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

Emily Vogtmann1,2,3, 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 Shu1,*

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

Submitted 23 December 2013: Final revision received 10 June 2014: Accepted 19 June 2014: First published online 7 August 2014

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\% and 74\%, 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 described\(^{(19,20)}\). 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 (0–25 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 \(\mu\)mol/l. For undetectable ITC levels (\(n = 350\) for SWHS; \(n = 8\) for SMHS), the value was set to 0.1 \(\mu\)mol/l divided by the square root of two. Urinary creatinine was measured using the Jaffé alkaline picate 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 (METs 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 >17,593 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 \( \beta \) 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 \( (P = 0.3897) \).
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 GSTT1 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 1.52 (95 % CI 1.22, 1.60) for never, past and current smokers, respectively, after adjustment of smoking status, and urinary ITC levels of 1.29 (95 % CI 1.22, 1.26) and 1.30 (95 % CI 1.24, 1.27) 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 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 ($P=0.036$) and the combination GSTM1/GSTT1 gene category ($P=0.038$) 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 1 Demographic characteristics of the included participants from the Shanghai Women’s and Men’s Health Studies

<table>
<thead>
<tr>
<th></th>
<th>SWHS (n 3589)</th>
<th></th>
<th>SHMS (n 1015)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Median or %</td>
<td>IQR</td>
<td>Median or %</td>
<td>IQR</td>
</tr>
<tr>
<td>59.5</td>
<td>49.1–65.6</td>
<td>65.6</td>
<td>56.2–70.6</td>
<td></td>
</tr>
<tr>
<td>Educational level (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>35.7</td>
<td>–</td>
<td>14.0</td>
<td>–</td>
</tr>
<tr>
<td>Middle school</td>
<td>29.8</td>
<td>–</td>
<td>32.3</td>
<td>–</td>
</tr>
<tr>
<td>High school</td>
<td>22.8</td>
<td>–</td>
<td>27.0</td>
<td>–</td>
</tr>
<tr>
<td>College</td>
<td>11.8</td>
<td>–</td>
<td>26.7</td>
<td>–</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>96.6</td>
<td>–</td>
<td>38.4</td>
<td>–</td>
</tr>
<tr>
<td>Past</td>
<td>0.6</td>
<td>–</td>
<td>18.2</td>
<td>–</td>
</tr>
<tr>
<td>Current</td>
<td>2.9</td>
<td>–</td>
<td>43.4</td>
<td>–</td>
</tr>
<tr>
<td>Alcohol consumption (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>97.3</td>
<td>–</td>
<td>68.4</td>
<td>–</td>
</tr>
<tr>
<td>Ever</td>
<td>2.7</td>
<td>–</td>
<td>31.6</td>
<td>–</td>
</tr>
<tr>
<td>Tea consumption (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>74.3</td>
<td>–</td>
<td>37.8</td>
<td>–</td>
</tr>
<tr>
<td>Ever</td>
<td>25.7</td>
<td>–</td>
<td>62.2</td>
<td>–</td>
</tr>
<tr>
<td>Ginseng use (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>66.1</td>
<td>–</td>
<td>63.7</td>
<td>–</td>
</tr>
<tr>
<td>Ever</td>
<td>33.9</td>
<td>–</td>
<td>36.3</td>
<td>–</td>
</tr>
<tr>
<td>Family history of cancer (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26.8</td>
<td>–</td>
<td>31.9</td>
<td>–</td>
</tr>
<tr>
<td>No</td>
<td>73.2</td>
<td>–</td>
<td>68.1</td>
<td>–</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3</td>
<td>22.1–26.7</td>
<td>23.9</td>
<td>21.8–26.1</td>
</tr>
<tr>
<td>Leisure-time physical activity (MET x h/d)</td>
<td>0.0</td>
<td>0.0–1.23</td>
<td>0.3</td>
<td>0.0–2.9</td>
</tr>
<tr>
<td>Total energy intake (kJ/d)</td>
<td>6822</td>
<td>5816–7902</td>
<td>7644</td>
<td>6520–8887</td>
</tr>
<tr>
<td>Total energy intake (kcal/d)</td>
<td>1630.6</td>
<td>1390.1–1888.6</td>
<td>1826.9</td>
<td>1558.2–2147.9</td>
</tr>
<tr>
<td>Cruciferous vegetable intake (g/d)</td>
<td>82.5</td>
<td>50.7–131.8</td>
<td>90.9</td>
<td>56.6–147.0</td>
</tr>
<tr>
<td>Dietary ITC (µmol/d)</td>
<td>7.0</td>
<td>4.4–11.2</td>
<td>7.9</td>
<td>4.7–13.0</td>
</tr>
<tr>
<td>Urinary ITC (nmol/mg creatinine)</td>
<td>1.7</td>
<td>0.7–4.0</td>
<td>1.5</td>
<td>0.7–3.8</td>
</tr>
</tbody>
</table>

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.
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).
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\(^2\).\(^3\).\(^4\). 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\(^4\).\(^5\). 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\(^1\), do not cover every cruciferous vegetable that produces ITC\(^2\),\(^3\) and do not account for variability in actual ITC exposure affected by growing conditions, cooking methods\(^4\),\(^5\) and storage conditions\(^6\).\(^7\). We used ITC data from vegetables in Asia; however, this calculation excluded white turnip/radish because data on ITC content were not available\(^2\).\(^3\).\(^4\). 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; 1.4%) 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

### 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

<table>
<thead>
<tr>
<th></th>
<th>Morning sample</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Mean</td>
<td>95 % CI</td>
<td>(P) value*</td>
<td>(n)</td>
<td>Mean</td>
<td>95 % CI</td>
<td>(P) value*</td>
<td></td>
</tr>
<tr>
<td><strong>GSTM1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>1188</td>
<td>1·42</td>
<td>1·32, 1·54</td>
<td>0·9217</td>
<td>1478</td>
<td>1·58</td>
<td>1·47, 1·70</td>
<td>0·0436</td>
<td></td>
</tr>
<tr>
<td>Carrier</td>
<td>857</td>
<td>1·43</td>
<td>1·31, 1·57</td>
<td></td>
<td>1038</td>
<td>1·77</td>
<td>1·63, 1·93</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GSTT1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>1011</td>
<td>1·43</td>
<td>1·31, 1·55</td>
<td>0·9405</td>
<td>1270</td>
<td>1·61</td>
<td>1·49, 1·74</td>
<td>0·4140</td>
<td></td>
</tr>
<tr>
<td>Carrier</td>
<td>1045</td>
<td>1·43</td>
<td>1·32, 1·56</td>
<td></td>
<td>1266</td>
<td>1·69</td>
<td>1·56, 1·83</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GSTM1/GSTT1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both null</td>
<td>586</td>
<td>1·43</td>
<td>1·28, 1·60</td>
<td>0·9737</td>
<td>707</td>
<td>1·61</td>
<td>1·45, 1·78</td>
<td>0·0328</td>
<td></td>
</tr>
<tr>
<td>One null and one carrier</td>
<td>1014</td>
<td>1·42</td>
<td>1·30, 1·54</td>
<td></td>
<td>1317</td>
<td>1·59</td>
<td>1·48, 1·72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both carrier</td>
<td>440</td>
<td>1·44</td>
<td>1·27, 1·64</td>
<td></td>
<td>485</td>
<td>1·93</td>
<td>1·70, 2·18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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\(^2\).\(^3\).\(^4\). 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\(^2\).\(^3\). The correlations observed in our study were all statistically significant (\(P<0·0001\)), but the correlations were weak. Relatively weak correlations (\(r=0·16\), \(P<0·01\)) 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\(^2\).

Downloaded from https://www.cambridge.org/core. IP address: 54.191.40.80, on 12 May 2017 at 04:20:26, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S1368980014001505
genes on the urinary output of ITC. One study found that
urinary ITC was higher among participants with the GSTM1
null genotype\(^{29}\), 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 vege-
table 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 cruci-
ferous vegetable intake and dietary ITC, the FFQ had rela-
tively 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\(^ {19}\), 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 challeng-
ing 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
genomes, such as GSTP1 or GSTA1, or other potential meta-
bolizing 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.

**Acknowledgements**

Acknowledgements: The authors would like to thank the
participants and the staff from the Shanghai Women’s and
Men’s Health Studies. They also would like to thank Dr
Hui Cai for statistical assistance and Regina Courtney for
laboratory support from Vanderbilt University Medical
Center.

Financial support: This work was supported by
funds from the National Institutes of Health (NIH; grant
numbers R01 CA082729 and R37 CA070867) and the
Vanderbilt Clinical and Translational Science Award from
the National Center for Research Resources (grant number
UL1 RR024975-01) which is now at the National Center
for Advancing Translational Sciences (grant number UL1
TR000445-06). E.V. and H.-L.L. were supported by the
Fogarty International Research Scholars and Fellows Program at Vanderbilt University (grant number
R24 TW007988); and E.V. was supported by the Cancer
Prevention and Control Training Program at the University
of Alabama at Birmingham funded through the NIH
(grant number 5R25 CA047888). The content is solely the
responsibility of the authors and does not necessarily
represent the official views of the NIH. The funders had no
role in the design, analysis or writing of this article.

Conflict of interest: None. Authorship: G.Y., Y.T.G., W.Z.,
Y.-B.X., and X.-O.S. designed the study. E.V., G.Y., H.-L.L.,
B.Z., Y.-B.X. and X.-O.S. generated, collected, analysed
and/or interpreted the data. E.V., G.Y., H.-L.L. and
Y.-B.X. drafted or revised the initial manuscript. All authors
read and approved the final manuscript. Ethics of human subject participation: The
SWHS and SMHS were reviewed by the Institutional
Review Boards at the Shanghai Cancer Institute and
Vanderbilt University Medical Center.

**Supplementary material**

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

**References**

sinigrin (2-propenyl glucosinolate) by the human colonic
microflora in a dynamic in vitro large-intestinal model.
Carcinogenesis 23, 1009–1016.
anti-proliferative activity and mechanism of action. Proc Nutr
Soc 65, 68–75.


33. Steck SE, Gammon MD, Hebert JR et al. (2007) GSTM1, GSTT1, GSTP1, and GSTA1 polymorphisms and urinary isothiocyanate metabolites following broccoli consumption in humans. J Nutr 137, 904–909.
