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Cruciferous vegetables, glutathione S-transferase polymorphisms and the risk of colorectal cancer among Chinese men

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Abstract

Purpose—To assess the associations between cruciferous vegetable (CV) intake, *GST* gene polymorphisms and colorectal cancer (CRC) in a population of Chinese men.

Methods—Using incidence density sampling, CRC cases (N = 340) diagnosed prior to December 31, 2010 within the Shanghai Men's Health Study were matched to non-cases (N = 673). CV intake was assessed from a food frequency questionnaire and by isothiocyanate (ITC) levels from spot urine samples. *GSTM1* and *GSTT1* were categorized as null (0 copies) versus non-null (1 or 2 copies). Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between CV intake and *GST* gene variants with CRC and statistical interactions were evaluated.

Results—CRC risk was not associated with CV intake, whether measured by self-report or by urinary ITC, nor with *GST* gene variants. No statistical interactions were detected between CV intake and *GST* gene variants on the odds of CRC. Stratifying by timing of urine sample collection and excluding CRC cases diagnosed in the first two years did not materially alter the results.

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Conclusions—This study provides no evidence supporting the involvement of CV intake in the development of CRC in Chinese men.

Keywords

brassicaceae; China; colorectal neoplasms; glutathione S-transferase M1; glutathione S-transferase T1; men

INTRODUCTION

Cruciferous vegetable (CV) intake has long been studied for protective effects against cancer. The preventive benefit has been proposed to be related to the presence of isothiocyanate (ITC), which has been observed to reduce oxidative stress, induce differentiation and decrease inflammation [1, 2]. Recent evidence has suggested that ITCs, specifically sulforaphane, may also play a role in epigenetic changes [3]. However, epidemiologic studies investigating CVs or urinary ITC and the risk of colorectal cancer (CRC) have found weak or null associations [4–13].

ITC induces glutathione S-transferase (GST) activity and then is metabolized by GST enzymes for elimination [14]. Individuals with a homozygous deletion of both copies of the *GSTM1* or *GSTT1* gene do not produce the GSTM1 or GSTT1 enzyme, respectively [14]. The absence of these enzymes could lead to decreased activity of GST and lengthened exposure to ITC, increasing the anti-carcinogenic effects of ITC. Previous epidemiological research has suggested that GST gene polymorphisms interact with CV intake or urinary ITC to modify the risk of CRC, but evidence is inconsistent [4, 6–8, 11, 13, 15, 16].

We evaluated the association between CV consumption, both estimated by self-report and urinary ITC, alone and in conjunction with *GST* gene polymorphisms on the risk of CRC using data from the Shanghai Men's Health Study (SMHS).

METHODS

Source population

The methodology for the SMHS has been described in detail [17]. Briefly, the SMHS is a prospective, population-based cohort study in Shanghai, China where men aged 40 to 74 years without a history of cancer were recruited between March 2002 and June 2006. A total of 61,483 men participated with a response rate of 74.1%. At enrollment, participants were asked to provide spot urine and blood samples. Buccal cell samples were requested from participants unwilling to provide blood samples. The SMHS received approval from the Institutional Review Board at Vanderbilt University and the Shanghai Cancer Institute.

Case ascertainment and control selection

Annual record linkage with the population-based Shanghai Cancer Registry and the Shanghai Municipal Vital Statistics Unit identified incident cancer cases and decedents, respectively. Incident cancer cases were verified through home visits and medical chart review. CRC cases, defined as a primary tumor having ICD-9 code 153 (malignant neoplasm of colon) or 154 (malignant neoplasm of rectum, rectosigmoid junction, and anus), diagnosed prior to December 31, 2010 were included.

Controls were identified from the SMHS using incidence density sampling with a 2:1 control to case selection ratio and matched on age (± 2 years), date of sample collection (± 30 days), time of sample collection (morning or afternoon), time after last meal (± 2 hours), recent vitamin use (yes or no) and the availability of the required biospecimen.

Because biological samples from SMHS participants were limited, subjects included in previous case-control studies were excluded from selection (N = 2,424). Median follow-up was 6 years.

Assessment of cruciferous vegetable intake

Usual dietary intake over the past 12 months was assessed at baseline using a validated food frequency questionnaire (FFQ). The FFQ captured about 89% of the average food intake in this population and was tested for validity and reliability [18]. The FFQ assessed how often participants consumed specific foods or food groups and the amount consumed for that time period. The average amounts of each food group were calculated by summing the intake for each component food. Nutrient intake was calculated using the Chinese Food Composition Tables [19].

The CVs included greens/Chinese greens, green cabbage, Chinese cabbage/bok choy cabbage, cauliflower, and white turnip. Total CV intake was categorized into tertiles based on the distribution in controls.

Measurement of urinary ITC

High-performance liquid chromatography was used to determine total urinary ITC from baseline spot urine samples [20, 21]. Laboratory staff was blinded to the case status of the samples. For each laboratory run, two representative standards and a reagent blank were included. Each week, a standard curve was created from samples of *N*-acetyl-L-cysteine conjugates of phenethyl ITC (0.2–25 mmol/L) in urine from subjects on a controlled diet. All urine samples and standards were assayed in duplicate and the average ITC level was calculated. If the standard deviation of the mean was greater than 10%, the individual values were checked and the sample was reanalyzed if necessary. The matched sets of cases and controls were included in the same analytic run. The limit of detection was 0.1 µmol/L, so for undetectable ITC levels (N = 8; 0.8%), the ITC value was set to 0.1 µmol/L divided by 2. The laboratory coefficient of variation for ITC was 4.3%. Urinary creatinine was measured using Jaffe' alkaline picrate procedure [22]. All ITC levels were adjusted for urine creatinine level and reported as nmol/mg creatinine. Urinary ITC was categorized into tertiles based on the distribution within the controls.

GST genotyping

DNA was extracted from blood (86.0%) and buccal cell (14.0%) samples. The *GSTM1* and *GSTT1* gene copy numbers (0, 1 or 2) were determined using duplex real-time quantitative polymerase chain reaction-based assays as described in the NCI SNP500 project including modifications [23]. The sequences for the assay design were obtained from GenBank (*GSTM1*, NM_000561 and *GSTT1*, NM_000853) and a 384-well plate in ABI PRISM 7900 Sequence Detection Systems was used (Applied Biosystems, Foster City, CA). The laboratory staff was blinded to the case status of the samples and 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 controls ($p = 0.25$ and $p = 0.69$, respectively).

GSTM1 and *GSTT1* were categorized as *GSTM1*-null or *GSTT1*-null versus non-null (0 versus 1 or 2 copies). A combined category of *GSTM1* and *GSTT1* was created with *GSTM1*-null and *GSTT1*-null, one null and one non-null, and both non-null.

Other covariates of interest

We selected potential confounders a priori based on the previous literature. Demographic variables of interest were age, education level, occupation, and annual per capita family income. Each participant's body mass index (BMI) was calculated from the interviewer-measured height and weight at the baseline visit (kg/m^2). Behavioral characteristics from the baseline questionnaire were cigarette smoking, alcohol consumption, and amount of leisure time physical activity (MET hours/day). We also considered history of diabetes and family history of cancer from the baseline questionnaire. Dietary characteristics of interest were red meat, total meat and total energy intake as derived from the FFQ. Since data was missing for only a small proportion of participants (2.1% overall), for simplicity, we categorized participants with missing data to the most common categories for the covariate. Participants with data missing on education ($N = 19$), income ($N = 1$) or occupation ($N = 1$) were assigned middle school education, 6,000 – 11,999 yuan annual per capita family income, and occupation in manual work. The two participants missing data on family history of cancer were classified as having no family history of cancer.

Statistical analysis

We excluded one control with extreme energy intake (< 500 or $> 4,200$ kcal/day), one case and three controls with missing data on both *GSTM1* and *GSTT1* copy number, and the two controls for the excluded case which left 340 cases and 673 matched controls for analysis. Seven cases had a selection ratio of 1:1 and 333 cases had a selection ratio of 2:1.

Descriptive statistics were calculated for cases and controls and differences were tested using two-way ANOVA for continuous variables and the Cochran-Mantel-Haenszel test for categorized variables stratified by matched pairs. Due to the non-normality of physical activity, the Friedman non-parametric test was used. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression for the association between CV intake, urinary ITC, and the *GST* gene variants with CRC and adjusted for potential confounders. Tests for trend were calculated by including the tertile as a continuous variable. Restricted cubic spline models with 3 knots placed at 10%, 50% and 90% with adjustment for confounders and matching variables were created for a 20 g/day change in CV intake or a 1 nmol/mg creatinine change in urinary ITC. We created stratified models by *GST* gene variants by breaking the matched pairs and adjusting for matching variables and confounders in logistic regression models. Statistical interactions between tertiles of CV intake and urinary ITC with *GST* gene variants were tested using the likelihood ratio test.

Sensitivity analyses included investigating the interaction between urinary ITC and *GST* genes with CRC separately for morning and afternoon urine samples since ITC is rapidly excreted after consumption with a peak in excretion from 2–6 hours after consumption [24]. Participants who provided an afternoon urine sample would be more likely to have had recent CV consumption and potentially higher levels of ITC. In order to assess whether undiagnosed CRC affected the association estimates, we conducted the analyses excluding CRC cases diagnosed within two years of baseline and their matched controls. SAS 9.3 was utilized for analyses and two-sided p-values are presented.

RESULTS

Descriptive statistics of the CRC cases and matched controls are presented in Table 1. In general, cases were similar to controls. However, cases were more likely to have a higher income (9.1% versus 7.0% with annual per capital family income $\geq 24,000$ yuan; $p =$

0.0561), a higher BMI (24.4 versus 23.8 kg/m²; $p = 0.0077$) and a family history of cancer (37.9% versus 28.8%; $p = 0.0031$) than controls.

Self-reported CV consumption was not associated with CRC with an adjusted OR of 0.85 (95% CI: 0.60, 1.22) for the 2nd tertile and 1.06 (95% CI: 0.76, 1.50) for the 3rd tertile compared to the lowest tertile of consumption. Similarly, urinary ITC levels were unrelated to CRC with an adjusted OR of 1.28 (95% CI: 0.90, 1.81) and 1.12 (95% CI: 0.79, 1.60) for the 2nd and the 3rd tertiles, respectively, compared with the 1st tertile (Table 2). The restricted cubic spline models did not show a significant non-linear association between CV intake or urinary ITC and CRC (results not shown). *GSTM1* and *GSTT1* genotype also did not appear to have an association with CRC. Compared to the null genotype, the adjusted OR of CRC for the *GSTM1* non-null genotype was 1.04 (95% CI: 0.78, 1.38) and for the *GSTT1* non-null genotype was 0.84 (95% CI: 0.63, 1.11). The combination category of *GSTM1* and *GSTT1* was not significantly associated with the odds of CRC (Table 3). In addition, no significant associations were detected by *GSTM1* or *GSTT1* copy number (results not shown).

Statistical interactions were not observed between self-reported CV intake or urinary ITC, and the *GST* gene variants on the odds of CRC (Table 4). Similarly, no statistical interactions were observed when CV intake and urinary ITC were analyzed as continuous variables (results not shown). When the analyses for interaction with urinary ITC were conducted separately for morning or afternoon urine samples, no statistical interactions were observed for the morning urine samples, but interactions were observed for the *GSTT1* gene ($p_{\text{interaction}} = 0.0248$) and the *GSTM1/GSTT1* combination ($p_{\text{interaction}} = 0.0919$) in the afternoon urine samples. However, within each genotype of the afternoon urine samples, the only potential trend observed was a positive association between urinary ITC and CRC among participants with a null genotype for both *GSTM1* and *GSTT1* ($p_{\text{trend}} = 0.0636$). When the cases diagnosed within 2 years of baseline were excluded, findings were similar (results not shown).

DISCUSSION

From this study of CRC cases and matched controls nested in the SMHS, no association was detected between CRC and CV intake, either from a FFQ or urinary level of ITC. No association was detected between *GSTM1* or *GSTT1* polymorphisms and CRC risk, and no interaction was seen between CVs with either *GST* gene variant on the odds of CRC. The findings were generally similar for the sensitivity analyses.

Previous studies investigating the association between self-reported CV consumption and CRC have been mixed, although a recent meta-analysis reported a pooled risk ratio (RR) of 0.82 (95% CI: 0.75, 0.90) for the highest category of CV intake compared to the lowest with a similar pooled RR for Asian populations (RR 0.82; 95% CI: 0.64, 1.05). The association was weaker for prospective cohort studies (RR 0.93; 95% CI: 0.87, 1.00) suggesting that the association may have been possibly related to recall bias [25]. Unlike self-reported consumption, urinary ITC measurements are unrelated to recall, but reflect more recent CV consumption since ITC metabolites are eliminated from the body within 48 hours of CV intake [24]. Studies on the association between ITC and CRC have also been variable. One study, also in Shanghai, did not detect an association overall between urinary ITC and CRC, but found an inverse association among cases whose urine samples were collected at least 5 years prior to diagnosis (2nd through 4th versus 1st quartile; OR 0.70; 95% CI: 0.49, 0.99) [12]. When we excluded cases that were diagnosed within two years of baseline, no association was detected between urinary ITC and CRC. Similar to our findings, the Shanghai Women's Health Study (SWHS) did not find an independent association between

ITC and CRC [13]. A case-control study in the United States found an inverse association between detectable ITC and the incidence of CRC (OR 0.59; 95% CI: 0.36, 0.98), but when the study was analyzed by the amount of ITC detected, the inverse association no longer remained significant [11]. Because only 8 men had undetectable ITC levels in our study, we were unable to analyze the effect of undetectable versus detectable ITC.

In general, most studies have not observed an independent association between *GSTM1* or *GSTT1* and the risk of CRC [6, 11, 13, 15, 16, 26]. One case-control study in the United States did find an inverse association between having 0 or 1 copies of the *GSTM1* gene versus 2 copies and left-sided advanced colorectal adenoma (OR = 0.6; 95% CI = 0.4, 0.9), but no independent association was observed with the *GSTT1* gene [23].

The literature on the interaction between CV intake and *GST* gene variants on the risk of CRC risk has been inconsistent. For instance, in a case-control study in Singapore, an inverse association was observed between dietary ITC intake and CRC among participants with the null genotype for both the *GSTM1* and *GSTT1* genes (high dietary ITC versus low OR 0.43; 95% CI: 0.20, 0.96), but when the genotypes were considered individually, no inverse association was observed [6]. Similarly, in the SWHS, the strongest inverse association between urinary ITC and CRC was observed among women with both *GSTM1* and *GSTT1* null genotypes (3rd versus 1st tertile OR 0.51; 95% CI: 0.27, 0.95), but individually, an inverse association was observed among women with the *GSTM1* null genotype, but not the *GSTT1* null genotype [13]. Other studies have found no indication of an interaction between CVs and *GST* genotypes, or interactions with *GSTM1* or *GSTT1* only [8, 11, 15, 16]. We did not detect any interaction between CV intake or urinary ITC level with either *GST* gene variant. The lack of association observed in this study within the SMHS compared to the SWHS could be related to gender differences or the prevalence of other risk factors, such as smoking. Future research could consider the potential interaction between smoking status and *GST* gene polymorphisms and consider additional chemopreventive mechanisms of CVs, such as epigenetic changes, on a population level.

Our study has a number of strengths. All of the self-reported data were obtained prior to diagnosis of cancer so any misreporting of dietary intake should be unrelated to case status. In addition, the blinding of laboratory staff during ITC sample processing and genotyping should decrease any potential bias within the laboratory. Some limitations should also be noted. First, there are limitations to the self-reported measures of CV intake; however the urinary ITC analysis yielded similar results. Laboratory errors in the assessment of urinary ITC and the *GST* gene variants could occur, however these would likely be non-differential. There are also limitations in the use of spot urine samples since ITC is rapidly excreted after consumption, with maximum excretion 2–6 hours after intake [24], but the results from the FFQ and urinary ITC were similar. In addition, the duration of follow-up may not have been long enough to observe an association between baseline CV exposure and colorectal cancer; however, self-reported CV intake represented habitual exposure and over 88% of participants reported to not have greatly increased or decreased consumption of fresh vegetables over the past 5 years (results not shown). It is possible that there was insufficient variation in CV intake or urinary ITC to detect an effect of these exposures. If the effect of CVs has a low threshold and does not follow a dose-response relationship, we may have been unable to detect an association due to the relatively high levels of CV consumption with a median intake of 90.6 (interquartile range 56.6–146.6) g/day and median urinary ITC levels of 1.5 (interquartile range 0.7–3.8) nmol/mg creatinine. Our study was also likely underpowered for the interaction analyses, although, a significant interaction between urinary ITC and *GST* gene variants was observed in the SWHS which had similar cut-points for the ITC tertiles (< 0.95, 0.95–2.98, > 2.98 nmol/mg creatinine) and slightly fewer cases (N = 322), but more controls (N = 1,251) [13]. Also, we only considered two

polymorphisms related to GST enzymes, while other enzymes are involved in metabolizing ITC [27] and we did not consider other mechanisms through which ITC may relate to colorectal cancer, such as the proposed association between sulforaphane and DNA methylation [3]. Finally, although we have carefully adjusted for multiple potential confounders, residual confounding may be present.

In conclusion, in this prospective study of middle aged and elderly Chinese men, we did not find that CV intake or urinary level of ITC was significantly associated with the risk of CRC. Further, there was no indication of an interaction between CV intake and *GST* gene variants on the risk of CRC.

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List of abbreviations and acronyms

BMI	Body mass index
CI	Confidence interval
CRC	Colorectal cancer
CV	Cruciferous vegetable
FFQ	Food frequency questionnaire
GST	Glutathione <i>S</i> -transferase
ITC	Isothiocyanate
OR	Odds ratio
SMHS	Shanghai Men's Health Study
SWHS	Shanghai Women's Health Study

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Table 1

Baseline characteristics of the colorectal cancer cases and matched controls from the Shanghai Men's Health Study

	Colorectal cancer cases	Matched controls	p value
Number of subjects	340	673	
Age			
< 55 years	22.4%	21.4%	0.2106
55 – 59 years	10.9%	11.6%	
60 – 64 years	15.6%	14.6%	
65 – 69 years	23.5%	23.5%	
70 years	27.6%	29.0%	
Educational level (%)			
Elementary school	12.4%	14.9%	0.5244
Middle school	33.8%	31.6%	
High school	25.9%	27.6%	
College	27.9%	25.9%	
Annual per capita family income (%)			
< 6,000 yuan	5.3%	10.0%	0.0561
6,000 – 11,999 yuan	48.5%	45.5%	
12,000 – 23,999 yuan	37.1%	37.6%	
24,000 yuan	9.1%	7.0%	
Occupation			
Professional	35.6%	37.4%	0.4410
Clerical	20.6%	17.1%	
Manual worker	43.8%	45.5%	
Cigarette smoking (%)			
Never	37.1%	38.9%	0.1816
Past	16.2%	19.3%	
Current	46.8%	41.8%	
Alcohol consumption (%)			
Never	68.2%	68.4%	0.9219
Ever	31.8%	31.6%	
BMI (kg/m ²)	24.4 ± 3.4	23.8 ± 3.1	0.0077
Leisure time physical activity (MET hours/day)	1.7 ± 2.7	1.7 ± 2.7	0.5124
Total energy intake (Kcal/day)	1913.7 ± 440.6	1849.1 ± 446.4	0.0289
Red meat intake (g/day)	57.5 ± 36.4	55.7 ± 42.3	0.4995
Total meat intake (g/day)	122.6 ± 69.3	115.7 ± 75.8	0.1327
History of diabetes (%)	11.2%	9.2%	0.3226
Family history of cancer (%)	37.9%	28.8%	0.0031

Continuous variables are presented as mean ± standard deviation.

Association between cruciferous vegetable consumption, isothiocyanate and colorectal cancer for cases and matched controls

Table 2

	Cases/controls	Unadjusted		Adjusted	
		OR	(95% CI)	OR	(95% CI)
Cruciferous vegetable consumption					
< 66.8 g/day (median: 44.3)	113/224	1.00		1.00	
66.8–122.0 g/day (median: 90.0)	98/224	0.88	(0.64, 1.23)	0.85	(0.60, 1.22)
122.1 g/day (median: 175.5)	129/225	1.14	(0.83, 1.55)	1.06	(0.76, 1.50)
<i>p trend</i>		0.3897		0.6679	
Increase of 20 g/day of cruciferous vegetables	340/673	1.01	(0.98, 1.04)	1.00	(0.97, 1.04)
Urinary ITC					
< 0.8 nmol/mg creatinine (median: 0.4)	105/224	1.00		1.00	
0.8–2.4 nmol/mg creatinine (median: 1.5)	121/224	1.17	(0.85, 1.62)	1.28	(0.90, 1.81)
2.5 nmol/mg creatinine (median: 5.5)	114/225	1.09	(0.78, 1.52)	1.12	(0.79, 1.60)
<i>p trend</i>		0.6119		0.5379	
Increase of 1 nmol/mg creatinine of urinary ITC	340/673	1.00	(0.97, 1.02)	0.99	(0.97, 1.01)

Adjusted models included age, BMI, leisure time physical activity, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking, alcohol consumption and family history of cancer.

Table 3
Association between glutathione S-transferase gene variants and colorectal cancer

	Cases/controls	Unadjusted		Adjusted	
		OR	(95% CI)	OR	(95% CI)
<i>GSTM1</i>					
Null	201/379	1.00		1.00	
Non-null	134/259	0.99	(0.76, 1.30)	1.04	(0.78, 1.38)
<i>GSTT1</i>					
Null	173/318	1.00		1.00	
Non-null	164/350	0.86	(0.66, 1.12)	0.84	(0.63, 1.11)
<i>GSTM1/GSTT1</i>					
Both null	106/169	1.00		1.00	
One null/One non-null	159/336	0.75	(0.55, 1.03)	0.76	(0.54, 1.06)
Both non-null	67/128	0.80	(0.55, 1.18)	0.83	(0.55, 1.24)
<i>p trend</i>		0.1977		0.2793	

Adjusted models included age, BMI, leisure time physical activity, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking, alcohol consumption and family history of cancer.

Table 4

Evaluation of associations between cruciferous vegetable consumption, isothiocyanate, and colorectal cancer by glutathione S-transferase genotype

	Cases/controls	Tertile of cruciferous vegetable consumption						Tertile of ITC									
		1		2		3		1		2		3					
		OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	p trend	p int	OR	(95% CI)	OR	(95% CI)	p trend	p int		
<i>GSTM1</i>																	
Null	201/379	1.00	0.67	(0.42, 1.07)	1.04	(0.66, 1.63)	0.7792	0.2867	1.00	1.13	(0.72, 1.77)	1.06	(0.68, 1.65)	0.8111	0.9546		
Non-null	134/259	1.00	1.04	(0.59, 1.84)	1.28	(0.73, 2.23)	0.3786		1.00	1.27	(0.72, 2.24)	1.11	(0.63, 1.94)	0.7629			
<i>GSTT1</i>																	
Null	173/318	1.00	0.78	(0.48, 1.28)	1.11	(0.68, 1.81)	0.6831	0.8807	1.00	0.80	(0.49, 1.30)	1.01	(0.63, 1.63)	0.9392	0.1061		
Non-null	164/350	1.00	0.90	(0.54, 1.50)	1.11	(0.67, 1.82)	0.6457		1.00	1.50	(0.92, 2.43)	0.95	(0.57, 1.59)	0.8302			
<i>GSTM1/GSTT1</i>																	
Both null	106/169	1.00	0.43	(0.21, 0.87)	0.81	(0.42, 1.57)	0.6302	0.4721	1.00	0.98	(0.50, 1.90)	1.44	(0.76, 2.73)	0.2531	0.1375		
One null/One non-null	159/336	1.00	0.97	(0.59, 1.61)	1.42	(0.86, 2.36)	0.1746		1.00	1.08	(0.67, 1.74)	0.71	(0.43, 1.17)	0.1919			
Both non-null	67/128	1.00	1.16	(0.45, 2.96)	0.95	(0.41, 2.24)	0.8496		1.00	2.45	(0.99, 6.04)	1.79	(0.74, 4.34)	0.2342			

All models adjusted for age, BMI, leisure time physical activity, total energy intake, red meat intake, total meat intake, education, income, occupation, smoking, alcohol consumption, family history of cancer, and matching variables including sample type (blood/buccal cell), collection time (morning/afternoon), time (hours) between last meal and sample collection, recent antibiotic use, and time (days) between sample collection and assays for ITC.