

Correlates of self-reported dietary cruciferous vegetable intake and urinary isothiocyanate from two cohorts in China

Emily Vogtmann^{1,2,3}, Gong Yang¹, Hong-Lan Li³, Jing Wang³, Li-Hua Han³, Qi-Jun Wu³, Li Xie³, Quiyin Cai¹, Guo-Liang Li¹, John W Waterbor², Emily B Levitan², Bin Zhang⁴, Yu-Tang Gao³, Wei Zheng¹, Yong-Bing Xiang³ and Xiao-Ou Shu^{1,*}

¹Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, 2525 West End Avenue 6th floor, Nashville, TN 37232-8300, USA; ²Department of Epidemiology, School of Public Health, University of Alabama at Birmingham, Birmingham, AL, USA; ³Department of Epidemiology and State Key Laboratory of Oncogene and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, People's Republic of China; ⁴Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

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Abstract

Objective: To assess correlations between cruciferous vegetable intake and urinary isothiocyanate (ITC) level, in addition to glutathione *S*-transferase (*GST*) genotypes and other individual factors.

Design: The study included cohort participants whose urinary ITC levels had been previously ascertained. Urinary ITC was assessed using HPLC. Usual dietary intake of cruciferous vegetables was assessed using a validated FFQ and total dietary ITC intake was calculated. Recent cruciferous vegetable intake was determined. *GST* genotypes were assessed using duplex real-time quantitative PCR assays. Spearman correlations were calculated between the covariates and urinary ITC levels and linear regression analyses were used to calculate the mean urinary ITC excretion according to *GST* genotype.

Setting: Urban city in China.

Subjects: The study included 3589 women and 1015 men from the Shanghai Women's and Men's Health Studies.

Results: Median urinary ITC level was 1.61 nmol/mg creatinine. Self-reported usual cruciferous vegetable intake was weakly correlated with urinary ITC level ($r_s = 0.1149$; $P < 0.0001$), while self-reported recent intake was more strongly correlated with urinary ITC ($r_s = 0.2591$; $P < 0.0001$). Overall, the *GST* genotypes were not associated with urinary ITC level, but significant differences according to genotype were observed among current smokers and participants who provided an afternoon urine sample. Other factors, including previous gastrectomy or gastritis, were also related to urinary ITC level.

Conclusions: The study suggests that urinary secretion of ITC may provide additional information on cruciferous vegetable intake and that *GST* genotypes are related to urinary ITC level only in some subgroups.

Keywords

Brassicaceae
China

Glutathione *S*-transferase M1
Glutathione *S*-transferase T1

When cruciferous vegetables are consumed, the internal glucosinolates are converted to isothiocyanate (ITC)^(1,2), a potential chemopreventive compound that inhibits phase-I enzymes (carcinogen activating) and activates phase-II enzymes (carcinogen detoxifying)⁽²⁾. Glutathione *S*-transferases (*GST*) are phase-II enzymes induced by ITC that catalyse conjugation of ITC for urinary excretion^(3,4) and *GST* gene deletions alter enzymatic *GST* activity^(5,6). Interactions of cruciferous vegetable consumption and/or urinary ITC levels with *GST* gene variants have been

considered in the risk of breast, colorectum, lung and gastric cancers^(7–13).

Cruciferous vegetable consumption is typically assessed using dietary recall such as FFQ. However, FFQ data on cruciferous vegetables have limitations, particularly recall errors⁽¹⁴⁾. There are additional limitations when calculating dietary ITC intake values, which combine FFQ with laboratory data on the ITC content of specific vegetables. Actual ITC content varies between vegetables, so the calculated laboratory content could be different from that

in the actual cruciferous vegetable consumed. A biomarker of cruciferous vegetable intake, like urinary ITC excretion, would not share these limitations. However, ITC and its metabolites are eliminated within 48 h after cruciferous vegetable consumption⁽¹⁵⁾. Therefore, urinary ITC level reflects very recent intake, whereas an FFQ assesses intake over longer periods of time.

Although intake of cruciferous vegetables and *GST* gene polymorphisms are likely the strongest determinants of urinary ITC excretion, factors such as age, gender, BMI and kidney function have all been associated with urinary excretion of metabolites^(16–18). Therefore, our goal was to identify factors associated with urinary ITC levels using data from the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS).

Methods

Source population

The SWHS and SMHS are prospective, population-based cohort studies in Shanghai, China^(19,20). In brief, for the SWHS, 74 941 women living in Shanghai aged 40–70 years were recruited from 1996 to 2000. For the SMHS, 61 483 men aged 40–74 years were recruited from 2002 to 2006. Participation rates for the SWHS and SMHS were 92.7% and 74.1%, respectively. Trained interviewers administered surveys and obtained anthropometric measurements and biological samples (spot urine, blood and/or buccal cells). Both studies were conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Boards at the Shanghai Cancer Institute and Vanderbilt University Medical Center. Written informed consent was obtained from all participants.

Nested case–control study participants

Data included in the current study were drawn from three nested SWHS case–control studies and one SMHS study that were conducted to assess the association between urinary ITC and cancer. All urine samples were collected prior to diagnosis of any cancer. The methods for the colorectal cancer and lung cancer studies in the SWHS have been published^(9,10). From the SWHS, 430 breast cancer cases diagnosed before February 2007, 328 colorectal cancer cases diagnosed before January 2006 and 232 lung cancer cases diagnosed before February 2003 were selected. From the SMHS, 341 colorectal cancer cases diagnosed before January 2011 were selected. In both cohorts, incidence density sampling was used to select controls that were free of cancer at the time of the case's cancer diagnosis. Since controls could have developed cancer after selection we considered participants who developed cancer (up to 31 December 2010) to be cases and all others to be non-cases. We combined data from all four nested case–control studies with 3603 women and 1020 men.

Assessment of cruciferous vegetable and dietary isothiocyanate intakes

Usual dietary intake over the past 12 months was assessed at baseline. The FFQ have good validity and reliability^(21,22) and assessed intake of common cruciferous vegetables, including Chinese greens, green cabbage, Chinese cabbage/bok choy cabbage, cauliflower and white turnip/radish. Average intake of dietary ITC was derived by multiplying the reported amount of consumption of each cruciferous vegetable by the ITC content in Asia^(23,24). At baseline urine sample collection, participants were asked about recent cruciferous vegetable intake including the total number of times they consumed green cabbage, Chinese cabbage and cauliflower during the past week and past 24 h, and how many days or hours before sample collection they last consumed cruciferous vegetables.

Measurement of urinary isothiocyanate

Pre-diagnosis urine samples were measured for ITC level in all four studies following an identical laboratory protocol. HPLC was used to determine total urinary ITC as previously described^(10,25). Laboratory staff were blinded to the samples' case–control status and all urine samples and standards were assayed in triplicate for the SWHS and duplicate for the SMHS. In each laboratory run, representative standards and a reagent blank were included. A standard curve was created weekly using data from samples of *N*-acetyl-L-cysteine conjugates of phenethyl ITC (0.2–25 mmol/l) in urine from individuals on a controlled diet. The average of the ITC measurements for each participant was used for analysis. If the standard deviation of the mean was greater than 10%, the sample was reanalysed. The limit of detection for urinary ITC was 0.1 µmol/l. For undetectable ITC levels (n 350 for SWHS; n 8 for SMHS), the value was set to 0.1 µmol/l divided by the square root of two. Urinary creatinine was measured using the Jaffé alkaline picrate procedure⁽²⁶⁾. All ITC levels were adjusted for urine creatinine level and reported as nmol/mg creatinine.

For the SWHS, urinary ITC analysis was conducted in three batches. Batch 1 was completed in July 2006, batch 2 in August 2007 and batch 3 in August 2008. The SMHS analysis was completed in August 2012. Time between sample collection and sample processing was adjusted in the analysis to account for storage time. The within-batch and between-batch CV were 15.1% and 13.7%, respectively.

GST genotyping

DNA was extracted from blood (86.1%) and buccal cell (13.9%) samples. For both the SWHS and SMHS, copy numbers (0, 1 or 2 gene copies) of the *GSTM1* and *GSTT1* genes were assessed using duplex real-time quantitative PCR-based assays using methods previously described⁽²⁷⁾. The sequences used in assay design were obtained from GenBank (*GSTM1*, NM_000561 and *GSTT1*, NM_000853). Real-time PCR were conducted in a 384-well plate in an ABI PRISM 7900 Sequence Detection System (Applied

Biosystems, Foster City, CA, USA). Laboratory staff were blinded to the samples' case-control status. Coriell DNA samples containing 0, 1 or 2 copies of the *GSTM1* and *GSTT1* genes were included for internal quality control. The concordance rate for quality control samples, including water, Coriell DNA and blinded DNA samples was 100%. *GSTM1* and *GSTT1* genotypes were within Hardy-Weinberg equilibrium among the non-cases from the SWHS ($P=0.1143$ and $P=0.6924$ for *GSTM1* and *GSTT1*, respectively) and SMHS ($P=0.1897$ and 0.8361 for *GSTM1* and *GSTT1*, respectively).

Other covariates of interest

Additional variables available for study included age and education, dietary, behavioural and medical factors assessed at baseline. Participants with missing data for education (four women and nineteen men) were set as the most common category, high-school education. BMI was calculated from the interviewer-measured height and weight. Behavioural characteristics included cigarette smoking, alcohol consumption, tea consumption, ginseng intake, amount of exercise per day ($\text{MET} \times \text{h/d}$, where MET = metabolic equivalent of task) and menopausal status for women. The two women with missing menopausal status were classified as premenopausal because they were younger than the median age of menopause (49.5 years). Self-reported prevalent conditions (pulmonary tuberculosis, chronic bronchitis, asthma, chronic gastritis, chronic hepatitis, gallstones, diabetes, high blood pressure, CHD, stroke and polyps) as well as previous surgical interventions (gastrectomy and cholecystectomy) were considered. Because urinary ITC levels may be altered by diminished kidney function, we categorized participants as having a history of chronic kidney disease (International Classification of Diseases, ninth revision 9 (ICD-9) code: 403, 404 and 585; n 3), nephritis (ICD-9 code: 580-589; n 31), other urinary disorders (ICD-9 code: 590-599; n 148) or 'any urinary disorder' (ICD-9 code: 403, 404 and 580-599; n 177), and assessed the effect of 'any urinary disorder' in stratified analyses.

Statistical analysis

We excluded four women who had cancer prior to baseline, four with extreme reported total energy intake (<2092 or $>14\,644$ kJ/d (<500 or >3500 kcal/d)), five with missing data for both *GST* genes and one woman with missing BMI data. We excluded one man with extreme reported total energy intake (<2092 or >17593 kJ/d (<500 or >4200 kcal/d)) and four with missing data on both *GST* genes. After these exclusions, 3589 women (1071 cases and 2518 non-cases) and 1015 men (350 cases and 665 non-cases) remained for analysis.

Descriptive statistics were calculated for the SWHS and SMHS participants. Spearman correlations (r_s) were calculated between covariates and urinary ITC and adjusted for sex and batch effects. Spearman correlations of usual cruciferous vegetable and dietary ITC intakes and recent

cruciferous vegetable intake with urinary ITC levels were calculated adjusted for batch, sex and total energy intake.

Linear regression analysis was applied to evaluate the association between the *GST* gene variants and smoking history with urinary ITC levels. Urinary ITC was natural log transformed to approximate normality and the β estimate and 95% confidence intervals were back-transformed to the original scale for presentation. Since smoking is a *GST* inducer and since participants who provided urine samples in the afternoon would have been more likely to have recently consumed cruciferous vegetables, we evaluated effect modification of the association between *GST* gene variants and urinary ITC by smoking status and morning v. afternoon urine sample collection using stratified linear regression models. A linear prediction model of the natural log of ITC was created using backwards selection. Variables with a P value <0.10 remained in the model. Since it is possible the individuals who developed a cancer over follow-up may have had subclinical diseases or conditions at study enrolment that may affect dietary intake and analyses of the study, we conducted additional analyses restricted to those cancer-free individuals. Similar association patterns were observed. Therefore, results from the analyses of all samples were reported. The SAS 9.3 statistical software package was used for all analyses and a two-sided P value <0.05 was considered statistically significant.

Results

Women and men from the SWHS and SMHS differed on several baseline characteristics including age, education, smoking history, alcohol and tea consumption, family history of cancer, BMI and leisure-time physical activity. The participants reported different intakes of cruciferous vegetables with a median of 82.5 g/d for women and 90.9 g/d for men. However, urinary ITC levels were similar for women and men with a median of 1.7 and 1.5 nmol/mg creatinine, respectively (Table 1). Urinary ITC levels ranged from undetectable to 602.6 nmol/mg creatinine, with a median of 1.61 nmol/mg creatinine.

No strong correlations were observed between baseline sociodemographic, behavioural or physical characteristics and urinary ITC. Similarly, baseline prevalent conditions and prior surgeries were generally uncorrelated with urinary ITC. And no strong correlations were detected between the matching variables and urinary ITC levels (see online supplementary material, Supplemental Table 1).

Urinary ITC was significantly, although weakly, correlated with baseline usual consumption of cruciferous vegetables ($r_s=0.1149$, $P<0.0001$) and dietary ITC ($r_s=0.1172$, $P<0.0001$). The correlations for total cruciferous vegetables appeared to be stronger in men ($r_s=0.1733$) than women ($r_s=0.0988$). However, no interaction was observed between cruciferous vegetable intake and sex ($P=0.7845$) or between cruciferous vegetable intake and batch ($P=0.3897$)

Table 1 Demographic characteristics of the included participants from the Shanghai Women's and Men's Health Studies

	SWHS (<i>n</i> 3589)		SHMS (<i>n</i> 1015)	
	Median or %	IQR	Median or %	IQR
Age (years)	59.5	49.1–65.6	65.6	56.2–70.6
Educational level (%)				
≤Elementary school	35.7	–	14.0	–
Middle school	29.8	–	32.3	–
High school	22.8	–	27.0	–
≥College	11.8	–	26.7	–
Cigarette smoking (%)				
Never	96.6	–	38.4	–
Past	0.6	–	18.2	–
Current	2.9	–	43.4	–
Alcohol consumption (%)				
Never	97.3	–	68.4	–
Ever	2.7	–	31.6	–
Tea consumption (%)				
Never	74.3	–	37.8	–
Ever	25.7	–	62.2	–
Ginseng use (%)				
Never	66.1	–	63.7	–
Ever	33.9	–	36.3	–
Family history of cancer (%)				
Yes	26.8	–	31.9	–
No	73.2	–	68.1	–
BMI (kg/m ²)	24.3	22.1–26.7	23.9	21.8–26.1
Leisure-time physical activity (MET × h/d)	0.0	0.0–1.2	0.3	0.0–2.9
Total energy intake (kJ/d)	6822	5816–7902	7644	6520–8987
Total energy intake (kcal/d)	1630.6	1390.1–1888.6	1826.9	1558.2–2147.9
Cruciferous vegetable intake (g/d)	82.5	50.7–131.8	90.9	56.6–147.0
Dietary ITC (μmol/d)	7.0	4.4–11.2	7.9	4.7–13.0
Urinary ITC (nmol/mg creatinine)	1.7	0.7–4.0	1.5	0.7–3.8

SWHS, Shanghai Women's Health Study; SMHS, Shanghai Men's Health Study; IQR, interquartile range; MET, metabolic equivalent of task; ITC, isothiocyanate.

Median and IQR are presented for continuous variables.

for associations with urinary ITC. Stronger correlations were observed for the measures of recent cruciferous vegetable intake with $r_s = 0.2591$ ($P < 0.0001$) for the number of times cruciferous vegetables were consumed in the past week, $r_s = 0.2400$ ($P < 0.0001$) for the number of times cruciferous vegetables were consumed in the past 24 h and $r_s = -0.2877$ ($P < 0.0001$) for the number of hours since the last intake of cruciferous vegetables (Table 2). Usual cruciferous vegetable consumption was not strongly correlated with the measures of recent intake, with r_s of 0.0421 for the number of times cruciferous vegetables were consumed in the past week, 0.0239 for the number of times cruciferous vegetables were consumed in the past 24 h and -0.0240 for the number of hours since the last intake of cruciferous vegetables. Additional adjustment for other covariates, including morning or afternoon urine sample collection and smoking, did not materially alter the correlations (results not shown). When analyses were restricted to non-cases, correlations were similar (results not shown). When analyses were restricted to participants with 'no urinary disorder' the correlations were similar, but in participants with 'any urinary disorder' the correlations were attenuated; however, only 177 participants were categorized as 'any urinary disorder' (results not shown).

Mean levels of urinary ITC were lower among participants with the *GST* null genotypes; however, the observed

differences were not statistically significant (Table 3). Adjustment for baseline covariates that were significantly associated with ITC level did not alter associations. Similarly, inclusion of self-reported usual cruciferous vegetable intake did not materially change results (results not shown). Smokers had lower urinary ITC levels, with geometric mean urinary ITC levels of 1.59 (95 % CI 1.52, 1.66), 1.29 (95 % CI 1.04, 1.59) and 1.40 (95 % CI 1.22, 1.61) nmol/mg creatinine for never, past and current smokers, respectively, after adjustment for batch and sex.

When the analyses were stratified by smoking status, current smokers with the carrier genotype for both the *GSTM1* and *GSTT1* genes had a significantly higher urinary level of ITC than the other combinations, with a geometric mean of 1.91 (95 % CI 1.52, 2.42) nmol/mg creatinine. Current smokers with the carrier genotype of the *GSTM1* gene also had a slightly higher urinary excretion of ITC, with a geometric mean of 1.66 (95 % CI 1.40, 1.96) nmol/mg creatinine (Table 4). When stratified by morning or afternoon urine sample collection, a difference in urinary ITC level was detected for the *GSTM1* gene ($P = 0.0436$) and the combination *GSTM1/GSTT1* gene category ($P = 0.0328$) among participants who provided a urine sample in the afternoon (Table 5). However, no differences in self-reported recent cruciferous vegetable consumption (past 24 h or week) were

Table 2 Spearman correlations between reported dietary intakes and urinary isothiocyanate (ITC) levels among the included participants from the Shanghai Women's and Men's Health Studies

	Women		Men		Women and men	
	Model 1		Model 1		Model 2	
	r_s	P value	r_s	P value	r_s	P value
Cruciferous vegetables (g/d)	0.0988	<0.0001	0.1733	<0.0001	0.1149	<0.0001
Greens, Chinese greens (g/d)	0.0809	<0.0001	0.1320	<0.0001	0.0921	<0.0001
Green cabbage (g/d)	0.0970	<0.0001	0.1011	0.0013	0.0975	<0.0001
Chinese cabbage/bok choy cabbage (g/d)	0.0384	0.0217	0.0682	0.0299	0.0433	0.0033
Cauliflower (g/d)	0.0505	0.0025	0.0977	0.0018	0.0607	<0.0001
White radish/turnip (g/d)	0.0882	<0.0001	0.1903	<0.0001	0.1104	<0.0001
Dietary ITC (μ mol/d)	0.1055	<0.0001	0.1617	<0.0001	0.1172	<0.0001
Cruciferous vegetables (times/past week)	0.2530	<0.0001	0.2839	<0.0001	0.2591	<0.0001
Cruciferous vegetables (times/past 24 h)	0.2416	<0.0001	0.2365	<0.0001	0.2400	<0.0001
Hours since last cruciferous vegetable intake	-0.2839	<0.0001	-0.3048	<0.0001	-0.2877	<0.0001

Model 1 adjusted for assay batch and total energy intake.

Model 2 adjusted for assay batch, total energy intake and sex.

Table 3 Geometric mean urinary isothiocyanate levels (nmol/mg creatinine) by glutathione S-transferase gene (*GST*) copy number among the included participants from the Shanghai Women's and Men's Health Studies

	n	Mean	95 % CI	P value
<i>GSTM1</i>				
Null	2666	1.51	1.43, 1.59	0.1209
Carrier	1895	1.61	1.51, 1.71	
<i>GSTT1</i>				
Null	2281	1.53	1.44, 1.62	0.5225
Carrier	2311	1.57	1.48, 1.66	
<i>GSTM1/GSTT1</i>				
Both null	1293	1.52	1.41, 1.64	0.1455
One null and one carrier	2331	1.51	1.43, 1.60	
Both carrier	925	1.68	1.53, 1.84	

The model adjusted for assay batch and sex.

Table 4 Geometric mean urinary isothiocyanate levels (nmol/mg creatinine) by glutathione S-transferase gene (*GST*) copy number, stratified by smoking status, among the included participants from the Shanghai Women's and Men's Health Studies

	Never smokers				Past smokers				Current smokers			
	n	Mean	95 % CI	P value*	n	Mean	95 % CI	P value*	n	Mean	95 % CI	P value*
<i>GSTM1</i>												
Null	2241	1.54	1.45, 1.63	0.2987	123	1.32	1.04, 1.68	0.7802	302	1.36	1.18, 1.58	0.0861
Carrier	1600	1.62	1.51, 1.73		75	1.39	1.02, 1.89		220	1.66	1.40, 1.96	
<i>GSTT1</i>												
Null	1931	1.55	1.45, 1.65	0.4488	100	1.42	1.09, 1.84	0.5476	250	1.44	1.23, 1.69	0.8300
Carrier	1918	1.60	1.50, 1.71		103	1.26	0.97, 1.64		290	1.48	1.28, 1.71	
<i>GSTM1/GSTT1</i>												
Both null	1105	1.53	1.41, 1.67	0.3523	52	1.26	0.87, 1.82	0.5243	136	1.55	1.25, 1.92	0.0200
One null and one carrier	1949	1.55	1.46, 1.65		113	1.43	1.12, 1.84		269	1.29	1.11, 1.51	
Both carrier	780	1.68	1.52, 1.85		31	1.06	0.66, 1.71		114	1.91	1.52, 2.42	

All models adjusted for assay batch and sex.

* P value tests the difference in means within each genotype stratum.

observed between participants who provided morning or afternoon urine samples (results not shown).

The model to predict urinary ITC level using backwards selection to select from all covariates included previous gastrectomy, leisure-time physical activity, history of diabetes, history of chronic gastritis, ever consuming ginseng, history of high blood pressure, history of CHD, the number

of times cruciferous vegetables were consumed in the past 24 h, morning sample collection, providing a blood sample, usual cruciferous vegetable intake, sample batch and hours since last intake of cruciferous vegetables. However, this model was able to predict only 11.7% ($R^2 = 0.117$) of the variation in urinary ITC (see online supplementary material, Supplemental Table 2).

Table 5 Geometric mean urinary isothiocyanate levels (nmol/mg creatinine) by glutathione *S*-transferase gene (*GST*) copy number, stratified by timing of the urine sample, among the included participants from the Shanghai Women's and Men's Health Studies

	Morning sample				Afternoon sample			
	<i>n</i>	Mean	95 % CI	<i>P</i> value*	<i>n</i>	Mean	95 % CI	<i>P</i> value*
<i>GSTM1</i>								
Null	1188	1.42	1.32, 1.54	0.9217	1478	1.58	1.47, 1.70	0.0436
Carrier	857	1.43	1.31, 1.57		1038	1.77	1.63, 1.93	
<i>GSTT1</i>								
Null	1011	1.43	1.31, 1.55	0.9405	1270	1.61	1.49, 1.74	0.4140
Carrier	1045	1.43	1.32, 1.56		1266	1.69	1.56, 1.83	
<i>GSTM1/GSTT1</i>								
Both null	586	1.43	1.28, 1.60	0.9737	707	1.61	1.45, 1.78	0.0328
One null and one carrier	1014	1.42	1.30, 1.54		1317	1.59	1.48, 1.72	
Both carrier	440	1.44	1.27, 1.64		485	1.93	1.70, 2.18	

All models adjusted for assay batch and sex.

**P* value tests the difference in means within each genotype stratum.

Discussion

In the present study of Chinese men and women, self-reported intake of cruciferous vegetables was only weakly correlated with urinary ITC levels measured in a spot urine sample. The strongest correlations were observed between self-reported recent cruciferous vegetable intake and urinary ITC. Overall, urinary ITC did not appear to be related to *GST* gene polymorphisms, but when the data were stratified by smoking status, some differences by genotype were observed among current smokers. Additionally, among participants who provided an afternoon urine sample, those with the *GSTM1*-null genotype had lower urinary ITC excretion than the carrier genotype. A number of individual factors were associated with urinary ITC levels, but the linear prediction model was only able to explain approximately 12% of the variation.

In a previous study of postmenopausal women in the USA, a relatively weak correlation (Pearson correlation = 0.22) was observed between cruciferous vegetable intake from an FFQ and urinary dithiocarbamate, another biomarker of cruciferous vegetable intake⁽²⁸⁾. This observed correlation was stronger than the correlation with usual intake in our study; however, the study assessed cruciferous vegetable intake only during the week prior to a cruciferous vegetable intervention and the correlation in our study for recent intake was similar to this finding. Another study among a Chinese population in Singapore noted statistically significant associations between consumption of cruciferous vegetables and urinary ITC ($P=0.0004$) and between dietary ITC and urinary ITC ($P=0.0003$), but did not report the strength of the associations⁽²³⁾. The correlations observed in our study were all statistically significant ($P<0.0001$), but the correlations were weak. Relatively weak correlations ($r_s=0.16$, $P<0.01$) were observed in a population of female controls from the Shanghai Breast Cancer Study between both usual cruciferous vegetable intake and dietary ITC and urinary levels of ITC⁽²⁹⁾.

Feeding studies have shown that urinary ITC is a useful biomarker of dietary exposure to ITC, with a Spearman correlation of 0.93 between cruciferous vegetable dose and 24 h urinary output of ITC^(15,25). These results are expected since urinary ITC has a peak excretion between 2 and 6 h after consuming cruciferous vegetables with low to no presence after 24 to 48 h⁽¹⁵⁾. Thus, urinary ITC levels are good markers for recent cruciferous vegetable intake. Our FFQ assessed usual intake over the past year and a single urine sample from each participant was available for our study. Since urinary ITC reflects recent intake and the FFQ only ascertained usual intake, the weak correlations were expected. Supporting this point, we found stronger correlations of urinary ITC with recent cruciferous vegetable intake, although the correlation was still relatively weak. Measurement error in cruciferous vegetable intake data derived from the FFQ could also attenuate the correlations. In general, FFQ data are prone to dietary recall errors⁽¹⁴⁾, do not cover every cruciferous vegetable that produces ITC⁽³⁰⁾ and do not account for variability in actual ITC exposure affected by growing conditions, cooking methods⁽³¹⁾ and storage conditions⁽³²⁾. We used ITC data from vegetables in Asia; however, this calculation excluded white turnip/radish because data on ITC content were not available^(23,24). Some of the variation between cruciferous vegetable intake and urinary ITC appeared to be affected by chronic conditions, such as gastrointestinal disease, since in the linear prediction model both a history of chronic gastritis (n 938; 20.4%) and previous gastrectomy (n 52; 1.1%) were statistically associated with urinary ITC. Although this is an intriguing finding, all chronic condition data were collected by self-report and it is beyond the scope of the study to investigate potential mechanisms such as whether gastrointestinal disease changed urinary ITC levels or if participants with gastrointestinal disease changed intake of cruciferous vegetables.

ITC induces *GST* enzymes that in turn catalyse the conjugation of ITC⁽²⁾, but previous research has been inconsistent regarding the effect of the *GSTM1* and *GSTT1*

genes on the urinary output of ITC. One study found that urinary ITC was higher among participants with the *GSTM1* null genotype⁽³³⁾, another found that urinary ITC was slightly higher among participants with the *GSTT1* null genotype⁽²⁹⁾ and two other studies observed no difference by genotype^(34,35). We found no indication for a difference by genotype in our entire sample, but *GSTM1* and the combination of *GSTM1* and *GSTT1* genotypes appeared to be associated with urinary ITC levels among current smokers. Smoking is a GST inducer and in our sample, urinary ITC level was lower for participants having a null genotype for *GSTM1* or for both *GSTM1* and *GSTT1*. The *GSTM1* and the combination of *GSTM1* and *GSTT1* genotypes also were associated with urinary ITC levels for participants who provided an afternoon urine sample, suggesting that these genotypes may be relevant to ITC excretion rates when exposure levels are higher, since afternoon samples are presumably collected sooner following consumption of cruciferous vegetables than morning samples would be, although this may be a chance finding. Additional research is needed on the relationship between cruciferous vegetable consumption and *GST* gene variants including the effect of smoking and timing of urine sample collection.

Our study has a number of important strengths including the relatively large sample size, high response rate and the population-based study design of the parent study. Although there are limitations to the self-reported measures of cruciferous vegetable intake and dietary ITC, the FFQ had relatively high validity and reliability measures^(21,22). The urinary ITC measurement is limited because only a single, spot urine sample was available. Given that urinary ITC appears to have a peak excretion between 2 and 6 h after consumption of cruciferous vegetables⁽¹⁵⁾, our study is limited by relying on a spot urine sample. A 24 h urine sample would likely give a better estimate of recent cruciferous vegetable intake, but collection of a 24 h urine sample in a large-scale epidemiological study would be extremely challenging and may lead to increased missing data and selection bias. Confounding due to unmeasured confounders could be an issue. For example, we did not assess other *GST* genes, such as *GSTP1* or *GSTA1*, or other potential metabolizing genes on the urinary excretion of ITC. Finally, laboratory errors in the assessment of urinary ITC and the *GST* gene variants could occur; however, quality control procedures minimized errors and any bias would likely be non-differential.

Conclusion

In conclusion, self-reported usual intake of cruciferous vegetables was weakly correlated with urinary level of ITC while self-reported recent intake was more strongly correlated with urinary ITC, which suggests that urinary ITC measured in a spot urine sample is a better biomarker for recent cruciferous vegetable intake. *GST* gene variants,

particularly *GSTM1*, may be important in ITC metabolism and excretion among current smokers and shortly after intake. Future research on urinary ITC should take into consideration the influence of genotype, smoking and upper gastrointestinal diseases.

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Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S1368980014001505>

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