

## Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study

Adeline Seow<sup>1,3</sup>, Jian-Min Yuan<sup>2</sup>, Can-Lan Sun<sup>2</sup>,  
David Van Den Berg<sup>2</sup>, Hin-Peng Lee<sup>1</sup> and Mimi C.Yu<sup>2</sup>

<sup>1</sup>Department of Community, Occupational and Family Medicine, Faculty of Medicine, National University of Singapore, Singapore and <sup>2</sup>USC/Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

<sup>3</sup>To whom correspondence should be addressed  
Email: cofseowa@nus.edu.sg

**Dietary intake of cruciferous vegetables (*Brassica* spp.) has been inversely related to colorectal cancer risk, and this has been attributed to their high content of glucosinolate degradation products such as isothiocyanates (ITCs). These compounds act as anticarcinogens by inducing phase II conjugating enzymes, in particular glutathione S-transferases (GSTs). These enzymes also metabolize ITCs, such that the protective effect of cruciferous vegetables may predicate on GST genotype. The Singapore Chinese Health Study is a prospective investigation among 63 257 middle-aged men and women, who were enrolled between April 1993 and December 1998. In this nested case-control analysis, we compared 213 incident cases of colorectal cancer with 1194 controls. Information on dietary ITC intake from cruciferous vegetables, collected at recruitment via a semi-quantitative food frequency questionnaire, was combined with GSTM1, T1 and P1 genotype from peripheral blood lymphocytes or buccal mucosa. When categorized into high (greater than median) and low (less than/equal to median) intake, dietary ITC was slightly lower in cases than controls but the difference was not significant [odds ratio (OR) 0.81, 95% confidence interval (CI) 0.59–1.12]. There were no overall associations between GSTM1, T1 or P1 genotypes and colorectal cancer risk. However, among individuals with both GSTM1 and T1 null genotypes, we observed a 57% reduction in risk among high versus low consumers of ITC (OR 0.43, 95% CI 0.20–0.96), in particular for colon cancer (OR 0.31, 0.12–0.84). Our results are compatible with the hypothesis that ITCs from cruciferous vegetables modify risk of colorectal cancer in individuals with low GST activity. Further, this gene–diet interaction may be important in studies evaluating the effect of risk-enhancing compounds in the colorectum.**

### Introduction

The role of diet in the aetiology of colorectal cancer is widely accepted. Among the various components of diet, epidemiologic studies have been fairly consistent in demonstrating an inverse association with intake of cruciferous vegetables (1,2), and this is supported by recent findings from animal studies (3). A distinctive feature of these vegetables is their relatively high content of glucosinolates, which are

converted *in vivo* to isothiocyanates (ITCs), indoles and nitriles by the enzyme myrosinase (4,5). Apart from the colorectum, cruciferous vegetables have been shown to possess chemopreventive activity against a variety of other cancers such as those of the lung and prostate (6,7), and there is accumulating evidence from laboratory studies that this occurs primarily through their effects on the metabolism of pro-carcinogens. Specifically, glucosinolate degradation products from cruciferous vegetables are believed to inhibit phase I activating enzymes, and induce phase II detoxification enzymes (8,9). ITCs, in particular, exert their effects through the latter pathway. Induction of phase II detoxification enzymes reduces exposure of the target tissue to DNA damage, thus exerting a 'blocking effect' on the initiation stage of chemical carcinogenesis (5).

One of the most important detoxification enzyme systems is the glutathione S-transferase (GST) family of enzymes. These enzymes are expressed in a wide variety of human tissue, including both normal and malignant colonic mucosa (10). Conjugation with glutathione by GST is an important step in the metabolism and subsequent detoxification of carcinogens like polycyclic aromatic hydrocarbons, among others. They are also known to metabolize ITCs, resulting in the formation of *N*-acetylcysteine conjugates, which are excreted in the urine (11,12). Human GSTs comprise several subfamilies of isoenzymes: principally GSTM, GSTP and GSTT. Deletions in the GSTM1 and GSTT1 gene produce the null genotypes, which lead to absence of activity of these enzymes; similarly, reduced activity of GSTP1 has been attributed to the low activity B allele (13–15). Epidemiologic studies of GST and colorectal cancer risk have been suggestive of a deleterious effect of the null or low activity genotype (16,17), but findings have been inconsistent (18,19).

Recent findings, both laboratory and epidemiological, suggest that this somewhat equivocal relationship may be explained by a more complex gene–environment interaction. Dietary ITCs induce GST enzyme activity (20,21), and their potency as enzyme inducers has been shown to be related to their total intracellular concentrations (22), and more recently to the formation of intracellular reactive oxygen species (23). On the other hand, the GST enzymes metabolize ITC. The beneficial effect of ITC is therefore dependent in part on the presence or absence of GST activity; individuals with low activity would metabolize these compounds at a slower rate, allowing the protective effects to be exerted to a greater extent at the target tissue level. Similarly, the observed effect of GST on disease risk needs to be viewed in the light of dietary elements known to induce its activity.

The Singapore Chinese population has rising rates of colorectal cancer, particularly cancer of the colon, the age-standardized incidence in males doubling from 10.4 to 20.9 per 100 000 per year in the last three decades (24). Their rates are significantly higher than those in China and similar to Chinese populations in developed countries (25). This population consumes high amounts of cruciferae relative to those in other

**Abbreviations:** CI, confidence interval; GST, glutathione S-transferase; ITC, isothiocyanates; OR, odds ratio.

developed countries. Within the Singapore Chinese Health Study, we demonstrated previously that at all levels of consumption of cruciferous vegetables; urinary excretion of ITC was dependent on GSTT1 genotype (26).

In this report, we use data from the Singapore Chinese Health Study to examine the association between dietary ITC, and its interaction with GSTM1, T1 and P1 genotypes, and colorectal cancer risk in this population.

## Materials and methods

### Study population

The subjects were participants of the Singapore Chinese Health Study, a population-based, prospective investigation of diet and cancer risk (27). Briefly, between April 1993 and December 1998, we recruited 63 257 Chinese men and women from two major dialect groups in Singapore (Hokkien and Cantonese). These were between the ages of 45 and 74 years, residing in government housing estates, of which 86% of the population are resident. Each completed a structured questionnaire administered in person by a trained fieldworker.

In April 1994, 1 year after the initiation of the cohort study, we began collection of blood and single-void urine specimens from a random 3% sample of study participants. A 20 ml blood sample was obtained from each subject. Immediately after blood collection, the tubes were put on ice during transport from the subjects' homes to the laboratory. The specimens were then separated into their various components (plasma, serum, red blood cells and buffy coat). These were subsequently stored in a liquid nitrogen tank at  $-180^{\circ}\text{C}$  until August 2001, when they were moved to  $-80^{\circ}\text{C}$  freezers, which were more economical for long-term storage. Subjects who were unwilling to donate blood were asked to donate buccal cells through the use of a mouthwash protocol based on published methods (28,29). These subjects were provided with a new toothbrush and asked to clean their teeth thoroughly. After an interval of 20 min, during which no food or drink was consumed, they were given 10 ml of commercially purchased 'Listerine' mouthwash and asked to swish the liquid vigorously in their mouths for 60 s. The mouthwash was then collected in a sterile 50 ml polypropylene tube, put on ice and brought back to the laboratory within 5 h, where it was stored at  $-30^{\circ}\text{C}$ . For analysis, the specimens were anonymized and shipped on dry ice to the University of Southern California.

As of July 1999, blood ( $n = 908$ ) or buccal cells ( $n = 286$ ) were collected from 678 female and 516 male cohort subjects without a history of colorectal cancer, who comprised the comparison group for this nested case-control analysis.

### Case ascertainment

We identified incident colorectal cancer cases through the population-based Singapore Cancer Registry (24,30). As of December 2000, 482 cases of incident colorectal cancer (ICDO C18-C20) had developed among cohort subjects. Blood ( $n = 162$ ) or buccal ( $n = 55$ ) specimens were available on 45% (217/482) of the colorectal cancer cases. Histological information of each colorectal cancer diagnosis was confirmed by reviewing the pathology report of each case that was identified. Compared with those who had no formal education, a higher proportion of subjects who had primary school or higher education donated a blood or buccal cell specimen (50 versus 36%). More male cases donated specimens (51%) compared with females (38%). Similar proportions of Cantonese and Hokkien cases donated specimens (46 versus 44%). The average age at diagnosis of cancer was comparable between cases with and without specimen (65.0 versus 65.6 years).

Of these 217 cases, we excluded four cases: lymphoma (one), carcinoma in-situ (one), carcinoid (one) and unknown histologic type (one). Therefore, the present study included 213 colorectal carcinomas.

The study protocol was approved by the Institutional Review Boards of the National University of Singapore and the University of Southern California. All participants gave written, informed consent at the time of recruitment and at collection of blood (or buccal cells) and urine specimens.

### Information on diet and other background variables

The development and validation of the Singapore Chinese Health Study food frequency questionnaire (FFQ) have been described previously (27). At recruitment, information on usual diet over the last year was obtained via this semi-quantitative FFQ, which was administered in person at the subject's home. The questionnaire listed 165 food items, and the respondent was asked to select from eight frequency categories (ranging from 'never' to 'two or more times a day') and three portion sizes with accompanying photographs. The vegetable section included nine cruciferous vegetables commonly consumed by

Chinese in Singapore (see Appendix 1). Average daily intake of 96 nutrient and non-nutrient compounds, including ITC and ethanol, was computed for each study subject via linkage to the Singapore Food Composition Database. The dietary component of the questionnaire was subsequently validated against a series of 24 h diet recalls (27). In the analysis, all foods and nutrients were expressed as weight per 1000 kcal to adjust for total energy intake. Apart from dietary histories, the questionnaire also elicited information on lifetime tobacco use, usual physical activity (weekly hours of strenuous sports, vigorous activity and moderate activity), medical history, family history of cancer and reproductive history (women only).

### GSTM1, T1 and P1 determination

Genomic DNA was isolated using a PureGene Blood Kit (Gentra Systems, Minneapolis, MN) or a QIAamp 96 DNA Blood Kit (Qiagen, Valencia, CA). Genotyping for GSTM1, T1 and P1 was performed using the fluorogenic 5'-nuclease assay (TaqMan Assay) (31).

The TaqMan assays were performed using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. The oligonucleotide primers for amplification of the polymorphic region of GSTP1 were GC070for (5'-CCTGGTGGACATGGTGAATG-3') and GC070rev (5'-TGCTCACACCATAGTTGGTGTAGATGA-3'). In addition, the fluorogenic MGB oligonucleotide probes used to detect each of the alleles were GC070F (5'-TGCAAATACGTCCTCCCT-3') labeled with 6-FAM and GC070V (5'-TGCAAATACATCTCCCT-3') labeled with VIC (Applied Biosystems). PCR amplification using ~10 ng of genomic DNA was performed in a thermal cycler (MWG Biotech, High Point, NC) with an initial step of  $95^{\circ}\text{C}$  for 10 min followed by 50 cycles of  $95^{\circ}\text{C}$  for 25 s and  $60^{\circ}\text{C}$  for 1 min. The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System (Applied Biosystems) and the results analyzed with Sequence Detection Software (Applied Biosystems). Experimental samples were compared with 12 controls to identify the three genotypes at each locus. Any samples that were outside the parameters defined by the controls were identified as non-informative and were retested.

Genotyping of the GSTT1 and GSTM1 loci using the TaqMan assay consisted of separate assays for GSTT1, GSTM1 and the albumin (ALB) control gene. The oligonucleotide primers for amplification of the GSTT1, GSTM1 and ALB genes were GC003for (5'-GTGCAAACCTCCTGGAGAT-3') and GC003rev (5'-AGTCCTTGGCCTTCAGAATGA-3'), GC004for (5'-CTTGGAGGAACTCCCTGAAAAG-3') and GC004rev (5'-TGGAACCTCCATAACACGTGA-3'), GC005for (5'-CGATTTTCTTTTATGGGCAGTAGC-3') and GC005rev (5'-TGGAAACTTCTGCAAACTCAGC-3'), respectively. Fluorescent oligonucleotide probes, for detection of PCR reaction products, were synthesized to contain the dye 6-FAM (BioSearch Technologies, Novato, CA). The probes for the GSTT1, GSTM1 and ALB genes were GC003FAM (5'-ATGCTGCCATCCCTGCC-3'), GC004FAM (5'-AAGCGGCCATGGTTTGCAGG-3') and GC005FAM (5'-CGCCTGAGCCAGAGATTCCCA-3'), respectively. PCR amplification using ~10 ng of genomic DNA was performed in an ABI 7900HT Sequence Detection System (Applied Biosystems) with an initial step of  $95^{\circ}\text{C}$  for 10 min followed by 50 cycles of  $95^{\circ}\text{C}$  for 25 s and  $60^{\circ}\text{C}$  for 1 min. The fluorescence profile of each well was measured in real-time during the PCR amplification and the results analyzed with Sequence Detection Software (Applied Biosystems). Any sample with a fluorescence signal that crossed a threshold of 0.2  $\Delta\text{Rn}$  before cycle 40 was considered positive for the loci analyzed. Samples negative for both GSTT1 and GSTM1 must be positive for ALB to be called, otherwise, the sample was designated non-informative and retested. All analyses were carried out blind to case or control status.

### Statistical analysis

Matched sets of cases and controls were defined jointly by gender, year of birth (1917–1925, 1926–1930, 1931–1935, 1936–1940, 1941–1945, 1946–1954), year of recruitment (1993–1995, 1996–1998) and dialect group (Cantonese, Hokkien). Conditional logistic regression analysis (32) was used to obtain odds ratios (ORs) and their 95% confidence intervals (CI) for the associations between ITC intake, GST genotype and colorectal cancer. Subjects were categorized into high and low ITC intake based on the median value for the entire cohort. For analysis of genotype, we compared null genotypes of GSTM1 and T1 with the non-null, and the GSTP1 AB and BB genotypes against the genotype expected to have highest activity (AA). We subsequently stratified by GST genotype and examined the effect of ITC intake using the same conditional models. For the subgroup analyses, matched sets were only defined by sex and dialect group in order to maximize the number of subjects available for analysis. Age and year of interview were included in the model as covariates. In addition to matching factors, all results presented were adjusted for potential confounding factors, including level of education (no formal education, primary school, secondary school or higher), body mass index ( $<20$ , 20 to  $<24$ , 24 to  $<28$ , 28+  $\text{kg/m}^2$ ), cigarette smoking (never,

**Table I.** Dietary ITC intake in relation to risk of colorectal cancer, Singapore Chinese Health Study

Dietary ITC intake level <sup>a</sup>	Controls	Colorectal cancer		Colon cancer		Rectal cancer	
		Cases	OR (95% CI) <sup>b</sup>	Cases	OR (95% CI) <sup>b</sup>	Cases	OR (95% CI) <sup>b</sup>
<i>Total subjects</i>							
Low ITC	599	127	1.00	76	1.00	51	1.00
High ITC	595	86	0.81 (0.59–1.12)	54	0.83 (0.56–1.22)	32	0.76 (0.46–1.25)
<i>Never smokers</i>							
Low ITC	402	71	1.00	46	1.00	25	1.00
High ITC	462	57	0.89 (0.58–1.36)	38	0.94 (0.57–1.53)	19	0.83 (0.41–1.66)
<i>Ever smokers</i>							
Low ITC	197	56	1.00	30	1.00	26	1.00
High ITC	133	29	0.77 (0.44–1.36)	16	0.81 (0.39–1.68)	13	0.74 (0.34–1.63)

<sup>a</sup>Low (or high) ITC levels were defined as lower (or higher) than the median dietary ITC intake (5.16 µmol/1000 kcal) among all cohort members.

<sup>b</sup>ORs and 95% CIs were derived from conditional logistic regression models including education, BMI, cigarette smoking, weekly strenuous sports/vigorous work, alcohol drinking, and saturated fat as covariates; each risk set of cases and controls was formed according to sex, dialect group (Cantonese, Hokkien), year of recruitment (1993–1995, 1996–1998), and year of birth (1917–1925, 1926–1930, 1931–1935, 1936–1940, 1941–1945, 1946–1953).

ever), weekly strenuous sports/vigorous work (yes, no), alcohol drinking (g ethanol/day in four categories) and saturated fat (% kcal in quartiles).

Statistical analysis was carried out using the SAS software version 8.2 (SAS Institute, Cary, NC) and Epilog for Windows version 1.0 (Epicenter Software, Pasadena, CA). All *P* values reported are two-sided, and *P* values of <0.05 were considered statistically significant.

## Results

Altogether, 213 cases, and 1194 controls were included in this analysis. Of the cases, 130 (61%) had cancers of the colon, and the remaining 83 (39%) had rectal carcinomas.

The mean age of cases at time of diagnosis was 65.1 (SD 7.7) years. The proportion of males among cases was 60% and among controls 43%. Slightly more than half (58 and 51%, respectively) of cases and controls were Hokkien in dialectal group origin.

Among males, 60% (76) of cases and 56% (287) of controls had ever smoked, and 32% of both cases and controls were current smokers at baseline interview. Among females, the corresponding figures were 10 (9) and 6% (43) for ever smokers, and 7 and 5% for current smokers, respectively. Overall, there was no association between a history of smoking and colorectal cancer risk (OR 0.99, 95% CI 0.69–1.43), nor among smokers was there any increase in risk with increasing duration, intensity or with currency of smoking (data not shown).

The mean energy-adjusted dietary intake of cruciferous vegetables among cases was 26.0 g/1000 kcal (SD 15.4), and among controls 28.9 g/1000 kcal (SD 16.9). Dietary intake of ITC was also slightly lower among cases (mean 5.4 µmol/1000 kcal, SD 3.9) than among controls (6.0 µmol/1000 kcal, SD 3.9).

When grouped into high (greater than median) and low (less than/equal to median) intake (Table I), high ITC intake was associated with a reduced risk of colorectal cancer, although this was not statistically significant (OR 0.81, 95% CI 0.59–1.12). There was no evidence of a modification effect by smoking on the ITC-colorectal cancer association; the OR among lifetime non-smokers was 0.89 (95% CI 0.58–1.36) for high versus low dietary ITC intake, and among ever smokers 0.77 (95% CI 0.44–1.36). The associations between dietary ITC intake and risk of colon or rectal cancer alone were similar to both sites combined (Table I).

The prevalence of the GSTM1 and GSTT1 null genotype

among controls was 45 and 40%, respectively, and that of the GSTP1 AB and BB genotypes was 30 and 5%. Overall, there was no association between GSTM1, T1 and P1 genotypes and colorectal cancer risk (Table II). The OR for GSTM1 null genotype was 1.22 (95% CI 0.90–1.67), relative to GSTM1 non-null, and that for the GSTT1 null genotype was 0.88 (95% CI 0.64–1.21) relative to GSTT1 non-null genotype. Results were similar for both colon and rectal cancers. The OR for GSTP1 AB and BB genotypes, relative to the high activity AA genotype, were 0.94 (95% CI 0.67–1.33) and 0.54 (95% CI 0.20–1.41).

No significant associations were observed, and odds ratios were fairly uniform across strata, when dietary ITC intake was examined among subjects grouped by GSTM1 or GSTP1 genotype (Table III). There was a slight difference between GSTT1 null and non-null individuals. Among GSTT1 null subjects, the OR for high versus low ITC intake was 0.63 (95% CI 0.37–1.07) compared with an OR of 0.97 (95% CI 0.64–1.47) among GSTT1 non-null subjects. However, among individuals null for both GSTM1 and T1, high dietary ITC conferred a 57% reduction in risk which was statistically significant (OR 0.43, 95% CI 0.20–0.96). There were too few cases (*n* = 9) who were null for GSTM1 and T1, and GSTP1 AB/BB genotypes for meaningful analysis. On further analysis, this effect was confined to the subjects with colon cancer (OR 0.31, 95% CI 0.12–0.84) and not seen among those with rectal cancers (Table IV).

We also examined the effect of duration of follow-up on the ITC-colorectal cancer association. When analyses were restricted to colorectal cancers with >3 years of follow-up (*n* = 20), the OR for high versus low ITC intake among subjects with both GSTM1 and GSTT1 null genotypes was similar to that derived from the entire dataset (data not shown).

## Discussion

In summary, in this Asian population with high cruciferous vegetable intake and colorectal cancer rates, we observe an interaction between GST genotype and dietary ITC such that high dietary ITC is associated with a significantly lower risk of colorectal cancer among individuals who are both GSTM1 and T1-null, and hence metabolize and excrete these compounds at a slower rate. The association is not seen in those who are positive for one or both of these metabolic enzymes.

**Table II.** GSTM1, GSTT1 and GSTP1 genotypes in relation to risk of colorectal cancer, Singapore Chinese Health Study

Genotype	Controls <sup>a</sup>	Colorectal cancer		Colon cancer		Rectal cancer	
		Cases <sup>a</sup>	OR (95% CI) <sup>b</sup>	Cases	OR (95% CI) <sup>b</sup>	Cases	OR (95% CI) <sup>b</sup>
GSTM1							
Non-null	653	105	1.00	62	1.00	43	1.00
Null	537	108	1.22 (0.90–1.67)	68	1.24 (0.85–1.82)	40	1.20 (0.75–1.92)
GSTT1							
Non-null	710	133	1.00	83	1.00	50	1.00
Null	480	80	0.88 (0.64–1.21)	47	0.86 (0.58–1.27)	33	0.94 (0.58–1.51)
GSTP1							
AA <sup>c</sup>	779	148	1.00	90	1.00	58	1.00
AB	352	59	0.94 (0.67–1.33)	35	0.91 (0.59–1.39)	24	1.01 (0.60–1.69)
BB	54	5	0.54 (0.20–1.41)	4	0.77 (0.26–2.26)	1	0.23 (0.03–1.76)

<sup>a</sup>Sum of cases and controls was fewer than the total number of cases (218) and controls (1194) as subjects with missing GSTM1, GSTT1, or GSTP1 genotype were excluded from this analysis.

<sup>b</sup>ORs and 95% CIs were derived from conditional logistic regression models including education, BMI, cigarette smoking, weekly strenuous sports/vigorous work, alcohol drinking, and saturated fat as covariates; each risk set of cases and controls was formed according to sex, dialect group (Cantonese, Hokkien), year of recruitment (1993–1995, 1996–1998), and year of birth (1917–1925, 1926–1930, 1931–1935, 1936–1940, 1941–1945, 1946–1953).

<sup>c</sup>A is the high activity allele of the GSTP1 gene.

**Table III.** Dietary ITC intake in relation to risk of colorectal cancer stratified by GST genotypes, Singapore Chinese Health Study

GST genotype	Low ITC <sup>a</sup>			High ITC <sup>a</sup>		
	Cases <sup>b</sup>	Controls <sup>b</sup>	OR <sup>c</sup>	Cases <sup>b</sup>	Controls <sup>b</sup>	OR (95% CI) <sup>c</sup>
GSTM1 non-null	67	335	1.00	38	318	0.71 (0.45–1.14)
GSTM1 null	60	261	1.00	48	276	0.85 (0.54–1.35)
GSTT1 non-null	79	377	1.00	54	333	0.97 (0.64–1.47)
GSTT1 null	48	219	1.00	32	261	0.63 (0.37–1.07)
GSTP1 AA	90	393	1.00	58	386	0.75 (0.51–1.11)
GSTP1 AB or GSTP1 BB	36	200	1.00	28	206	0.92 (0.50–1.69)
GSTM1 non-null or GSTT1 non-null	104	499	1.00	70	467	0.92 (0.64–1.32)
GSTM1 null and GSTT1 null	23	97	1.00	16	127	0.43 (0.20–0.96)

<sup>a</sup>Low (or high) ITC levels were defined as lower (or higher) than the median dietary ITC intake (5.16 μmol/1000 kcal) among all cohort members.

<sup>b</sup>Sum of cases and controls was fewer than the total number of cases (218) and controls (1194) as subjects with missing GSTM1, GSTT1, or GSTP1 genotype were excluded from this analysis.

<sup>c</sup>ORs and 95% CIs were derived from conditional logistic regression models including education, BMI, cigarette smoking, weekly strenuous sports/vigorous work, alcohol drinking, and saturated fat as covariates; each risk set of cases and controls was formed according to sex, dialect group (Cantonese, Hokkien), year of recruitment (1993–1995, 1996–1998), and year of birth (1917–1925, 1926–1930, 1931–1935, 1936–1940, 1941–1945, 1946–1953).

**Table IV.** Dietary ITC intake in relation to risk of colorectal cancer risk stratified by GSTM1 and GSTT1 genotype, Singapore Chinese Health Study

	Controls <sup>a</sup>	Colon cancer		Rectal cancer	
		Cases	OR (95% CI) <sup>b</sup>	Cases	OR (95% CI) <sup>b</sup>
GSTM1 non-null or GSTT1 non-null					
Low ITC <sup>c</sup>	499	60	1.00	44	1.00
High ITC	467	46	1.00 (0.64–1.53)	24	0.78 (0.45–1.35)
GSTM1 null and GSTT1 null					
Low ITC	97	16	1.00	7	1.00
High ITC	127	8	0.31 (0.12–0.84)	8	0.84 (0.27–2.63)

<sup>a</sup>Sum of controls was fewer than the total number of controls (1194) since subjects with missing GSTM1 or GSTT1 genotype were excluded from this analysis.

<sup>b</sup>ORs and 95% CIs were derived from conditional logistic regression models including age, year of interview, education, BMI, cigarette smoking, weekly strenuous sports/vigorous work, alcohol drinking, and saturated fat as covariates; each risk set of cases and controls was formed according to sex and dialect group.

<sup>c</sup>Low (or high) ITC levels were defined as lower (or higher) than the median dietary ITC intake (5.16 μmol/1000 kcal) among all cohort members.

In an earlier study among Singapore Chinese, Lee *et al.* (33) demonstrated a significant inverse association between cruciferous vegetable intake and colorectal cancer (OR 0.48,

95% 0.23–1.01 for highest versus lowest tertile, *P* for trend <0.05). Among the dietary factors examined, this was the most consistent effect observed in that study. The mean intake

of eight cruciferous vegetables at the time of that study (1985–1987) was 62.5 g/day among males and 67.7 g/day among female controls. When cruciferous vegetable intake (nine varieties) was assessed for the current study approximately one decade later, the mean intakes were 42.1 and 43.4 g/day, respectively. Although study methodology was not uniform, the data suggest a fall in the intake of cruciferous vegetables that appears to parallel rising rates of colorectal cancer in the Singapore population.

While there is now a body of evidence that supports the association between cruciferous vegetables and colon cancer (1,2), the present study provides new information on the effect of GST, the main metabolic enzymes, on this relationship, and is the first to demonstrate this using ITC values calculated from the full range of cruciferous vegetables consumed in the population. Our results are in agreement with those of Lin *et al.* (34) who reported a protective effect of broccoli against colorectal adenomas only among GSTM1 null individuals. While the highest quartile of broccoli intake was itself protective (OR 0.47, 95% CI 0.30–0.73), among those who were also GSTM1 null, the OR was 0.36 (0.19–0.68), compared with 0.74 (0.40–0.99) (*P* for interaction 0.01) in those who were GSTM1 non-null. Slattery *et al.* (35) found that among US men and women aged 55 years and younger, risk of colon cancer decreased with increasing levels of cruciferous vegetable, and broccoli intake, and this effect was most marked among those with the GSTM1 null genotype. Among this group, the odds ratio for four or more servings per week versus no intake was 0.23 (95% CI 0.10–0.54).

There has been some uncertainty as to whether the protective effects of cruciferous vegetables can be attributed to individual compounds like ITC or indoles, or if they are due to the action of other unknown chemicals (5). The present study suggests that ITCs are indeed the major constituents in cruciferous vegetables that account for their chemopreventive activity in the colon and elsewhere. We also show that the ITC-colon cancer effect is strongest among individuals deficient for GST, the major metabolic pathway for elimination of ITCs. Similar relationships between GST, ITC and lung cancer have been demonstrated in diverse populations (36–38). Taken together, these results provide strong evidence that the inverse ITC–cancer relationship is a causal one.

The chemopreventive activity of ITCs through their ability to inhibit phase I enzymes and induce phase II enzymes in various target tissues has been demonstrated *in vivo* in relation to chemically induced tumorigenesis (5,8,39,40). In addition, ITCs may act as anticarcinogens through more than one pathway. Recent studies have shown that ITCs and other phase II enzyme inducers can also act as ‘suppressing agents’ during the post-initiation stage of carcinogenesis by promoting apoptosis, and suppressing malignant transformation, possibly through their effect on the cellular glutathione pool (19,41,42). Such induction of apoptosis has been demonstrated in colon cancer cell lines, and colonic crypts of dimethylhydrazine (DMH) treated rats (41,43). On the other hand, some studies have reported that benzyl ITC may increase resistance to apoptosis and promote carcinogenesis when administered post-initiation (44).

Since sufficient intracellular concentrations are required for a compound to exert its chemopreventive effect, the colon is a site where ITC chemoprevention is particularly significant. Data exist to suggest that concentrations of ITC shown to be active as enzyme inducers *in vitro* can be achieved in the colonic

mucosa from an average serving of cruciferous vegetable such as broccoli (9,41), and this is consistent with human feeding studies, which observe altered metabolizing enzyme activity in humans given diets rich in cruciferae (5).

A fuller understanding of the metabolic processes operating in the colon requires involvement of specific colon carcinogens (45), and in this regard the possible risk-enhancing effect of meat, particularly meat cooked at high temperatures, has been of interest (46,47). In a controlled feeding study (48), ingestion of cruciferous vegetables increased conjugated urinary mutagenicity among volunteers consuming a fried meat diet, whereas ingestion of non-cruciferous vegetables did not have this effect. This increase was 2-fold higher among GSTM1 null subjects relative to GSTM1 non-null, consistent with data from the present study, and other epidemiologic studies on GST-ITC interaction.

We did not observe an independent effect of GSTM1, T1 null, or P1 AB/BB genotype, on risk of colorectal cancer. While a lower total GST activity in blood lymphocytes has been observed among individuals at higher risk of colon cancer (49), and the GSTM1 and T1 genotypes have been related to somatic genetic changes (18) epidemiologic studies are inconsistent (19). Two studies on the relationship between GSTP1 and colorectal cancer risk reported no significant association (50,51). Similarly, there have been many studies evaluating the association between GSTM1/T1 with colorectal cancer, some supporting and others refuting this (16,17,52–54). The data from the present study do not support an independent effect of GSTM1, T1 or P1 polymorphism in the colon.

There are some issues that should be considered in evaluating GST genotype as an independent risk factor for colorectal cancer, and these may also explain the lack of consistency between studies. At the target tissue level, it is probable that biotransformation ultimately depends on a delicate balance between phase I and II enzymes. In addition, among the various isoenzymes of the GST family, each may compensate to some degree for reduced activity of another, such that the effect of individual genotypes is indiscernible. In the colon, GSTP1 is the most abundant isoform (55), and accounts for 80% of the total activity (19). An absence of the high activity allele (A) leads to a reduction in, rather than absence of, activity, which may again be difficult to demonstrate in epidemiological studies.

The strengths of our study are that dietary information was collected prospectively using a validated questionnaire which included all major cruciferae consumed in this population, allowed computation of ITC intake, and adjustment for total energy and other relevant variables. Three major GST isoenzymes were evaluated, including GSTP1, the most common isoform in the colon. The chief limitation is the relatively short follow-up of the cohort (the mean follow-up time per subject was 5 years). However, we intend to verify this set of novel findings when a longer duration of follow-up (with a correspondingly larger number of cases) has been achieved.

In conclusion, our results provide support for an inverse relationship between high intake of ITCs from cruciferous vegetables and colorectal cancer; and this effect is most clearly seen in GSTM1 and T1 null individuals, among whom these compounds are metabolized and excreted at a slower rate. Our results also suggest that consideration of metabolic genotypes in the investigation of risk-enhancing factors, dietary or other-

wise, in the colon, may lead to a more refined understanding of the etiology of the disease.

## Acknowledgements

We thank Ms Siew-Hong Low of the National University of Singapore for supervising the field work of the Singapore Chinese Health Study, and Ms Kazuko Arakawa of the University of Southern California for the development and management of the cohort study database. The Singapore Chinese Health Study has been supported by grants R01 CA55069, R35 CA53890 and R01 CA80205 from the National Cancer Institute, Bethesda, Maryland.

## References

- Potter, J.D. (1996) Nutrition and colorectal cancer. *Cancer Causes Control*, **7**, 127–146.
- Voorrips, L.E., Goldbohm, R.A., van Poppel, G., Sturmans, F., Hermus, J.R.R. and van den Brandt, P.A. (2000) Vegetable and fruit consumption and risks of colon and rectal cancer in a prospective cohort study. The Netherlands Cohort Study on Diet and Cancer. *Am. J. Epidemiol.*, **152**, 1081–1092.
- Chung, F.L., Conaway, C.C., Rao, C.V. and Reddy, B.S. (2000) Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethyl isothiocyanate. *Carcinogenesis*, **21**, 2287–2291.
- Wattenberg, L.W. (1992) Inhibition of carcinogenesis by minor dietary constituents. *Cancer Res.*, **52**, 2085S–2091S.
- Steinkellner, H., Rabot, S., Freywald, C., Nobis, E., Scharf, G., Chabicovsky, M., Knasmuller, S. and Kassi, F. (2001) Effects of cruciferous vegetables and their constituents on drug metabolizing enzymes involved in the bioactivation of DNA-reactive dietary carcinogens. *Mutat. Res.*, **480/481**, 285–297.
- Cohen, J.H., Kristal, A.R. and Stanford, J.L. (2000) Fruit and vegetable intakes and prostate cancer risk. *J. Natl Cancer Inst.*, **92**, 61–68.
- Verhoeven, D.T.H., Goldbohm, R.A., van Poppel, G., Verhagen, H. and van den Brandt, P. (1996) Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidemiol. Biomark. Prev.*, **5**, 733–748.
- Zhang, Y. and Talalay, P. (1994) Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res.*, **54**, 1976S–1986S.
- Hecht, S.S. (1999) Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. *J. Nutr.*, **129**, 768S–774S.
- De Bruin, W.C.C., Wagenmans, M.J.M., Board, P.G. and Peters, W.H.M. (1999) Expression of glutathione S-transferase q class isoenzymes in human colorectal and gastric cancers. *Carcinogenesis*, **20**, 1453–1457.
- Brusewitz, G., Cameron, B.D., Chasseaud, L.F., Gorler, K., Hawkins, D.R., Koch, H. and Mennicke, W.H. (1977) The metabolism of benzyl isothiocyanate and its cysteine conjugate. *Biochem. J.*, **162**, 99–107.
- Jiao, D., Ho, C.T., Foiles, P. and Chung, F.L. (1994) Identification and quantification of the N-acetylcysteine conjugate of alkyl isothiocyanate in human urine after ingestion of mustard. *Cancer Epidemiol. Biomark. Prev.*, **3**, 487–492.
- Hayes, J.D. and Pulford, D.J. (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.*, **30**, 445–600.
- Harries, L.W., Stubbins, M.J., Forman, D., Howard, G.C.W. and Wolf, C.R. (1997) Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis (Lond.)*, **18**, 641–644.
- Mannervik, B., Awasthi, Y.C., Board, P.G. et al. (1992) Nomenclature for human glutathione transferases. *Biochem. J.*, **282**, 305–308.
- Deakin, M., Elder, J., Hendrickse, C. et al. (1996) Glutathione S-transferase GSTT1 genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. *Carcinogenesis*, **17**, 881–884.
- Katoh, T., Nagata, N., Kuroda, Y., Itoh, H., Kawahara, A., Kuroki, N., Ookuma, R. and Bell, D.A. (1996) Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. *Carcinogenesis*, **17**, 1855–1859.
- Rebeck, T.R. (1997) Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. *Cancer Epidemiol. Biomark. Prev.*, **6**, 733–743.
- Grubben, M.J.A.L., Nagengast, F.M., Katan, M.B. and Peters, W.H.M. (2001) The glutathione biotransformation system and colorectal cancer risk in humans. *Scand. J. Gastroenterol.*, **36** (suppl. 234), 68–76.
- Bogaards, J.J.P., Verhagen, H., Willems, M.I., van Poppel, G. and van Bladeren, P.J. (1994) Consumption of Brussels sprouts results in elevated a-class glutathione S-transferase levels in human blood plasma. *Carcinogenesis*, **15**, 1073–1075.
- Nijhoff, W.A., Grubben, M.J.A.L., Nagengast, F.M., Jansen, J.B.M.J., Verhagen, H., van Poppel, G. and Peters, W.H.M. (1995) Effects of consumption of Brussel sprouts on intestinal and lymphocytic glutathione S-transferases in humans. *Carcinogenesis*, **16**, 2125–2128.
- Zhang, Y. and Talalay, P. (1998) Mechanism of differential potencies of isothiocyanates as inducers of anticarcinogenic phase 2 enzymes. *Cancer Res.*, **58**, 4632–4639.
- Nakamura, Y., Ohigashi, H., Masuda, S., Murakami, A., Morimitsu, Y., Kawamoto, Y., Osawa, T., Imagawa, M. and Uchida, K. (2000) Redox regulation of glutathione S-transferase induction by benzyl isothiocyanate: correlation of enzyme induction with the formation of reactive oxygen intermediates. *Cancer Res.*, **60**, 219–225.
- Chia, K.S., Seow, A., Lee, H.P. and Shanmugaratnam, K.S. (2000) Cancer incidence in Singapore 1993–1997. Singapore Cancer Registry, Singapore.
- Flood, D.M., Weiss, N.S., Cook, L.S., Emerson, J.C., Schwartz, S.M. and Potter, J.D. (2000) Colorectal cancer incidence in Asian migrants to the United States and their descendants. *Cancer Causes Control*, **11**, 403–411.
- Seow, A., Shi, C.-Y., Chung, F.-L., Jiao, D., Hankin, J.H., Lee, H.-P., Coetzee, G.A. and Yu, M.C. (1998) Urinary total isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione S-transferase M1/T1/P1 genotypes. *Cancer Epidemiol. Biomark. Prev.*, **7**, 775–781.
- Hankin, J.H., Stram, D.O., Arakawa, K., Park, S., Low, S.H., Lee, H.P. and Yu, M.C. (2001) Singapore Chinese Health Study: development, validation and calibration of the quantitative food frequency questionnaire. *Nutr. Cancer*, **39**, 187–195.
- Lum, A. and Le Marchand, L. (1998) A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. *Cancer Epidemiol. Biomarkers. Prev.*, **7**, 719–724.
- London, S.J., Xia, J., Lehman, T.A., Yang, J.H., Granada, E., Chunhong, L., Dubeau, L., Li, T., David-Beabes, G.L. and Li, Y. (2001) Collection of buccal cell DNA in seventh-grade children using water and a toothbrush. *Cancer Epidemiol. Biomarkers. Prev.*, **10**, 1227–1230.
- Parkin, D.M., Whelan, S.L., Ferlay, J., Raymond, L. and Young, J. (1997) *Cancer Incidence in Five Continents, Volume VII*. IARC Scientific Publications No. 143. IARC, Lyon.
- Lee, L.G., Connell, C.R. and Bloch, W. (1993) Allelic discrimination by nick-translation PCR with fluorogenic probes. *Nucleic Acids Res.*, **21**, 3761–3766.
- Hosmer, D. and Lemeshow, S. (1989) *Applied Logistic Regression*. John Wiley & Sons, Inc., New York, NY.
- Lee, H.P., Gourley, L., Duffy, S.W., Esteve, J., Lee, J. and Day, N.E. (1989) Colorectal cancer and diet in an Asian population—a case-control study among Singapore Chinese. *Int. J. Cancer*, **43**, 1007–1016.
- Lin, H.J., Probst-Hensch, N.M., Louie, A.D., Kau, I.H., Witte, J.S., Ingles, S.A., Frankl, H.D., Lee, E.R. and Haile, R.W. (1998) Glutathione transferase null genotype, broccoli and higher prevalence of colorectal adenomas. *Cancer Epidemiol. Biomarkers. Prev.*, **7**, 647–652.
- Slatery, M.L., Kampman, E., Samowitz, W., Caan, B.J. and Potter, J.D. (2000) Interplay between dietary inducers of GST and the GSTM-1 genotype in colon cancer. *Int. J. Cancer*, **87**, 728–733.
- London, S.J., Yuan, J.-M., Chung, F.-L., Gao, Y.-T., Coetzee, G.A., Ross, R.K. and Yu, M.C. (2000) Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms and lung cancer risk: a prospective study of men in Shanghai, China. *Lancet*, **356**, 724–729.
- Spitz, M.R., Duphorne, C.M., Detry, M.A., Pillow, P.C., Amos, C.I., Lei, L., de Andrade, M., Gu, X., Hong, W.K. and Wu, X. (2000) Dietary intake of isothiocyanates: evidence of a joint effect with glutathione S-transferase polymorphisms in lung cancer risk. *Cancer Epidemiol. Biomarkers. Prev.*, **9**, 1017–1020.
- Zhao, B., Seow, A., Lee, E.J.D., Poh, W.-T., The, M., Eng, P., Wang, Y.-T., Tan, W.-C., Yu, M.C. and Lee, H.-P. (2001) Dietary isothiocyanates, glutathione S-transferase, -M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. *Cancer Epidemiol. Biomarkers. Prev.*, **10**, 1063–1067.
- Hecht, S.S. (1995) Chemoprevention by isothiocyanates. *J. Cell Biochem.*, **22**, 195–209.
- Chung, F.L., Morse, M.A. and Eklind, K.I. (1992) New potential chemopreventive agents for lung carcinogenesis of tobacco-specific nitrosamines. *Cancer Res.*, **52**, 2719S–2722S.
- Kirlin, W.G., Cai, J., DeLong, M.J., Patten, E.J. and Jones, D.P. (1999) Dietary compounds that induce cancer preventive phase 2 enzymes activate

- apoptosis at comparable doses in HT29 colon carcinoma cells. *J. Nutr.*, **129**, 1827–1835.
42. Bonnesen, C., Eggleston, I.M. and Hayes, J.D. (2001) Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. *Cancer Res.*, **61**, 6120–6130.
43. Smith, T.K., Lund, E.K. and Johnson, I.T. (1998) Inhibition of dimethylhydrazine-induced aberrant crypt foci and induction of apoptosis in rat colon following oral administration of the glucosinolate sinigrin. *Carcinogenesis*, **19**, 267–273.
44. Samaha, H.S., Kelloff, G.J., Steele, V., Rao, C.V. and Reddy, B.S. (1997) Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. *Cancer Res.*, **57**, 1301–1305.
45. Ketterer, B. (1998) Dietary isothiocyanates as confounding factors in the molecular epidemiology of colon cancer (Editorial). *Cancer Epidemiol. Biomarkers Prev.*, **7**, 645–646.
46. Chen, J., Stampfer, M.J., Hough, H.L., Garcia-Closas, M., Willett, W.C., Hennekens, C.H., Kelsey, K.T. and Hunter, D.J. (1998) A prospective study of *N*-acetyltransferase genotype, red meat intake and risk of colorectal cancer. *Cancer Res.*, **58**, 3307–3311.
47. Sinha, R., Kulldorff, M., Chow, W.H., Denobile, J. and Rothman, N. (2001) Dietary intake of heterocyclic amines, meat-derived mutagenic activity and risk of colorectal adenomas. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 559–562.
48. DeMarini, D.M., Hastings, S.B., Brooks, L.R., Eischen, B.T., Bell, D.A., Watson, M.A., Felton, J.S., Sandler, R. and Kohlmeier, L. (1997) Pilot study of free and conjugated urinary mutagenicity during consumption of pan-fried meats: possible modulation by cruciferous vegetables, glutathione *S*-transferase-M1 and *N*-acetyltransferase-2. *Mutat. Res.*, **381**, 83–96.
49. Szarka, C.E., Pfeiffer, G.R., Hum, S.T. *et al.* (1995) Glutathione *S*-transferase activity and glutathione *S*-transferase m-expression in subjects with risk for colorectal cancer. *Cancer Res.*, **55**, 2789–2793.
50. Harris, M.J., Coggan, M., Langton, L., Wilson, S.R. and Board, P.G. (1998) Polymorphism of the Pi class glutathione *S*-transferase in normal populations and cancer patients. *Pharmacogenetics*, **8**, 27–31.
51. Welfare, M., Monesola, A.A., Bassendine, M.F. and Daly, A.K. (1999) Polymorphisms in GSTP1, GSTM1 and GSTT1 and susceptibility to colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 289–292.
52. Gertig, D.M., Stampfer, M., Haiman, C., Hennekens, C.H., Kelsey, K. and Hunter, D.J. (1998) Glutathione *S*-transferase GSTM1 and GSTT1 polymorphisms and colorectal cancer risk: a prospective study. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 1001–1005.
53. Slattery, M.L., Potter, J.D., Samowitz, W., Bigler, J., Caan, B. and Leppert, M. (1998) NAT2, GSTM-1, cigarette smoking and risk of colon cancer. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 1079–1084.
54. Zhang, H., Ahmadi, A., Arbman, G., Zdolsek, J., Carstensen, J., Nordenskjold, B., Soderkvist, P. and Sun, X.-F. (1999) Glutathione *S*-transferase T1 and M1 genotypes in normal mucosa, transitional mucosa and colorectal adenocarcinoma. *Int. J. Cancer (Pred. Oncol.)*, **84**, 135–138.
55. Pool-Zobel, B.L., Abrahamse, S.L., Collins, A.R., Kark, W., Gugler, R., Oberreuther, D., Siegel, E.G., Lishaut, S.T. and Rechkemmer, G. (1999) Analysis of DNA strand breaks, oxidized bases and glutathione *S*-transferase P1 in human colon cells from biopsies. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 609–614.

Received June 27, 2002; revised and accepted September 11, 2002

## Appendix 1

### Cruciferous vegetables listed in the Singapore Chinese Health Study questionnaire

Common name	Local name, if applicable	Botanical name
Chinese white cabbage	Pak choi, siew pak choi	<i>B.chinensis</i>
Chinese mustard	Kai choi	<i>B.juncea</i> var. <i>rugosa</i>
Chinese flowering cabbage	Choi sum	<i>B.chinensis</i> var. <i>parachinensis</i>
Watercress		<i>N.officinale</i>
Chinese kale	Kai lan	<i>B.alboglabra</i>
Head cabbage		<i>B.oleracea</i> var. <i>capitata</i>
Celery cabbage	Wong nga pak	<i>B.pekinensis</i> var. <i>cylindrical</i>
Broccoli		<i>B.oleracea</i> var. <i>italica</i>
Cauliflower		<i>B.oleracea</i> var. <i>botrytis</i>

Head cabbage and celery cabbage were grouped as one item in the questionnaire.