Isothiocyanate exposure, glutathione *S*-transferase polymorphisms, and colorectal cancer risk[1–4]

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ABSTRACT

Background: Isothiocyanates, compounds found primarily in cruciferous vegetables, have been shown in laboratory studies to possess anticarcinogenic activity. Glutathione *S*-transferases (GSTs) are involved in the metabolism and elimination of isothiocyanates; thus, genetic variations in these enzymes may affect in vivo bioavailability and the activity of isothiocyanates.

Objective: The objective was to prospectively evaluate the association between urinary isothiocyanate concentrations and colorectal cancer risk as well as the potential modifying effect of *GST* genotypes on the association.

Design: A nested case-control study of 322 cases and 1251 controls identified from the Shanghai Women's Health Study was conducted.

Results: Urinary isothiocyanate concentrations were inversely associated with colorectal cancer risk; the inverse association was statistically significant or nearly significant in the *GSTM1*-null (P for trend = 0.04) and the *GSTT1*-null (P for trend = 0.07) genotype groups. The strongest inverse association was found among individuals with both the *GSTM1*-null and the *GSTT1*-null genotypes, with an adjusted odds ratio of 0.51 (95% CI: 0.27, 0.95), in a comparison of the highest with the lowest tertile of urinary isothiocyanates. No apparent associations between isothiocyanate concentration and colorectal cancer risk were found among individuals who carried either the *GSTM1* or *GSTT1* gene (P for interaction < 0.05).

Conclusion: This study suggests that isothiocyanate exposure may reduce the risk of colorectal cancer, and this protective effect may be modified by the *GSTM1* and *GSTT1* genes. *Am J Clin Nutr* 2010;91:704–11.

INTRODUCTION

Cruciferous vegetables, including cabbage, broccoli, bok choy, Brussels sprouts, kale, and cauliflower, are unique dietary sources of glucosinolates, which are the parent compounds of isothiocyanates and indoles (1). These glucosinolate break-down products are capable of inhibiting carcinogen-activating enzymes and regulating apoptosis and cell proliferation in cultured tumor cells (2, 3). Administration of crucifers or isothiocyanates to experimental animals has been shown to inhibit the development of colonic aberrant crypt foci (4, 5) and to reduce the incidence and multiplicity of chemical-induced tumors, including tumors of the colon and forestomach (6–8). Isothiocyanates are also potent inducers of phase II enzymes, which are involved in detoxifying potential endogenous and exogenous carcinogens (9, 10). It has been shown that intake of cruciferous vegetables effectively increases the urinary excretion of potential carcinogens such as the heterocyclic amines found in well-done meat (11, 12). This suggests that cruciferous vegetables or their constituent isothiocyanates may confer cancer chemopreventive effects in humans.

Exposure to isothiocyanates in vivo depends not only on dietary intake and absorption of isothiocyanates but on inherent capacity in isothiocyanate metabolism and excretion as well. Glutathione *S*-transferases (GSTs), the primary enzymes involved in isothiocyanate metabolism, catalyze the conjugation of isothiocyanates with glutathione (13–15), giving rise, in most cases, to less reactive metabolites that are more readily excreted with urine. It has been hypothesized that individuals that are homozygous for deletion of either the *GSTM1* or *GSTT1* gene may metabolize and eliminate isothiocyanates at a slower rate and therefore may be more intensely exposed to isothiocyanates after consumption of cruciferous vegetables (16). A few epidemiologic studies have recently evaluated this hypothesis and suggest that the anticancer effect of isothiocyanates may differ by *GST* genotype (17–19).

Urine is the principal disposal route for isothiocyanates and their metabolites. Because there are no endogenous sources of urinary isothiocyanates in humans (20), urinary isothiocyanate concentrations are considered to be an aggregate measure of the

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level of isothiocyanate intake, absorption, and metabolism and, thus, to reflect the cumulative internalized dose biologically available from multiple sources of dietary exposures. Total urinary isothiocyanates and their metabolites can be quantified with high sensitivity and accuracy by using an HPLC-based method by cyclocondensation reaction (9, 21).

In this report we describe a comprehensive evaluation of the association of colorectal cancer risk with isothiocyanate exposure, as assessed by both dietary crucifer intake and by prediagnostic measurements of urinary isothiocyanates, in a case-control study nested within the Shanghai Women's Health Study—a large cohort study of Chinese women who are known to habitually consume large amounts of crucifers and have a low incidence of colorectal cancer (22). We also evaluated whether *GST* genotypes interact with isothiocyanates to modify colorectal cancer risk.

SUBJECTS AND METHODS

Cohort of the Shanghai Women's Health Study

The design and methods of the Shanghai Women's Health Study were described in detail elsewhere (23). Briefly, the cohort includes 74,942 women who were recruited between 1996 and 2000 from 7 urban communities of Shanghai and were 40–70 y of age at study enrollment. The participation rate was 92.7%. All women completed a detailed baseline survey that collected information on demographic characteristics, lifestyle and dietary habits, medical history, family history of cancer, and other exposures. Anthropometric measurements, including weight, height, and circumferences of the waist and hips, were also taken.

Usual dietary intake over the 12 mo before the interview was assessed at baseline for all cohort members and was reassessed 2–3 y after the baseline survey for ≈91% of cohort members using a comprehensive, quantitative, food-frequency questionnaire (FFQ). Five cruciferous vegetables commonly consumed in this population were listed as separate items on the questionnaire, including Chinese greens (bok choy), green cabbage, Chinese cabbage (nappa), cauliflower, and white turnip/radish. Nutrient intakes were calculated by multiplying the amount of each food consumed by the nutrient content of the specific food derived from the Chinese food composition tables (24).

At enrollment, most cohort members donated a urine sample (n = 65,755; 88%) and a blood sample (n = 56,832; 76%) (23). Urine samples were collected into a sterilized cup containing 125 mg ascorbic acid to prevent oxidation of labile metabolites. A 10-mL blood sample was drawn into an EDTA-containing evacuated tube. For those who did not donate a blood sample at baseline, a sample of exfoliated buccal cells (n = 8,934) was collected during the first follow-up survey by using a modified mouthwash method (23). After collection, the samples were kept in a portable styrofoam box with ice packs (at ≈0–4°C) and processed within 6 h for long-term storage at –70°C. A biospecimen collection form was completed for each woman, which included information on the date and time of sample collection, time of last meal, and day of last menstruation (for premenopausal women) as well as intake of selected foods, cigarette smoking, and medication use over the previous 24 h and during the previous week. The study was approved by the relevant Institutional Review Boards for human research in both China and the United States, and written informed consent was obtained from all study participants.

Outcome ascertainment

The cohort was followed for occurrence of cancer and other chronic diseases by a combination of biennial home visits and annual record linkage to the Shanghai Cancer Registry and Shanghai Vital Statistics database. Nearly all cohort members were successfully followed; the response rates for the first (2000–2002), second (2002–2004), and third (2004–2007) in-person follow-up surveys were 99.8%, 98.7%, and 96.7%, respectively. All possible incident cancer cases were verified by home visits. Medical charts from the diagnostic hospital were reviewed to verify the diagnosis.

Nested case-control design

The nested case-control study described in this report included 322 incident colorectal cancer cases who provided a urine sample at baseline and in whom cancer was diagnosed before 31 December 2005. We included only participants who donated a urine sample before any cancer diagnosis. The incidence-density method was used for case-control matching. Controls were selected from women who donated a urine sample at baseline and were free of any cancer at the time of cancer diagnosis for the index case. Cases and controls were individually matched for age at baseline (±2 y), date (≤30 d) and time (morning or afternoon) of urine collection, interval since last meal (≤2 h), menopausal status (before or after), and antibiotic use (yes or no) in the week before sample collection. For most cases (n = 302), we identified 4 controls for each case. For the remaining cases, for whom 4 matched controls could not be found, fewer controls were included. Three cases had a case-to-control ratio of 1:1, 4 cases had a ratio of 1:2, and 13 cases had a ratio of 1:3. A total of 1258 controls were selected.

Urinary isothiocyanate measurement and *GST* genotyping

Total urinary isothiocyanates and their metabolites were assayed by using an HPLC-based method by the cyclocondensation reaction (9, 21). Urine samples and standards were assayed in triplicate. Three representative standards and a reagent blank were included in all analytic runs. Samples of *N*-acetyl-L-cysteine conjugates of phenethyl isothiocyanate (0.2–25 mmol/L) in urine from subjects on a controlled diet were analyzed weekly for a standard curve. To control for batch-to-batch variability, samples for each case-control set were analyzed in the same laboratory run. All laboratory assays were performed in 2007–2008. Laboratory staff were blinded to the case-control status of the urine samples and the identity of the quality control samples. The limit of detection for the urinary isothiocyanates was 0.1 μmol/L. This assay showed a high degree of interday and intraday precision, with CVs of 1.4% and 0.5%, respectively (21). Urinary creatinine was measured by using a test kit from Sigma Company (St Louis, MO), and isothiocyanate measurements were reported as nmol/mg creatinine. The average of 3 measurements for each participant was used in the analysis.

After DNA was extracted from blood (86.4%) or exfoliated buccal cells (13.6%), the copy number for the *GSTM1* and *GSTT1* genes was determined by a duplex real-time quantitative

polymerase chain reaction (PCR)–based assay according to the method described in the NCI SNP500 project, with modifications (25). The assay was designed to detect whether an individual has 0, 1, or 2 copies of the *GSTM1* and *GSTT1* genes. All sequences used in the assay design were obtained from GenBank (*GSTM1*, NM_000561 and *GSTT1*, NM_000853). Real-time PCR was performed in a 384-well plate with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA). Laboratory staff were blinded to the case-control status of the samples. Coriell DNA samples containing 0, 1, or 2 copies of the *GSTM1* and *GSTT1* genes were included to serve as internal quality controls. The concordance rate for quality control samples, including water, Coriell DNA, and blinded DNA samples was 100%. There were no differences in genotyping success rate and genotype distribution of these 2 genes between DNA extracted from blood and buccal cells.

Statistical analyses

We excluded women with a prior history of familial adenomatous polyposis (n = 3) and missing data on urinary isothiocyanate concentrations (n = 4). The final analytic data set included 322 colorectal cancer cases and 1251 individually matched controls.

For samples with undetectable isothiocyanate values (n = 140; 8.9%), we estimated values by dividing the lowest detectable value of the assay by the square root of 2 (26). To better estimate usual dietary intake, we used the average intake of the first FFQ at baseline and the second FFQ conducted 2–3 y after the baseline survey. Pairwise comparisons for urinary isothiocyanate concentrations and other continuous variables were conducted by using Wilcoxon's signed-rank test. The Wald test from a conditional logistic regression model was used to compare the frequency of categorical variables between cases and controls. Wilcoxon's signed-rank test or the Kruskal-Wallis test was also used to examine the difference in urinary isothiocyanate concentrations across *GST* genotype categories.

Urinary isothiocyanate concentrations and dietary crucifer intakes were grouped based on tertile distributions in the controls; the lowest tertile served as the reference. Conditional logistic regression modeling was used to estimate the odds ratios (ORs) of developing colorectal cancer and their 95% CIs associated with urinary isothiocyanate concentration or dietary crucifer intake and to adjust for potential confounders. Potential confounders adjusted for in multivariable models included age at enrollment (continuous), education (4 categories), household income (4 categories), body mass index (calculated as weight in kilograms divided by the square of height in meters, continuous), physical activity level [measured by metabolic equivalent (MET)-hours per week per year, continuous], colorectal cancer in first-degree relatives (yes or no), and intakes (continuous) of total energy, calcium, fruit, noncruciferous vegetables, and red meat. Tests for trend were performed by entering categorical variables as continuous variables in the model.

Potential effect modification by *GST* genotypes was evaluated in stratified analyses by breaking the matching and using unconditional logistic regression models. In addition to all of the covariates listed above, all matching variables were included in the model. Multiplicative diet-gene interactions were determined based on the likelihood ratio test comparing models that included only the main effect terms with models that included both the main effect and the interaction terms. Statistical analyses were carried out by using SAS version 9.1 (SAS Institute, Cary, NC). All statistical tests were based on 2-sided probability.

RESULTS

The distribution of baseline characteristics in this study population is presented in **Table 1**. Cases and controls were well matched for age and menopausal status at enrollment. Cases and controls did not differ significantly with regard to socioeconomic status, most lifestyle characteristics, or potential risk factors for colorectal cancer. However, compared with controls, cases appeared to have a lower household income, consumed slightly less red meat, and were less likely to be a cigarette smoker.

The *GSTM1* and *GSTT1* genotypes were within Hardy-Weinberg equilibrium for both cases and controls. There were no significant case-control differences in the copy number of the *GSTM1* and *GSTT1* genes (Table 1). The proportion of individuals with the null genotype for both genes was comparable with that of other Asian populations (27). Neither the *GSTM1* nor *GSTT1* genotypes were associated with colorectal cancer risk (data not shown).

Urinary isothiocyanate concentrations by *GST* genotype are shown in **Table 2**. Because only a small number of women carried both copies of the *GSTM1* or *GSTT1* gene and because there was no significant allelic dosage effect (1 compared with 2 copies) of the *GSTM1* or *GSTT1* gene on urinary isothiocyanate concentrations (data not shown), women with 1 and 2 copies of these genes were combined into a single group in this analysis. Compared with women homozygous for *GSTM1* deletion, women with at least one copy of the *GSTM1* gene had a higher level of urinary isothiocyanate excretion (P = 0.04). Women who carried both the *GSTM1* and *GSTT1* genes had the highest level of urinary isothiocyanate excretion, whereas women with the double null genotype had the lowest level of urinary isothiocyanate excretion (P = 0.02). This association persisted in a model mutually adjusted for both *GST* genotypes and dietary intake (P = 0.01) and was similar when cases and controls were analyzed separately. No significant differences were observed in dietary intake of cruciferous vegetables according to *GSTM1* or combined *GSTM1* and *GSTT1* genotypes.

Baseline urinary isothiocyanate concentrations in colorectal cancer cases were 50.1% (median) lower than in controls in a pairwise comparison, with a median case-control difference of −0.81 nmol/mg creatinine (Table 1). When analyzed as categorical variables in conditional logistic regression models, urinary isothiocyanate concentrations were inversely associated with overall colorectal cancer risk, but the results were not statistically significant (**Table 3**). The inverse association, however, was statistically significant among women with the *GSTM1*-null genotype (P for trend = 0.04) and was nearly significant among women with the *GSTT1*-null genotype (P for trend = 0.07). The strongest inverse association was found in women null for both the *GSTM1* and *GSTT1* genes (OR for the comparison of extreme tertiles: 0.51; 95% CI: 0.27, 0.95; P for trend = 0.03). No associations were found for women who carried either the *GSTM1* or *GSTT1* gene.

TABLE 1
Baseline characteristics of colorectal cancer cases and controls: Shanghai Women's Health Study (1997–2005)[1]

	Cases (n = 322)	Controls (n = 1251)	P
Education, college and above (%)	9.3	12.1	0.14[2]
Household income, >30,000 Yuan/y (%)	10.6	15.7	0.01[2]
Cigarette smoking (%)	1.9	4.4	0.04[2]
Alcohol consumption (%)	2.2	2.4	0.72[2]
Physical activity, >100 MET-h/wk per year (%)	58.1	55.2	0.34[2]
Family history of colorectal cancer (%)	2.8	2.3	0.57[2]
Postmenopausal at enrollment (%)	78.9	79.0	0.97[2]
GSTM1 (%)			
Null	58.7	58.4	0.61[2]
1 copy	36.3	34.8	—
2 copies	5.0	6.9	—
GSTT1 (%)			
Null	50.9	48.9	0.72[2]
1 copy	38.8	41.4	—
2 copies	10.3	9.7	—
Age at enrollment (y)	62 (54, 66)[3]	62 (54, 66)	0.21[4]
BMI (kg/m^2)	24.6 (22.4, 26.6)	24.5 (22.2, 26.9)	0.79[4]
Total energy intake (kcal/d)	1646 (1392, 1889)	1618 (1372, 1893)	0.67[4]
Cruciferous vegetable intake (g/d)	84.9 (48.8, 137.5)	84.0 (49.5, 135.7)	0.34[4]
Intake of all vegetables (g/d)	261.9 (175.4, 371.2)	250.7 (170.6, 361.5)	0.62[4]
Red meat intake (g/d)	38.3 (21.9, 62.2)	39.9 (24.0, 61.1)	0.05[4]
Urinary ITCs (nmol/mg creatinine)[5]	1.57 (0.58, 3.70)	1.76 (0.72, 4.04)	<0.0001[4]

[1] MET-h, metabolic equivalent hours; Yuan, Chinese currency (1 US dollar = ≈8 Yuan at recruitment); ITCs, isothiocyanates.
[2] Derived from Wald test from conditional logistic regression.
[3] Median; interquartile range (between the 25th and the 75th percentiles) in parentheses (all such values).
[4] Derived from Wilcoxon's signed-rank test for case-control differences within each case-control set.
[5] The median (interquartile range) difference between cases and controls (urinary ITCs of cases – mean ITCs of controls in the case-control set) was −0.81 (−2.60, 0.96).

The associations between urinary isothiocyanates and colorectal cancer did not vary by lifestyle characteristics, including body mass index, physical activity, or consumption of red meat, fruit, or noncruciferous vegetables (data not shown); nor did the associations vary appreciably by anatomic subsite (colon compared with rectum) or clinical stage of tumors. Very few women in this cohort ever smoked cigarettes (2.7%) (23), and the results were similar between nonsmokers and all subjects included in the study (data not shown). Furthermore, results from analyses that excluded cases diagnosed in the first year of follow-up were similar to the results presented in Table 3. For example, among individuals null for both the *GSTM1* and *GSTT*1 genes, the multivariable OR for the highest compared with the lowest tertile of urinary isothiocyanates was 0.52 (95% CI: 0.28, 0.995; P for trend = 0.04).

No apparent associations were found between dietary crucifer intake and colorectal cancer risk, either overall or by *GST* genotype (**Table 4**). Analyses of the correlation between urinary

TABLE 2
Baseline urinary isothiocyanate (ITC) excretion and crucifer intake by glutathione *S*-transferase genotype: Shanghai Women's Health Study (1997–2005)[1]

	Urinary ITCs		Crucifer intake	
	Median (IQR)	P[2]	Median (IQR)	P[2]
	nmol/mg creatinine		*g/d*	
GSTM1				
Null (n = 845)	1.84 (0.85, 4.18)	0.04	83.7 (47.2, 135.3)	0.25
Present (n = 588)	2.16 (1.01, 4.26)	—	84.9 (53.5, 137.5)	—
GSTT1				
Null (n = 709)	1.82 (0.87, 4.03)	0.08	87.6 (54.2, 138.8)	0.03
Present (n = 724)	2.09 (0.92, 4.65)	—	80.4 (45.3, 133.7)	—
GSTM1 and *GSTT1*				
Double null (n = 387)	1.60 (0.88, 3.84)	0.02	86.2 (50.5, 135.9)	0.61
Either one null (n = 780)	1.99 (0.84, 4.38)	—	84.9 (49.0, 137.4)	—
Double present (n = 266)	2.25 (1.13, 4.68)	—	77.0 (48.5, 133.0)	—

[1] Subjects with undetectable urinary ITC concentrations (n = 140) or missing data on *GSTM1* (n = 2) or *GSTT1* (n = 4) were excluded from the analyses. IQR, interquartile range (between the 25th percentile and the 75th percentile).
[2] Derived from Wilcoxon's signed-rank test or the Kruskal-Wallis test.

TABLE 3
Association of urinary isothiocyanate (ITC) concentrations with colorectal cancer risk by glutathione *S*-transferase genotype: Shanghai Women's Health Study (1997–2005)

	Tertile of urinary ITCs (nmol/mg creatinine)				
	1 (<0.95)	2 (0.95–2.98)	3 (>2.98)	*P* for trend	*P* for interaction
Overall					
No. of cases/controls	117/413	105/414	100/427	—	—
Crude OR (95% CI)	1.00	0.88 (0.65, 1.20)	0.81 (0.58, 1.12)	0.20	—
Multivariable OR (95% CI)[1]	1.00	0.87 (0.63, 1.18)	0.77 (0.55, 1.07)	0.12	—
GSTM1 null					
No. of cases/controls	77/242	58/242	54/245	—	—
Multivariable OR (95% CI)[2]	1.00	0.75 (0.50, 1.11)	0.66 (0.44, 1.00)	0.04	0.10
GSTM1 present					
No. of cases/controls	40/168	47/170	46/180	—	—
Multivariable OR (95% CI)[2]	1.00	1.22 (0.75, 1.99)	1.12 (0.68, 1.83)	0.68	—
GSTT1 null					
No. of cases/controls	66/199	50/215	48/196	—	—
Multivariable OR (95% CI)[2]	1.00	0.66 (0.42, 0.99)	0.67 (0.44, 1.05)	0.07	0.33
GSTT1 present					
No. of cases/controls	51/211	55/197	52/229	—	—
Multivariable OR (95% CI)[2]	1.00	1.13 (0.73, 1.75)	0.92 (0.59, 1.44)	0.71	—
GSTM1 and *GSTT1* null					
No. of cases/controls	40/105	32/119	24/102	—	—
Multivariable OR (95% CI)[2]	1.00	0.61 (0.35, 1.08)	0.51 (0.27, 0.95)	0.03	<0.05
GSTM1 or *GSTT1* null					
No. of cases/controls	63/231	44/219	54/237	—	—
Multivariable OR (95% CI)[2]	1.00	0.75 (0.48, 1.16)	0.84 (0.55, 1.28)	0.40	—
GSTM1 and *GSTT1* present					
No. of cases/controls	14/74	29/74	22/86	—	—
Multivariable OR (95% CI)[2]	1.00	2.16 (0.98, 4.78)	1.25 (0.56, 2.78)	0.73	—

[1] Odds ratios (ORs) were estimated by using multivariable conditional logistic regression models, adjusted for age, education, household income, physical activity, cigarette smoking, alcohol consumption, BMI, family history of colorectal cancer, and intakes of total energy, fruit, noncruciferous vegetables, red meat, and calcium.

[2] ORs were estimated by using multivariable unconditional logistic regression model, adjusted for all covariates included in the model above and all matching variables including menopausal status at sample collection, time of sample collection, time interval (h) between last meal and sample collection, antibiotic use in the past week, and time interval (y) between sample collection and ITC measurement.

isothiocyanate excretion and dietary intake of crucifers showed that the mean concentrations of urinary isothiocyanates increased from 1.48 to 1.80 and 1.90 nmol/mg creatinine with increasing tertiles of dietary crucifer intake (P for trend = 0.003). In a mutually adjusted model, both *GST* genotypes and dietary intake were significantly associated with urinary isothiocyanate concentrations. The correlation at the individual level, however, was very weak, either overall (Spearman correlation coefficient = 0.08, P = 0.001) or by *GST* genotype (Spearman correlation coefficients ranged from 0.05 to 0.11).

DISCUSSION

In this nested case-control study, the largest urine-based biomarker study to date of isothiocyanate exposure and colorectal cancer, we found an inverse association between urinary isothiocyanates and colorectal cancer risk. The protective effect of isothiocyanates was seen among individuals with homozygous deletion of *GSTM1* and particularly with deletion of both *GSTM1* and *GSTT1*. However, no apparent association between isothiocyanate concentration and colorectal cancer risk was found among individuals who carried either the *GSTM1* or *GSTT1* gene. We also found that urinary isothiocyanate concentrations were lower in women who had a homozygous deletion of either the *GSTM1* or *GSTT1* gene than in women who carried these *GST* genes and dietary intake was likely independent of *GST* genotypes, suggesting that the isothiocyanate metabolic clearance rate differs significantly by *GST* genotypes. These findings support the notion that individuals with *GST* deletion may metabolize isothiocyanates less efficiently and, therefore, may have a higher exposure to isothiocyanates, which allows them to benefit more from consumption of cruciferous vegetables in terms of reduction of cancer risk.

Our findings on the interactive effect of isothiocyanate exposure and *GST* genotype on colorectal cancer risk are supported by several previous epidemiologic studies of other cancer types (17, 28). For example, London et al (17) reported in a cohort study of men in Shanghai that high urinary isothiocyanate excretion levels were associated with a reduced risk of lung cancer, predominantly among men with the *GSTM1*-null and/or *GSTT1*-null genotypes. In general, studies of dietary isothiocyanate/crucifer intake and colorectal cancer, mostly with a case-control study design (19, 29–31), have reported an inverse relation between isothiocyanate exposure and risk of colorectal adenoma (19) or cancer (18, 29, 30) among individuals with null/low-activity *GST* genotypes, although the results are not entirely consistent (31). To date, only 2 nested case-control studies have prospectively evaluated the association between

TABLE 4
Association of cruciferous vegetable intake with colorectal cancer risk by glutathione *S*-transferase genotype: Shanghai Women's Health Study (1997–2005)

	Tertile of cruciferous intake				
	1	2	3	*P* for trend	*P* for interaction
Overall					
No. of cases/controls	113/414	104/412	105/425	—	—
Multivariable OR (95% CI)[1]	1.00	0.94 (0.69, 1.28)	0.93 (0.66, 1.31)	0.66	—
Multivariable OR (95% CI) by *GSTM1*[2]					
Null	1.00	0.95 (0.63, 1.44)	0.93 (0.59, 1.49)	0.77	0.96
Present	1.00	0.96 (0.58, 1.58)	1.00 (0.58, 1.72)	0.98	—
Multivariable OR (95% CI) by *GSTT1*[2]					
Null	1.00	0.97 (0.62, 1.51)	0.92 (0.56, 1.52)	0.75	0.72
Present	1.00	0.99 (0.63, 1.56)	0.96 (0.58, 1.59)	0.88	—
Multivariable OR (95% CI) by *GSTM1* and *T1*[2]					
Double null	1.00	0.87 (0.48, 1.57)	0.70 (0.35, 1.39)	0.31	0.82
Either one null	1.00	0.99 (0.63, 1.56)	1.15 (0.70, 1.88)	0.61	—
Double present	1.00	1.14 (0.55, 2.36)	0.86 (0.38, 1.96)	0.76	—

[1] Odds ratios (ORs) were estimated by using multivariable conditional logistic regression models, adjusted for age, education, household income, physical activity, cigarette smoking, alcohol consumption, BMI, family history of colorectal cancer, and intakes of total energy, fruit, noncruciferous vegetables, red meat, and calcium.

[2] ORs were estimated by using multivariable unconditional logistic regression model, adjusted for all covariates included in the model above and all matching variables including menopausal status at sample collection, time of sample collection, time interval (h) between last meal and sample collection, antibiotic use in the past week, and time interval (y) between sample collection and isothiocyanate measurement.

urinary isothiocyanate concentrations and colorectal cancer. Moy et al (32) reported recently in the Shanghai Men's Cohort that urinary isothiocyanate concentrations were inversely associated with the risk of colorectal cancer; however, this finding was observed only among men whose urine samples were collected $\geq$5 y before disease diagnosis, and the interaction with related genes was not evaluated. A similar protective effect of isothiocyanates was also reported in the Multiethnic Cohort Study (33), with a reduction in colorectal cancer risk of $\approx$40% among individuals with detectable concentrations of urinary isothiocyanates. The effect was more pronounced among individuals with the *G* allele (encoding a lower-activity allozyme) for the *GSTP1* Ile105Val polymorphism (*P* for interaction = 0.09). As acknowledged by the authors (33), that study, however, was limited by insufficient statistical power, particularly in stratified analyses and a short follow-up time (the median interval between the time of collection of urine samples and disease diagnosis was 1.4 y). The association between dietary intake of cruciferous vegetables and colorectal cancer risk was also evaluated in the Multiethnic Cohort Study, with no evidence of a protective effect of cruciferous vegetable intake as assessed by FFQ (34), similar to the results reported in the present study.

Several case-control studies, including our previous studies (35), have evaluated the association of colorectal cancer with dietary crucifer intake (18, 19, 29–31, 36). Results have been mixed but generally point to an inverse association (18, 19, 29, 30, 36). However, no such preventive effect was found in any of the recent cohort studies conducted in the United States, including the Iowa Women's Health Study (37), the Nurses' Health Study (38), the Health Professionals Follow-Up Study (38), the Multiethnic Cohort Study (34), and the Cancer Prevention Study II Nutrition Cohort (39) or in cohort studies conducted in Japan (40) and Finland (41). One exception was a case-control study nested within the Singapore Chinese Health Study (18). Higher intakes of cruciferous vegetables were associated with a reduced risk of colorectal cancer among individuals null for both the *GSTM1* and *GSTT1* genes. In a recent pooled analysis of 14 cohort studies (42), the pooled multivariable relative risk of the highest compared with the lowest tertile of crucifer intake was 0.99 (95% CI: 0.93, 1.06), similar to the findings of our study.

These studies may have been limited by possible misclassification errors in dietary assessment. The level of glucosinolates varies considerably in vegetables depending on their species, growing conditions, and maturity at the time of harvest (1). The formation of isothiocyanates is catalyzed by myrosinase, a thermosensitive enzyme in plant cells and in gastrointestinal microflora. Boiling cruciferous vegetables results in a 30–60% loss of intact glucosinolates due to thermal degradation and leaching (20). The bioavailability of isothiocyanates from fully cooked broccoli is 3-fold lower than in lightly cooked broccoli as a result of the inactivation of myrosinase by heat (43). Therefore, exposure levels of isothiocyanates can be further affected by many other factors, such as food-preparation methods and the status of colonic bacteria (44–46). Crucifer intake assessed by a food questionnaire is not an ideal measure of dietary exposure to isothiocyanates and related phytochemicals. In the present study, the mean concentration of urinary isothiocyanates increased with increasing intake of cruciferous vegetables, similar to a previous observation in Singapore Chinese (15); however, the correlation at the individual level was very weak. Such a weak correlation between urinary isothiocyanate concentrations and crucifer intakes was also reported previously (33, 47), which suggested that dietary crucifer intake assessed by FFQ may not adequately reflect the exposure level of isothiocyanates in vivo.

Although urinary isothiocyanate concentrations reflect crucifer intakes over the prior 24–48-h period (48), the current study was conducted in a population that is among the most frequent consumers of cruciferous vegetables in the world. This population consumes 84 g cruciferous vegetables/d on average, which is $\approx$6-fold higher than that in Western populations ($\approx$0.2

serving/d) (39). We showed previously that the within-person variation in urinary isothiocyanate measurements is relatively small in participants of the Shanghai Women's Health Study. The intraclass correlation coefficient was 0.50 for isothiocyanates measured in 4 urine samples collected over a 12-mo period, indicating that a single measurement in a spot urine sample reflects the level of isothiocyanate exposure over a longer period reasonably well in our study population. In addition, we matched cases and controls on the date of specimen collection to minimize potential seasonal and storage effects on concentrations of urinary isothiocyanates. Moreover, because recent antibiotic use can alter the status of gastrointestinal microflora that converts glucosinolates to isothiocyanates in the gut, we further matched cases and controls on antibiotic use. We also performed analyses of urinary isothiocyanates after excluding antibiotic users. The results did not differ appreciably. Other strengths of the study included the use of a prediagnostic urine sample, high participation rates at baseline, and a virtually complete follow-up of the cohort, which minimized potential biases inherent in case-control studies.

In summary, using dietary intake data and urine specimens from a large, population-based, prospective cohort study, we found a strong inverse association between urinary isothiocyanate concentrations and subsequent risk of developing colorectal cancer among women with the null genotype of the *GSTM1* and *GSTT1* genes. Given the long latency of cancer, continued follow-up is needed to provide the data necessary to further characterize the effect of isothiocyanates or crucifer intakes on colorectal cancer incidence.

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The authors' responsibilities were as follows—GY, X-OS, and WZ: study design, data collection, statistical analyses, and writing of manuscript; Y-TG, H-LL, and Y-BX: data collection and management; B-TJ, NR, and W-HC: data collection and revision of the manuscript; and QC, G-LL, MD, and F-LC: laboratory assays and revision of the manuscript. None of the authors had any financial conflicts of interest to declare.

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