shanghai Cancer Registry and Shanghai Municipal Vital Statistics. As of September 2002 (after an average of 12.5 years of follow-up), the cutoff date for case ascertainment of the present study, the cumulative losses to follow-up were 481 (2.6%) subjects (i.e. their vital status was unable to be determined via routine ascertainment methods).

Case Patients

As of September 2002, a total of 312 incident gastric cancer cases had been identified. Of the 312 cases, 269 (86.2%) were confirmed with histopathology, 5 (1.6%) were confirmed with cytology or surgery without biopsy, 30 (9.6%) were confirmed with radiology, and the remaining 8 (2.6%) were diagnosed based on clinical symptoms alone.

Control Subjects

For each case patient with gastric cancer, 3 control subjects were randomly selected from cohort members who were free of cancer and alive at the time of cancer diagnosis of the index case. Controls (n = 936) were matched to the index case by date of birth (within two years), date of biospecimen collection (within one month) and neighborhood of residence at recruitment.

Laboratory Methods

Total urinary ITC was measured as a cyclocondensation product of reaction with 1,2-benzenedithiol by high-performance liquid chromatography as previously described.32, 33 The intra-assay coefficient of variation within the batch was less than 5% and the limit of detection for the urinary isothiocyanate was 0.1 μmol/L. Urinary isothiocyanate concentrations are stable on long-term storage at −20°C.32, 33 Urinary creatinine (Cr) concentration was determined on each sample using a modified method as described previously.38 Urine samples on 5 cases were depleted after measurements of other urinary biomarkers; thus these 5 cases and their matched controls were excluded from the present study. We excluded additional 10 controls from the present analysis due to the depletion of their urine samples after measurements of other biomarkers. Thus, the present study included 307 cases and 911 matched controls with known ITC values.

An enzyme-linked immunosorbent assay (ELISA) based on local bacterium strains was used for the determination of the presence of *H. pylori* antibodies in serum collected before

diagnosis of cancer on 265 case patients and 879 control subjects. The ELISA method was previously described in detail.7

DNA was extracted from serum for the first 197 gastric cancer cases and their 591 matched control subjects. The method for DNA extraction from serum was previously described in detail.28 Sufficient amount of DNA was obtained from serum samples for 132 cases and 422 controls. For the latter case-control sets and those without sufficient serum DNA, we extracted DNA only from buccal cell samples to preserve serum samples for future measurement of biomarkers. The standardized method for DNA exaction from buccal cell samples was reported before.29, 39 The determination of *GSTM1* and *GSTT1* genotypes were carried out by using the TaqMan assays as described previously.29, 39 Informative *GSTM1* and *GSTT1* genotypes were obtained in 171 and 172 cases, respectively, and 749 and 752 controls, respectively. We examined and found no differences in the distributions by level of education, body mass index, cigarette smoking, alcohol consumption, seropositivity of antibodies to *H. pylori* between subjects with known and unknown GST genotypes among cases (170 versus 137) and controls (735 versus 176). The independent effects of *GSTM1* and *GSTT1* genotypes on the risk of gastric cancer was examined among the 170 cases and 735 controls with known genotypes of both *GST* genes. When examining the modifying effects of *GSTM1* and *GSTT1* genotypes on the ITC-gastric cancer risk association, 152 cases and 713 controls with both known serologic status of antibodies to *H. pylori* and *GSTM1* and *GSTT1* genotypes were included in statistical analysis.

Serum concentrations of specific carotenoids including α-carotene, β-carotene, β -cryptoxanthin, lycopene, and lutein/zeaxanthin were determined by high-performance liquid chromatography using methods previously described40, 41 while serum vitamin C was quantified using a method developed by Roe and Kuether42 with modification.43 In a previous analysis, we identified inverse associations between serum concentrations of β-carotene, lycopene and vitamin C and risk of gastric cancer.15

Statistical Analysis

The χ^2 test and the *t*-test were used to compare the distributions of selected demographics, cigarette smoking, alcohol consumption, and seropositivity of *H. pylori* antibodies between cases and controls. Urinary ITC levels were expressed in units of urinary creatinine (μmol/g Cr) to correct for varying water contents of individual spot urine samples. The distribution of urinary ITC levels was markedly skewed towards higher values, which was corrected to a large extent by transformation to logarithmic values. Therefore, formal statistical testing on continuous values of ITC was performed on logarithmically transformed values and the geometric means of ITC and their 95% confidence intervals (95% CI) are presented. The analysis of variance (ANOVA) method was used to examine the difference in urinary ITC concentrations between incident gastric cancer cases and their matched control subjects. Standard statistical methods for case-control studies were used in the present study. Conditional logistic regression models were used to calculate matched odds ratios (ORs), their corresponding 95% confidence intervals (CIs) and *P*-values. Study subjects were grouped by tertiles of total ITC defined by the distribution in all controls. As *H. pylori* is an established risk factor for gastric cancer, in order to adjust for the potential confounding effect of antibody status, matched case-control sets were broken to maximize the available sample size for analysis. Unconditional logistic regression models were used to calculate adjusted ORs and their associated 95% CIs and *P*-values in subjects with known antibody status. Unconditional logistic regression models included the matching variables age, number of years of sample storage and neighborhood of residence at baseline as well as the following covariates: level of education (no formal schooling or primary school, junior middle school, senior high school, and college or above), number of alcoholic drinks per day (none, <2, 2-<4, and 4 or more), smoking status (never, ever) and the presence of *H. pylori*

antibodies in serum (no, yes). We also examined the ITC-gastric cancer association with adjustment for serum levels of β-carotene, lycopene and vitamin C that were inversely related to the risk of developing gastric cancer in the study population.15 The adjustment for those serum antioxidants, which were available for 188 cases and 572 controls, did not materially change the inverse association between urinary total ITC and risk of gastric cancer and were not included in the final models.

To maximize the available sample size for assessing the modifying effect of *GSTM1* and *GSTT1* genotypes on the ITC-gastric cancer association, matched case-control sets were broken. Unconditional logistic regression models were used to estimate ORs and their corresponding 95% CIs. All unconditional logistic regression models included the matching variables and covariates listed above.

All statistical analyses were carried out using SAS software version 9.1 (SAS Institute, Cary, NC). All *P* values reported are two-sided, and those that were less than 0.05 were considered to be statistically significant.

Results

The present study included 307 case patients with gastric cancer and 911 matched controls. The mean age (± standard deviation) of case patients at cancer diagnosis was 65.30 (± 6.54) years. The corresponding figure for matched control subjects at the time of cancer diagnosis of index cases was 66.06 (± 6.88) years. The average time interval between biospecimen collection and cancer diagnosis was 7.59 (± 4.29) years, ranging from 1 month to 15.5 years.

Case patients had comparable body mass index (kg/m^2) to controls. Individuals who developed gastric cancer were less educated, consumed more cigarettes and alcohol, and were more likely to be infected with *H. pylori* than their counterparts who remained free of cancer (Table 1). Compared with never smokers, ever smokers had a statistically significant 51% increased risk of gastric cancer (OR=1.51, 95% CI=1.11–2.06). Heavy drinkers of alcoholic beverages (4 or more drinks/day) had a statistically significant 76% increased risk of gastric cancer (OR=1.76, 95% CI=1.09–2.85) compared to non-drinkers.

Of the 265 cases with known serologic status, 240 (91%) were positive for antibodies to *H. pylori*. The corresponding figure in the 879 control subjects with known status on *H. pylori* serology was 727 (83%) (Table 1). Compared with individuals negative for *H. pylori* antibodies, the OR (95% CI) of gastric cancer for *H. pylori* positive subjects was 1.95 (1.24, 3.07) (*P*-value = 0.004) (Table 2). The risk increased with increasing duration of follow-up; ORs (95% CIs) were 1.13 (0.60–2.14) for those with <4 years of follow-up and 2.85 (1.54–5.28) for those with 4 or more years of follow-up (Table 2). The difference in the two ORs were statistically different from each other (*P*-value = 0.03). There was no further increase in risk of gastric cancer associated with seropositivity of *H. pylori* with longer (8 or more years) duration of follow-up.

Of the 170 case patients with known *GST* genotypes, 98 (58%) possessed *GSTM1 null* genotype, 97 (57%) *GSTT1 null* genotype, and 55 (32%) both *GSTM1 null* and *GSTT1 null* genotypes. There were similar frequencies of *GSTM1* and/or *GSTT1 null* genotypes in control subjects (Table 1). There was no main effect of *GSTM1* and *GSTT1* genotypes on gastric cancer risk. The ORs (95% CIs) for men carrying *null* compared to *non-null* genotypes of *GSTM1* and *GSTT1* were 1.03 (0.72–1.46) and 1.04 (0.73–1.48), respectively. Similarly, the OR (95% CI) was 1.06 (0.63–1.78) for men carrying both *GSTM1 null* and *GSTT1 null* genotypes compared with those with non-null genotypes of both *GST* genes. The null association between *GST* genotypes and gastric cancer risk was present in both

smokers and nonsmokers (all *P* values for smoking × *GST* genotype interaction > 0.2) (data not shown).

The geometric means (95% CIs) of urinary total ITC in case patients and their matched controls were 1.18 (0–6.25) and 1.37 (0 –7.98), respectively (*P*=0.03). Urinary ITC was detectable in 285 (92.8%) cases and 856 (94.0%) controls, yielding an OR of 0.78 (95% CI = 0.43–1.41) as compared with undetectable ITC. High levels of ITC were associated with decreased risk of developing gastric cancer. Compared with the first tertile, the matched ORs (95% CI) of gastric cancer for the second and third tertiles were 0.83 (0.61–1.15) and 0.66 (0.47–0.94), respectively (*P* for trend = 0.02) (Table 3). Further adjustment for seropositivity of *H. pylori*, cigarette smoking, alcohol consumption, and level of education did not materially alter the inverse association between ITC and gastric cancer risk (Table 3). We also examined the ITC-gastric cancer association across time interval between urine collection and cancer diagnosis and found no decrease in risk of developing gastric cancer associated with high ITC within 4 years post enrollment; ORs (95% CIs) for the second and third tertiles of total ITC were 0.75 (0.41–1.37) and 1.02 (0.59–1.76), respectively (*P* for trend = 0.88). However, the greatest decrease in risk of developing gastric cancer associated with high levels of urinary ITC was observed in subjects with 4 or more years of follow-up; ORs (95% CIs) for the second and third tertiles of total ITC were 0.93 (0.65–1.33) and 0.58 (0.38–0.88), respectively (*P* for trend = 0.01). The inverse association between ITC and risk of developing gastric cancer remained in subjects with 8 or more years of follow-up; ORs (95% CIs) for the second and third tertiles of total ITC were 0.87 (0.56–1.35) and 0.63 (0.38–1.04), respectively, although the trend test was statistically borderline significant, due to the reduced number of cases included in the analysis (152 cases, *P* for trend = 0.08).

When we examined the modifying effect of *GST* genotypes on the ITC-gastric cancer association, we restricted our analyses to 152 cases and 713 controls with known *GST* genotypes and known serologic status of *H. pylori*. A more apparent inverse association between ITC and gastric cancer risk was present among individuals possessing null genotypes of *GSTM1* or *GSTT1*, especially among those with the null genotypes of both *GSTM1* and *GSTT1* genes (Table 4). Compared with the first tertile, the ORs (95% CIs) for the second and third tertiles of ITC were 0.43 (0.19–1.02) and 0.45 (0.19–1.09) (*P*-value for trend = 0.07), respectively. OR (95% CI) for the second and third tertiles combined was 0.44 (95% CI = 0.21–0.93) (*P*-value = 0.03).

We examined the differential effect of ITC on gastric cancer between individuals with and without antibodies to *H. Pylori*. The protective effect of ITC on gastric cancer was seen primarily in subjects with seropositive antibodies to *H. pylori*; the ORs (95% CIs) for the second and third tertiles of total ITC were 0.89 (0.62–1.27) and 0.63 (0.43–0.92), respectively (*P* for trend = 0.02) compared with the first tertile (data not shown). We also examined the ITC-gastric cancer association in subjects stratified by status of cigarette smoking and alcohol drinking. A statistically significant, inverse ITC-gastric cancer risk association was present in non-smokers or non-drinkers, but not in smokers or drinkers. Among never smokers, the ORs (95% CIs) for the second and third tertiles of total ITC were 0.78 (0.44–1.37) and 0.46 (0.25–0.84), respectively (*P* for trend = 0.01) relative to the first tertile. The corresponding ORs (95% CIs) among ever smokers were 0.89 (0.60–1.33) and 0.87 (0.57–1.33), respectively (*P* for trend = 0.50). Similarly, ORs (95% CIs) for the second and third tertiles of ITC were 0.72 (0.46–1.13) and 0.51 (0.32–0.86), respectively (*P* for trend = 0.006) among non-drinkers; and 1.03 (0.64–1.66) and 0.97 (0.59–1.61) among drinkers of alcoholic beverages (*P* for trend = 0.92).

Discussion

The present study demonstrated that high level of urinary total ITC was associated with a statistically significant, 30–35% decrease in risk of gastric cancer in middle-aged or older men in Shanghai, China. This protective effect of ITC on gastric cancer was stronger in men possessing the homozygous deletion polymorphisms of either *GSTM1* or *GSTT1* gene. These results showed a direct link between specific degradation products of cruciferous vegetables (i.e., ITCs) and reduced risk of developing gastric cancer in humans.

Few laboratory studies have examined the chemopreventive properties of ITC on gastric tumorigenesis. Feeding sulforaphane, an ITC that is abundant in broccoli, to mice following dosing of benzo[*a*]pyrene significantly reduced the number of carcinogen-induced gastric tumors in experimental animals.18 There are a limited number of epidemiologic studies that investigated the effect of cruciferous vegetables on gastric cancer risk in humans. The results are mixed.44 In a hospital-based case-control study conducted in Japan, high consumption of broccoli and Chinese cabbage was associated with a borderline statistically significant, reduced risk of gastric cancer.45 A case-cohort study in Hawaiian men of Japanese ancestry demonstrated a statistically nonsignificant, inverse association between cruciferous vegetable consumption and gastric cancer risk after adjusting for age and smoking.46 A recent case-control study in Hawaii failed to find a statistically significant inverse association between cruciferous vegetable consumption and gastric cancer in *H.pylori/*CagA-negative individuals, but suggested a decreasing risk with increasing intake of cruciferous vegetables among *H.pylori/*CagA-positive individuals (P_{trend}=0.12).47 In the present study, we used a validated urinary biomarker for ITC intake and demonstrated a statistically significant inverse association between urinary ITC and the risk of developing gastric cancer.

The modification of ITC chemoprevention of gastric cancer by polymorphisms of *GSTM1* and *GSTT1* is biologically plausible. GST-catalyzed conjugation with glutathione aids in the elimination of not only environmental carcinogens but also of anticarcinogenic substances in the diet, such as ITCs. Conjugation of ITC with glutathione, a reaction catalyzed by GSTs, constitutes the major route of ITC metabolism.33, 48 ITC are among the GST substrates that are most rapidly conjugated.48 Thus, individuals with abolished GST enzymes, resulting from the homozygous deletion of *GSTM1* and/or *GSTT1* gene, would derive more beneficial effect of dietary ITC on the inhibition of carcinogenesis. Within Chinese populations in Shanghai, China and Singapore, we have demonstrated the greatest cancer risk reduction associated with cruciferous vegetable consumption among individuals carrying null or low activity *GST* genotypes.28, 31 A recent feeding study showed a statistically significant, elevated plasma levels of sulphoraphane metabolites in *GSTM1 null* individuals compared to their *GSTM1 non-null* counterparts who consumed comparable amounts of broccoli, supporting the notion that subjects lacking functional enzymes have higher in vivo exposure to ITC and may derive greater beneficial effect from consumption of cruciferous vegetables. 49 Paradoxically, the same feeding study and another one-dose broccoli feeding study also showed higher urinary excretion of ITC in *GSTM1 null* subjects compared to *GSTM1 non-null* subjects, 49, 50 suggesting the complexity of ITC metabolism in humans.

The present study confirms our previous findings that history of infection with *H. pylori* is associated with a significantly increased risk of gastric cancer,7 particularly among individuals with relatively long duration of follow-up. These results were consistent with findings by others.51 Infection with *H. pylori* is an established risk factor for gastric cancer in humans7, 8, 13,51 and it is possible that the observed inverse association between urinary ITC levels and gastric cancer risk may be due to the bactericidal properties of ITC. Several laboratory studies have shown that ITC have various levels of antibacterial activity, and

sulforaphane, a specific ITC, possesses bactericidal properties against both antibiotic sensitive and resistant strains of *H. pylori* bacterium.18–20 Sulforaphane could inhibit both extracellular and intracellular forms of *H. pylori* in *in vitro* and *in vivo* studies.18, 19 While the exact mechanism of how ITC inhibits the *H. pylori* bacteria remains unknown, it is suspected that the inhibitory effects of ITC on *H. pylori* growth may be related to its intracellular accumulation in mammalian cells.18 Although not statistically significant, recent data from Hawaii suggests an inverse association between cruciferous vegetable intake and risk of gastric cancer among *H. pylori*/CagA-positive but not *H. pylori*/CagA-negative individuals,47 consistent with the notion of an antibacterial mechanism for the chemoprotection effect of cruciferous vegetables on gastric cancer.

In addition to its inhibition on *H. pylori*, ITC may protect against the development of gastric cancer through other mechanisms. ITC can influence the metabolism of procarcinogens via inhibition of phase I enzymes such as cytochrome P450 (CYP) enzymes, which are involved in the activation of procarcinogens.52 In NNK-treated rats, phenethyl ITC selectively inhibits specific CYP enzymes, most likely, CYP2A3 and CYP2A13.53, 54 Experimental studies have shown that ITC could block the formation of carcinogen-induced DNA adducts in rats and mice.55–57 Administration of benzyl ITC prior to dosing of carcinogen almost completely inhibited the development of benzo(*a*)pyrene-induced forestomach tumors in mice.58 Also, ITC can induce phase II enzymes such as GSTs, which can enhance the conjugation of activated carcinogens with glutathione, thus increasing their urinary excretion.59 Finally, the chemopreventive effect of ITC on cancer may be mediated through its ability to induce apoptosis. It has been demonstrated that dietary ITC can induce apoptosis in the gastrointestinal tract,60 thereby preventing colonal expansion of damaged cells and eventual formation of cancer in the stomach.

The present study had several strengths. We used a validated biomarker to assess total ITC exposure.33 We adjusted for the potential confounding effects of *H. pylori* infection, cigarette smoking, alcohol drinking, and serum antioxidants on the ITC-gastric cancer association. The prospective cohort study design ruled out the possibility of recall bias on exposure. For most cases, baseline blood and urine samples were collected many years prior to cancer diagnosis. Thus, the concern over a possible impact of clinical manifestation of cancer on the metabolism of ITC, resulting in altered levels of ITC in urine, among cases with a short follow-up duration is minimized. The long duration of follow-up (up to 16 years) allowed the present study to examine the long-term protective effect of ITC against the development of gastric cancer. The almost complete follow-up for incident cancer and death minimized the potential bias on results due to the loss to follow-up.

There are also several potential limitations to this study. One cannot presume that ITC level in a randomly-timed, single void urine sample correlates with usual intake of dietary ITC in an individual. However, we previously demonstrated among Chinese in Singapore, a population that shares a similar cultural and dietary heritage as our study population in Shanghai, a close and statistically significant correlation between dietary ITC ascertained from a validated food frequency questionnaire and total ITC levels in a randomly-timed spot urine.33 In the Shanghai Cohort Study, intake of specific cruciferous vegetables was not solicited at baseline. Therefore, the association between dietary ITC and gastric cancer risk cannot be assessed in this report. In a cohort study, it is advantageous to assess exposure at multiple time points prior to disease occurrence. However, in the case of biomarkers, it is rarely feasible due to the high cost and logistical complexity in collecting biospecimens from large numbers of cohort participants. Using this same biomarker approach based on a single spot urine, we have established an inverse association between dietary intake of ITC and lung cancer in this cohort study.28 Our findings were subsequently confirmed by others.30, 61 It is biologically plausible that the protective effect of ITC on gastric cancer is

mediated through the former's inhibitory actions on *H. pylori*. However, only 12 cases in the present analysis can be considered truly *H. pylori* negative (see Table 2). Thus, this study is incapable of examining whether there exists an interaction effect between ITC and *H. pylori* infection on gastric cancer.

Smokers consumed less amounts of cruciferous vegetables,62 and thus showed lower levels of urinary ITC than nonsmokers (0.97 versus 1.15 μmol/g creatinine; $P = 0.04$). The lower consumption of cruciferous vegetables in smokers could potentially explain the weaker inverse association between ITC and gastric cancer in this subgroup versus the never smokers.

Cruciferous vegetables contain precursors of ITC and other bioactive compounds including indole-3-carbinol.16 Indole-3-carbinol has shown a tumor inhibitory effect in mice forstomach.63 The observed inverse association between urinary total ITC and gastric cancer risk may merely reflect the chemoprotective effects of other compounds that are present in cruciferous vegetables.

In summary, dietary ITC, as measured by urinary biomarkers of total ITC, may protect against the development of gastric cancer. Individuals with homozygous deletion of *GSTM1* and *GSTT1* genes may derive a greater beneficial effect of ITC on gastric carcinogenesis.

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Abbreviations

ITC	Isothiocyanates
GSTs	glutathione *S*-transferases
ORs	odds ratios
CIs	95% confidence intervals

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Table 1

Demographic and lifestyle characteristics of gastric cancer cases and controls, Shanghai Cohort Study

Demographic or lifestyle factor	Cases *n* (%)	Controls *n* (%)	*P*[*]
Total subjects	307 (100.0)	911 (100.0)	
Age at interview (year)[†]	58.2 (5.3)	58.2 (5.2)	0.86
Body mass index (kg/m^2)[†]	22.4 (3.3)	22.2 (3.1)	0.39
Level of education			
No formal schooling or primary	118 (38.4)	275 (30.2)	0.004
Jr. middle school	98 (31.9)	283 (31.1)	
Senior high school	40 (13.0)	168 (18.4)	
College graduates or above	51 (16.6)	185 (20.3)	
Alcohol drinking on a weekly basis			
Non-Drinkers	156 (50.8)	519 (57.0)	0.06
Regular Drinkers	151 (49.2)	392 (43.0)	
No. of alcoholic beverages per day			
Non-Drinkers	156 (50.8)	519 (57.0)	<0.001
<2	71 (23.1)	236 (25.9)	
2–<4	36 (11.7)	95 (10.4)	
4+	44 (14.3)	61 (6.7)	
No. of years of alcohol drinking			
Non-Drinkers	156 (50.8)	519 (57.0)	0.09
<20	36 (11.7)	109 (12.0)	
20–<40	84 (27.4)	186 (20.4)	
40+	31 (10.1)	97 (10.6)	
Cigarette smoking			
Never Smokers	96 (31.3)	398 (43.70)	<0.001[‡]
Ever Smokers	211 (68.7)	513 (56.3)	
Former smokers	32 (10.4)	81 (8.9)	
Current smokers	179 (58.3)	432 (47.4)	<0.001[§]
No. of years of smoking			
Never smokers	96 (31.3)	398 (43.7)	<0.001
<30	79 (25.7)	187 (20.5)	
30+	132 (43.0)	326 (35.8)	
No. cigarettes per day			
Never smokers	96 (31.3)	398 (43.7)	0.007
<20	111 (36.2)	240 (26.3)	
20+	100 (32.6)	273 (30.0)	
H. pylori antibody serologic status[//]			
Negative	25 (9.4)	152 (17.3)	<0.001
Positive	240 (90.6)	727 (82.7)	
GSTM1[**]			

Demographic or lifestyle factor	Cases *n* (%)	Controls *n* (%)	*P*[*]
Non-null	72 (42.4)	320 (43.5)	0.78
Null	98 (57.6)	415 (56.5)	
GSTT1[**]			
Non-null	73 (42.9)	320 (43.5)	0.96
Null	97 (57.1)	415 (56.5)	
GSTM1 and *GSTT1*[**]			
Both or either non-null	115 (67.6)	504 (68.6)	0.82
Both null	55 (32.4)	231 (31.4)	

[*] *P*-values (2-sided) were derived from *t*-test (for means) or χ^2 (for frequencies) statistics.

[†] Means and standard deviations in parentheses.

[‡] P-value refers to the comparison between never and ever smoker.

[§] P-value refers to the comparison among never, former and current smokers.

[//] 42 cases and 32 controls with unknown status on antibodies to *H. pylori* were excluded from this analysis.

[**] 137 cases and 176 controls with unknown genotype of *GSTM1* and/or *GSTT1* were excluded from this analysis.

Table 2

Status on seropositivity of antibodies to *H. pylori* in relation to risk of gastric cancer stratified by follow-up time, Shanghai Cohort Study

	H. Pylori Negative		H. Pylori Positive		
	N	**OR**	**N**	**OR (95% CI)***	***P*-value**
Controls	152	---	727	---	---
Cases by number of years between blood draw and cancer diagnosis					
All combined	25	1.00	240	1.95 (1.24, 3.07)	0.004
<4 years	13	1.00	69	1.13 (0.60, 2.14)	0.70
4+ years	12	1.00	171	2.85 (1.54, 5.28)	<0.001
8+ years	8	1.00	110	2.63 (1.24, 5.59)	0.01

*Analysis includes 265 cases and 879 controls with known status on seropositivity of antibodies to *H. pylori.* Odds ratios (ORs) were derived from coefficient estimates of unconditional logistic regression models that included the following covariates: age, number of years of sample storage, neighborhood of residence at baseline, level of education (No formal schooling or primary school, junior middle school, senior high school, and college or above),number of alcoholic drinks per day (none, <2, 2–<4, and 4 or more), cigarette smoking (never, ever); OR, odds ratio;CI, confidence interval.

Table 3

Urinary levels of total isothiocyanates (ITC) in relation to risk of gastric cancer, Shanghai Cohort Study

ITC in tertiles (μmol/g Cr)*	Cases	Controls	Matched OR (95% CI) †	Fully Adjusted OR (95% CI) ‡
T1 (0.30)	122	312	1.00	1.00
T2 (1.01)	102	299	0.83 (0.61, 1.15)	0.88 (0.63, 1.24)
T3 (3.32)	83	300	0.66 (0.47, 0.94)	0.67 (0.47, 0.96)
P for trend			0.02	0.03

*Median values of ITC per tertile are reported. Range of ITC (μmol/g Cr) per tertile: T1 (≤0.60), T2 (0.60–≤1.9), T3 (>1.9)

†Odds ratios (ORs) were derived from coefficient estimates of conditional logistic regression models; cases and controls were matched on date of birth (within 2 years), year and month of urine sample collection, and neighborhood of residence at baseline; CI, confidence interval.

‡Included 265 cases and 879 controls with known status on seropositivity of antibodies to *H. Pylori*. Odds ratio derived from coefficient estimates of unconditional logistic regression models which included the following covariates: age, number of years of sample storage, neighborhood of residence at baseline, level of education (No formal schooling or primary school, junior middle school, senior high school, and college or above), number of alcoholic drinks per day (non, <2. 2–<4, and 4 or more), cigarette smoking (never, ever) and seropositivity of antibodies to *H. Pylori* (negative, positive); OR, odds ratio; CI, confidence interval.

Table 4

Urinary levels of total isothiocyanates (ITC) in relation to risk of gastric cancer stratified by *glutathione S-transferase (GST) M1* and *GSTT1* genotypes, Shanghai Cohort Study in those with H. Pylori info

	Urinary Total ITC in tertile									
	T1 (low)			T2			T3 (high)			*P* for trend
	Cases*	Controls*	OR†	Cases*	Controls*	OR (95% CI) †	Cases*	Controls*	OR (95% CI) †	
All	63	244	1.00	49	240	0.71 (0.45, 1.10)	40	229	0.53 (0.33, 0.84)	0.007
GSTM1										
Non-Null	25	99	1.00	23	106	0.82 (0.42, 1.62)	17	106	0.52 (0.25, 1.08)	0.08
Null	38	145	1.00	26	134	0.59 (0.32, 1.07)	23	123	0.50 (0.27, 0.93)	0.03
GSTT1										
Non-Null	26	116	1.00	21	89	0.93 (0.47, 1.84)	17	104	0.66 (0.32, 1.36)	0.27
Null	37	128	1.00	28	151	0.59 (0.33, 1.07)	23	125	0.47 (0.25, 0.88)	0.01
GSTM1 and *GSTT1*										
Non-Null of both genes	9	46	1.00	10	40	1.53 (0.49, 4.74)	7	45	0.79 (0.24, 2.65)	0.74
Null of one gene only	33	123	1.00	24	115	0.65 (0.35, 1.22)	20	120	0.53 (0.28, 1.04)	0.06
Null of both genes	21	75	1.00	15	85	0.43 (0.19, 1.02)	13	64	0.45 (0.19, 1.09)	0.07
Null of one or both genes	54	198	1.00	39	200	0.62 (0.38, 1.02)	33	184	0.50 (0.30, 0.84)	0.007

*Including 152 cases and 713 controls with known status on seropositivity of antibodies to *H. Pylori* and known genotypes of both *GSTM1* and *GSTT1*.

†Odds ratios (ORs) were derived from coefficient estimates of unconditional logistic regression models that also included the following covariates: age, number of years of sample storage, neighborhood of residence at baseline, level of education (No formal schooling or primary school, junior middle school, senior high school, and college or above), number of alcoholic drinks per day (none, <2, 2–<4, and 4 or more), cigarette smoking (never, ever) and seropositivity of antibodies to *H. Pylori* (negative, positive); OR, odds ratio; CI, confidence interval.