Urinary Isothiocyanates; Glutathione *S*-Transferase *M1*, *T1*, and *P1* Polymorphisms; and Risk of Colorectal Cancer: The Multiethnic Cohort Study

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Abstract

Although an association between diet, especially cruciferous vegetables, and colorectal cancer has been hypothesized, recent studies have been inconsistent with their findings. One possibility for the discrepant results is that the interaction with related genes has not generally been considered. The present study examined the associations among urinary isothiocyanates, glutathione *S*-transferase (*GST*) polymorphisms, and colorectal cancer risk in a case-control study nested within the Multiethnic Cohort Study, based in Hawaii and Los Angeles, California. We measured prediagnositic urinary isothiocyanate levels adjusted for creatinine and analyzed *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in 173 cases and 313 matched controls, with biospecimens collected between 2001 and 2006. Conditional logistic regression was used to compute odds ratios and 95% confidence intervals (95% CI). A detectable amount of urinary isothiocyanates was associated with a 41% decrease in colorectal cancer risk (95% CI, 0.36-0.98). No significant, main-effect associations were seen with a homozygous deletion of the *GSTM1* or *GSTT1* polymorphism, or with the AG or GG genotypes for *GSTP1* rs1695. There was a weak suggestion that for individuals with the *GSTP1* AG or GG genotype, a detectable amount of isothiocyanates further decreases one's risk of colorectal cancer compared with those with the *GSTP1* AA genotype, but the interaction term was not statistically significant (*P* = 0.09). This is only the second study published on the association between urinary isothiocyanates and colorectal cancer risk. The results suggest that further studies, with larger numbers, examining a possible interaction with the *GSTP1* polymorphisms are warranted. (Cancer Epidemiol Biomarkers Prev 2009;18(1):314−20)

Introduction

Diet has long been studied as a potentially important factor in the etiology of colorectal cancer (1). Intake of cruciferous vegetables, in particular, has been inversely associated with colorectal cancer risk (2-6). These vegetables are rich in glucosinolates, phytochemicals that are converted to isothiocyanates by plant myrosinase and by the gastrointestinal microbiota. A likely mechanism for an anticarcinogenic effect of isothiocyanates is the modulation of the activity of enzymes involved in the metabolism of carcinogens, especially the induction of phase 2 detoxification enzymes (7, 8). Indeed, isothiocyanates have been shown in a number of animal studies to exert anticancer effects by enhancing the activity of enzymes that block chemical carcinogenesis (9-12).

However, recent cohort studies have been inconsistent with their findings on the association between intake of cruciferous vegetables and colorectal cancer (13). One

possibility for the discrepant results is that the interaction with related genes has not been considered in these studies. The glutathione *S*-transferase (*GST*) enzymes are known to play an important role in the metabolism of isothiocyanates in humans, by conjugating isothiocyanates to glutathione, which then leads to excretion (14, 15). The hypothesis is that individuals with the null genotype of the *GSTM1* or *GSTT1* polymorphism, or the GG genotype for the *GSTP1* Ile[105]Val polymorphism, would have less conjugation activity (16, 17), and thus, in combination with high levels of isothiocyanates, would be better protected against colorectal cancer.

Of the six studies (18-23) that have investigated the association of cruciferous vegetables, *GSTM1, GSTT1*, and/or *GSTP1* polymorphisms, with colorectal cancer risk, only one found a significant interaction—individuals with a homozygous deletion of *GSTT1* and high cruciferous vegetable intake had a 60% reduced risk of colorectal cancer (95% CI, 0.2-0.8) compared with those with at least one copy of the gene and low intake (23). Three of the other studies found suggestions of modification, but all for different genotypes: One study found a decreased risk of colorectal cancer associated with a high intake of cruciferous vegetables only for individuals with both *GSTT1* null and *GSTM1* null genotypes (20); another found a decreased risk of colon cancer with high

cruciferous vegetable intake only for individuals of ages 55 years or younger, especially among those with a *GSTM1* deletion (21); and the last saw an increased risk for colorectal adenomas associated with higher cruciferous vegetable intake among those with the *GSTP1* GG genotype, but not for those with one or two copies of the common A allele (22).

In contrast with these six studies that assessed intake of cruciferous vegetables (or isothiocyanates, specifically) by self-reported diet only, we examined urinary isothiocyanates and related these measures to *GST* polymorphisms and colorectal cancer risk. In past studies examining urinary isothiocyanates and risk of other cancers, a significant inverse association was seen with lung cancer, especially among individuals with a homozygous deletion of *GSTM1* or of both *GSTM1* and *GSTT1* (24). Similarly, a significant trend for reduced risk of breast cancer with higher levels of urinary isothiocyanates was limited to individuals with a homozygous deletion of *GSTM1* or *GSTT1* (25).

The Multiethnic Cohort Study is composed of an unusually diverse population with a wide range of cruciferous vegetable intake, making it particularly suited to the study of the associations among urinary isothiocyanates, *GST* polymorphisms, and colorectal cancer risk.

Materials and Methods

Study Population. From 1993 to 1996, the Multiethnic Cohort Study recruited more than 215,000 individuals with the aim of studying the relationships among diet, genetic variation, and cancer in Hawaii and Los Angeles, California (26). The study targeted the five racial or ethnic groups of African Americans, Native Hawaiians, Japanese Americans, Latinos, and Whites, and required that participants be of ages 45 to 75 years in 1993. Participants completed a 26-page baseline questionnaire that included questions on quantitative food frequency with detailed information on portion sizes as well as questions on medical history and lifestyle. From that questionnaire, information on family history of colorectal cancer, body mass index, smoking history, physical activity, and alcohol use were incorporated into the analyses for the present study. Additionally, the dietary intake of specific food groups and nutrients, including the category of cruciferous vegetables, that consists primarily of consumption of broccoli, cabbage, and cauliflower as single items as well as in mixed dishes as measured by the food frequency questionnaire, was considered in the analyses.

The Biospecimen Subcohort. Recruitment of cohort members for prospective biospecimen collection largely took place between 2001 and 2006. They were contacted by letter, and then by phone, to request biological specimens (blood and urine). For those who agreed, a phone interview, which included a short screening questionnaire and an update of a few items from the baseline questionnaire, was administered. Blood samples were drawn at a clinical laboratory or at the subjects' homes and were processed within 4 h of collection. After centrifugation and separation, blood components were stored in 0.5-mL cryotubes in vapor phase of liquid nitrogen. Urine samples (overnight or first-morning) were mixed, measured, and aliquoted into 2-mL cryo-

tubes and frozen in −80°C freezers. The dispensation and location of all samples are stored through an informatic tracking system.

A total of 67,594 cohort members contributed to the biorepository, from which the cases and controls for this study were selected. Ninety-five percent of blood samples were fasting (≥8 h).

Selection of Cases and Controls. Incident colorectal cancer cases were identified through the Hawaii and California tumor registries of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute. For this nested case-control study, cases were defined as individuals who had contributed blood to the biorepository subcohort before their diagnoses of colorectal cancer and whose diagnoses were reported in the 2006 tumor linkage.

For each case, a pool of potential controls were selected from the individuals who contributed blood to the biorepository and were alive and free of colorectal cancer at the age of the case's diagnosis, and who matched the case on birth year (± 1 y), race or ethnicity, location (Hawaii or California), date of blood draw (± 6 mo), time of blood draw (± 2 h), hours of fasting before blood draw (0-<6, 6-<8, 8-<10, and 10+ h), and type of urine sample (overnight or first morning). From each pool, two controls were then randomly chosen.

Of the 263 eligible colorectal cancer cases and 526 eligible controls, 36 cases and 7 controls were not genotyped, and 23 cases and 54 controls did not have a urine sample available for the measurement of isothiocyanates. Our numbers were then reduced to 203 cases and 366 controls due to losses from conducting the matched analysis (163 cases were matched to 2 controls, 40 cases were matched to 1 control, and the remaining subjects existed in sets in which there were not at least 1 case and 1 control). Furthermore, 30 cases and 53 controls were lost due to missing information on one of the selected confounders. Thus, the study population for the present study included 173 cases and 313 matched controls.

Laboratory Assays

Urinary Isothiocyanates. The urine samples were analyzed for total isothiocyanates by cyclocondensation with 1,2-benzenedithiol, as previously described (27-29), with some minor modifications (Fowke, 2003, #29): We used an isothiocyanate-free urine for preparation of PEITC-NAC standards (0, 0.5, 1, 2, 5, 10, 15, 20, 25, and 50 mmol/L), instead of 20 mmol/L phosphate buffer (pH 5.0); for high-performance liquid chromatography assay, the mobile phase consisted of a mixture of methanol and water 5:2 (v/v) rather than previously reported methanol and water 7:3 (v/v). All other conditions were the same. All urine samples and standards were prepared and analyzed in triplicates. Samples from matched cases and controls were analyzed in the same analytic batch. Blind duplicate samples from quality control urine pools were analyzed with the study samples and the intrabatch coefficient of variation was 12%.

Urinary Creatinine. Urinary creatinine concentrations were measured with a Roche-Cobas MiraPlus chemistry analyzer using a kit from Randox Laboratories that is based on a kinetic modification of the Jaffé reaction with

a level of quantitation of <15 μmol. For creatinine levels of 739 to 2,151 mg/L, we found in this project mean inter- and intra-assay coefficient of variation values of 5.7% and 2.6%, respectively. Isothiocyanate levels were adjusted for creatinine level by taking the ratio of the two values.

DNA Extraction and Genotyping Assays. DNA was purified from blood buffy coat using QIAamp DNA Blood Kits (Qiagen). The DNA was plated on 384-well plates together with duplicate quality control pairs. All assays were run on an Applied Biosystems 7900HT Fast Real-Time System. Genotyping of the *GSTP1* missense single nucleotide polymorphism rs1695 was done using 10 ng of genomic DNA and the 5′ nuclease TaqMan allelic discrimination assay (C_3237198_20) from Applied Biosystems. The *GSTM1* and *GSTT1* copy numbers were analyzed on triplicate plates using 10 ng of genomic DNA and predesigned Applied Biosystems TaqMan copy number assays. Copy numbers were calculated using the ddCT approach. Briefly, the difference in Ct values (dCt) was calculated by subtracting the mean Ct of the reference (RNase P) triplicates run for each sample from the mean Ct of the triplicates for *GSTM1* or *GSTT1* for that sample. ddCT were calculated by subtracting the mean dCT of known two copy controls included in the analysis plates. Copy numbers were calculated from ddCT as follows: *GSTM1* or *GSTT1* copy number = 2^{-ddCT}. The final copy number (0, 1, or 2) was determined using a two-sided tolerance interval (30).

Ethnic-specific genotype frequencies for *GSTM1, GSTT1,* and *GSTP1* in the controls were compared against Hardy-Weinberg equilibrium. All frequencies met the Hardy-Weinberg equilibrium criteria at $P > 0.05$.

Statistical Analyses. To estimate the associations of urinary isothiocyanates, *GST* polymorphisms, and colorectal cancer, we used conditional logistic regression models to compute odds ratios (OR) and 95% confidence intervals (95% CI), in which the matched case-controls sets were the strata. Urinary isothiocyanate levels were categorized both as a binary variable (detectable versus undetectable) and as two dummy variables [using undetectable as the reference group, detectable but less than the median concentration of urinary total isothiocyanates (μmol/mg creatinine) in the control distribution, and detectable and equal to or greater than the median]. Linear trend in the logit of risk was tested by modeling urinary isothiocyanates as a trend variable assigned the median value of the appropriate category. The *GSTM1* and *GSTT1* deletion polymorphisms were modeled both as binary variables (homozygous present or heterozygous versus homozygous deletion), as dummy variables representing the number of copies (1 or 0) compared with no deletions (2 copies), and as the number of gene copies (2, 1, or 0). The *GSTP1* polymorphism was also modeled both as a binary variable (AA versus AG or GG genotype), as dummy variables (using AA as the reference group, AG and GG genotype), and the number of G alleles. For *GSTM1* and *GSTT1,* the linear trend in the logit of risk was tested based on the number of copies, whereas for *GSTP1,* the linear trend was based on the number of G alleles.

In addition to the initial crude models, secondary models were created to further adjust the risk estimates for potential residual confounding from the matching variables of age at blood draw and hours of fasting before blood draw as continuous variables to account for any variation within matched sets, as well as for the following risk factors for colorectal cancer that were found to be significantly associated in the data with both the exposure of urinary isothiocyanates and the outcome of colorectal cancer, without being associated with the *GST* polymorphisms (and thus possibly being an intermediate outcome): processed meat intake (density, g/kcal/d), ethanol consumption (g/d), obesity (as a binary variable, <30 kg/m² versus ≥30 kg/m²), and history of colorectal cancer screening (never versus ever). Total energy intake was also considered as a potential confounder, but was neither significant alone nor did it change the main association by more than 10%, and thus was not included in the final models. The likelihood ratio test was used to examine interactions between urinary isothiocyanates (modeled as a continuous variable) and *GST* polymorphisms in relation to colorectal cancer risk.

Results

As shown in Table 1, colorectal cancer cases were more likely to be obese, more likely to consume alcohol, and generally consume lesser fruits and vegetables and more red and processed meat than controls. Little to no difference was seen for a family history of colorectal cancer, smoking history, or moderate or vigorous physical activity.

Table 2 presents the OR for the association between urinary isothiocyanates and colorectal cancer risk. A detectable amount of isothiocyanates in the urine was associated with a 41% decrease in colorectal cancer risk (95% CI, 0.36-0.98) after adjusting for covariates. Grouped by concentration of urinary total isothiocyanates (μmol/mg creatinine), there was no suggestion of a trend with increasing amounts of urinary isothiocyanates and decreasing risk of colorectal cancer. Compared with those with undetectable isothiocyanates, individuals with a detectable but below-median concentration of isothiocyanates (>0.00-1.40 μmol/mg creatinine) had a 42% reduced risk of colorectal cancer (95% CI, 0.34-1.00), and individuals with an above-median concentration (>1.40 μmol/mg creatinine) had a 39% reduced risk of colorectal cancer (95% CI, 0.34-1.09).

Table 3 presents the OR for the association for *GSTM1, GSTT1,* and *GSTP1* genotypes and colorectal cancer risk. There were suggestions of an increase in risk for individuals with a homozygous deletion of the *GSTM1* polymorphism (OR, 1.31; 95% CI, 0.88-1.94) and a decrease in risk for individuals with a homozygous deletion of *GSTT1* (OR, 0.62; 95% CI, 0.39-1.00) or the AG or GG genotype for *GSTP1* (OR, 0.76; 95% CI, 0.49-1.18), but none of these associations were statistically significant. Similarly, the copy number analysis suggested an increased risk for subjects with increasing number of *GSTM1* deletions. No clear patterns emerged either when examining the *GSTT1* polymorphisms by number of allele copies or by the *GSTP1* polymorphism in the categories of AA, AG, and GG genotypes separately.

We examined the association of urinary isothiocyanates with colorectal cancer risk, stratified by *GST* genotypes (Table 4), and found a suggestion that for individuals with the *GSTP1* AG or GG genotype, a

Table 1. Baseline characteristics of the colorectal cancer cases and matched controls

	Cases (n = 173)	Controls (n = 313)
Age at blood draw (y), mean (±SD)	69.5 (±8.3)	69.2 (±8.2)
Hours of fasting before blood draw, mean (±SD)	12.4 (±3.6)	12.5 (±3.3)
Male, n (%)	106 (61)	187 (60)
Ethnicity, n (%)		
Japanese American	70 (40)	132 (42)
African American	32 (19)	55 (18)
Latino	34 (20)	59 (19)
White	26 (15)	47 (15)
Native Hawaiian	11 (6)	20 (6)
Education, n (%)*		
High school graduate or less	67 (39)	110 (35)
Some postsecondary education	54 (31)	96 (31)
College graduate	51 (30)	104 (34)
Had previous colonoscopy, n (%)*	62 (36)	142 (45)
1° family history of colorectal cancer, n (%)	21 (10)	29 (8)
Body mass index (kg/m^2), n (%)*		
<25.0	70 (40)	141 (45)
25.0-30.0	67 (39)	130 (42)
≥30.0	36 (21)	42 (13)
Smoking history (y), n (%)*		
None	68 (40)	143 (47)
<10	35 (21)	60 (30)
10-<20	38 (22)	51 (17)
≥20	29 (17)	48 (16)
Ethanol (g/d), n (%)*		
0	77 (45)	159 (51)
<8	45 (26)	85 (27)
≥8	51 (29)	69 (22)
Moderate or vigorous physical activity (h/wk), mean (±SD)*	1.29 (±1.52)	1.19 (±1.27)
Dietary variables, as density = g/kcal/d, mean (± SD)*		
Total vegetables	162.8 (±92.2)	167.1 (±83.2)
Cruciferous vegetables	24.3 (±25.5)	26.2 (±22.6)
Total fruit	170.2 (±134.2)	184.7 (±147.2)
Dietary fiber	11.5 (±4.6)	12.1 (±4.5)
Folate	175.3 (±75.1)	189.1 (±86.4)
Calcium	341.3 (±113.3)	367.2 (±138.1)
Processed meat	8.0 (±5.8)	7.4 (±6.4)
Red meat, not including processed meat	22.2 (±14.2)	18.7 (±12.0)

*Totals do not always sum to 173 and 313 because of missing values.

detectable amount of urinary isothiocyanates further decreases one's risk of colorectal cancer compared with those with the *GSTP1* AA genotype. However, none of the OR nor the interaction term were statistically significant (*P* for interaction of isothiocyanates with *GSTP1* polymorphism = 0.09). Given the strength of the inverse association, the lack of statistical significance probably reflects the relatively small sample sizes. An attempt was made to analyze the data for an interaction among those individuals who had homozygous deletions of both *GSTM1* and *GSTT1* versus all others, but the numbers of individuals with deletions for both was so small that the risk estimates were unstable (data not shown). Additionally, we found no significant associations among urinary isothiocyanate levels and any of the *GST* variants.

Table 2. Urinary isothiocyanate levels in relation to risk of colorectal cancer

	Cases n (%)	Controls n (%)	Crude OR*(95% CI)	Adjusted OR[†] (95% CI)
Urinary ITC				
Undetectable	42 (24)	57 (18)	1.00 (Reference)	1.00 (Reference)
Detectable	131 (76)	256 (82)	0.68 (0.42-1.09)	0.59 (0.36-0.98)
By amount detected (μmol/mg creatinine)				
0.0	41 (24)	57 (18)	1.00 (Reference)	1.00 (Reference)
>0.0-1.40	65 (38)	123 (39)	0.69 (0.42-1.15)	0.58 (0.34-1.00)
>1.40	66 (38)	133 (42)	0.66 (0.38-1.15)	0.61 (0.34-1.09)
P for trend[‡]			0.36	0.42

Abbreviation: ITC, isothiocyanate.
*Based on conditional logistic regression, using matched sets as strata.
[†]Further adjusted for age at blood draw, hours of fasting before blood draw, processed meat (density, g/kcal/d), ethanol (g/d), obesity, and history of colorectal cancer screening.
[‡]Group trend based on medians of isothiocyanate groupings (μmol/g creatinine) = 0.00, 0.58, and 4.08, respectively.

Table 3. *GSTM1*, *GSTT1*, and *GSTP1* genotypes in relation to risk of colorectal cancer

	Cases *n* (%)	Controls *n* (%)	Crude OR*(95% CI)	Adjusted OR[†] (95% CI)
GSTM1				
Non-null	82 (47)	166 (53)	1.00 (Reference)	1.00 (Reference)
Null	91 (53)	147 (47)	1.30 (0.89-1.89)	1.31 (0.88-1.94)
By number of copies				
2	13 (8)	36 (12)	1.00 (Reference)	1.00 (Reference)
1	69 (40)	130 (42)	1.49 (0.74-2.97)	1.38 (0.67-2.84)
0	91 (53)	147 (47)	1.77 (0.91-3.47)	1.68 (0.84-3.35)
P for trend			0.09	0.12
GSTT1				
Non-null	127 (73)	201 (64)	1.00 (Reference)	1.00 (Reference)
Null	46 (27)	112 (36)	0.60 (0.38-0.94)	0.62 (0.39-1.00)
By number of copies				
2	41 (24)	77 (25)	1.00 (Reference)	1.00 (Reference)
1	86 (50)	124 (40)	1.29 (0.79-2.12)	1.28 (0.76-2.14)
0	46 (27)	112 (36)	0.72 (0.40-1.28)	0.75 (0.41-1.37)
P for trend			0.20	0.27
GSTP1 rs1695				
AA	99 (57)	162 (52)	1.00 (Reference)	1.00 (Reference)
AG or GG	74 (43)	151 (48)	0.78 (0.51-1.19)	0.76 (0.49-1.18)
AA	113 (56)	188 (51)	1.00 (Reference)	1.00 (Reference)
AG	59 (34)	110 (35)	0.85 (0.55-1.30)	0.81 (0.52-1.28)
GG	15 (9)	41 (13)	0.54 (0.27-1.10)	0.57 (0.28-1.19)
P for trend			0.10	0.13

*Based on conditional logistic regression, using matched sets as strata.
†Further adjusted for age at blood draw, hours of fasting before blood draw, processed meat (density, g/kcal/d), ethanol (g/d), obesity, and history of colorectal cancer screening.

Discussion

In this case-control study nested in the Multiethnic Cohort Study, we found that an increased intake of cruciferous vegetables, as measured by urinary isothiocyanates, was associated with a decreased risk of colorectal cancer. When stratifying by *GST* polymorphisms, there was the suggestion that the association was more pronounced among individuals with the *GSTP1* GG or AG genotype, compared with individuals with the AA genotype. However, none of the findings in the stratified analyses were statistically significant. Our results are similar to those of two of the six previous studies that found no associations with the *GSTM1* or *GSTT1* polymorphisms (18, 19). Three of those six previous studies found suggestions of decreased risks of colorectal cancer or adenoma with increased intake of cruciferous vegetables or of isothiocyanates for individuals with a homozygous deletion of *GSTM1* (21), *GSTT1* (23), or a combination of deletions of both *GSTM1* and *GSTT1* (20), whereas the third found an increased risk of colorectal adenoma with higher cruciferous vegetable intake (22).

Only two of these studies examined the possible interaction between cruciferous vegetable intake and *GSTP1* polymorphisms in relation to colorectal cancer or adenomas. One found no association (20) and the other found a positive association between colorectal adenoma risk and higher cruciferous vegetable intake among individuals with the low-activity GG genotype (22).

Table 4. Urinary isothiocyanates in relation to risk of colorectal cancer by *GST* genotypes

	Cases *n* (%)	Controls *n* (%)	Crude OR*(95% CI)	Adjusted OR[†] (95% CI)	*P* for interaction[‡]
GSTM1					
GSTM1 non-null, undetectable ITC	19 (23)	31 (19)	1.00	1.00	
GSTM1 non-null, detectable ITC	63 (77)	135 (81)	0.59 (0.25-1.39)	0.49 (0.19-1.25)	
GSTM1 null, undetectable ITC	23 (25)	26 (18)	1.00	1.00	
GSTM1 null, detectable ITC	68 (75)	121 (82)	0.56 (0.21-1.46)	0.53 (0.15-1.93)	0.42
GSTT1					
GSTT1 non-null, undetectable ITC	32 (25)	39 (19)	1.00	1.00	
GSTT1 non-null, detectable ITC	95 (75)	162 (81)	0.63 (0.33-1.20)	0.56 (0.28-1.13)	
GSTT1 null, undetectable ITC	10 (22)	18 (16)	1.00	1.00	
GSTT1 null, detectable ITC	36 (78)	94 (84)	1.07 (0.32-3.57)	0.86 (0.17-4.43)	0.36
GSTP1					
GSTP1 AA, undetectable ITC	21 (21)	24 (15)	1.00	1.00	
GSTP1 AA, detectable ITC	78 (79)	138 (85)	0.53 (0.22-1.30)	0.58 (0.22-1.48)	
GSTP1 AG/GG, undetectable ITC	21 (28)	z33 (22)	1.00	1.00	
GSTP1 AG/GG, detectable ITC	53 (72)	118 (78)	0.57 (0.25-1.31)	0.47 (0.19-1.17)	0.09

*Based on conditional logistic regression, using matched sets as strata.
†Further adjusted for age at blood draw, hours of fasting before blood draw, processed meat (density, g/kcal/d), ethanol (g/d), obesity, and history of colorectal cancer screening.
‡Values based on adjusted models, with urinary isothiocyanates, adjusted for creatinine, as a continuous variable.

The primary strength of this study is the assessment of the exposure, intake of cruciferous vegetables, with a biomarker that is more objective and less prone to systematic measurement error than self-report of diet. In a recent human cross-over feeding study in Denmark, it was shown that levels of isothiocyanates in the urine collected 48 hours after dietary intervention showed a strong dose response with amount of intake of cruciferous vegetables [$r(s)$ = 0.90, P < 0.01; ref. 31]. Moreover, although not many epidemiologic studies have been done using urinary isothiocyanates, a recent study in Shanghai found that increasing levels of urinary isothiocyanates were associated with a decreasing risk of breast cancer, but intake of cruciferous vegetables, as measured by a food frequency questionnaire, were not (25). Similarly, another Shanghai study found an association between detectable urinary isothiocyanates and reduced risk of lung cancer, especially among those individuals with a homozygous deletion of both *GSTM1* and *GSTT1*, whereas a similar analysis using estimated isothiocyanate intake was not possible due to the limited vegetable items on the dietary instrument (24). In the present study, dietary intake of cruciferous vegetables is only mildly correlated with urinary isothiocyanates (Spearman correlation coefficient = 0.12, P = 0.002), although one should note that diet was assessed on average 7 years before biospecimen collection. In a forthcoming report on vegetable intake and colorectal cancer risk in the entire Multiethnic Cohort Study, dietary intake of cruciferous vegetables was not significantly associated with colorectal cancer risk among men (RR of highest versus lowest quintile: 0.87; 95% CI, 0.71-1.08; P for trend = 0.29) or women (RR of highest versus lowest quintile: 0.91; 95% CI, 0.73-1.14; P for trend = 0.79; ref. 32).

There are a number of limitations to the present study. It is possible that our population did not have a sufficiently high consumption of dietary isothiocyanates for us to detect a significant association with cancer risk, as the median urinary isothiocyanate level for all subjects in this study was 0.83 μmol/mg of creatinine (0.69 and 0.94 for cases and controls, respectively), compared with 1.71 for the Shanghai lung cancer study (24); 1.71 and 2.31 for cases and controls, respectively, for the Shanghai breast cancer study (25); and means of 2.32 and 2.75 for cases and controls, respectively, for a recent Shanghai colorectal cancer study (33). Others have noted that Shanghai Chinese tend to consume 300% more cruciferous vegetables than Americans in Los Angeles (33, 34). The group with the largest intake in our population, Japanese Americans, had median isothiocyanate levels of about one third less than the Shanghai Chinese in the colorectal study.

Additionally, we were able to obtain only a one-time urinary sample, reflecting intake in the last 12 to 24 hours, and measurement during such a limited time period may not well reflect usual diet. However, a pilot study we conducted before the present study, wherein 3 overnight urine samples were collected more than 3 weeks from 20 volunteers, resulted in an intra-class correlation coefficient of 0.43 for urinary total isothiocyanates, suggesting its acceptability as a biomarker to study in association with colorectal cancer. The first-morning samples are potentially more problematic. However, when comparing the direct association of

urinary isothiocyanate levels with risk of colorectal cancer in our population, the interaction between isothiocyanates and urinary collection method was not significantly different, although we found a slightly stronger reduction in risk for those with detectable isothiocyanates in their overnight samples than in the first-morning samples. Nonetheless, the issue of the representativeness of the biospecimen as a measure of long-term exposure would most likely result in an attenuation of the risk estimates rather than create a spurious association.

We also note that the median interval of time between collection of urine sample and diagnosis in the present study was short (1.4 years), whereas in the Shanghai colorectal cancer study, a significant association between urinary isothiocyanates and colorectal cancer risk was found only among those individuals from whom urine was collected 10 or more years before diagnosis (33). Finally, the relatively small numbers of subjects in our study allowed us to detect only strong modifying effects of the *GST* polymorphisms on the association between isothiocyanates and colorectal cancer risk and did not permit separate analyses for colon and rectal cancers.

In conclusion, these prospective findings add to the evidence for an inverse association between cruciferous vegetables and colorectal cancer risk by reporting that detectable urinary isothiocyanates are associated with a reduced risk of this malignancy. Significant interactions with the *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms were not found, but there was a weak suggestion of a further decrease in risk with increased urinary isothiocyanates for individuals with the *GSTP1* AG or GG genotype.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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