Dietary Isothiocyanates, Glutathione *S*-transferase -M1, -T1 Polymorphisms and Lung Cancer Risk among Chinese Women in Singapore[1]

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Abstract

Chinese populations consume a diet relatively high in isothiocyanates (ITCs), a derivative of cruciferous vegetables known to have cancer-protective effects. This class of compounds is metabolized by the glutathione *S*-transferase family of enzymes, which are also involved in the detoxification of tobacco-related carcinogens such as polycyclic aromatic hydrocarbons and alkyl halides. We evaluated the association between dietary isothiocyanate intake, *GSTM1* and *GSTT1* polymorphisms, and lung cancer risk in 420 Chinese women: 233 histologically confirmed lung cancer patients and 187 hospital controls. Among these, 58.8% of cases and 90.3% of controls were lifetime nonsmokers. An allele-specific PCR method was used to detect the presence or absence of the *GSTM1* and *GSTT1* genes in DNA isolated from peripheral blood. Higher weekly intake of ITCs (above the control median value of 53.0 μmol) reduced the risk of lung cancer to a greater extent in smokers [adjusted odds ratio (OR), 0.31; 95% confidence interval (CI), 0.10–0.98] than nonsmokers (OR, 0.70; 95% CI, 0.45–1.11). The inverse association was stronger among subjects with homozygous deletion of *GSTM1* and/or *GSTT1*. Among nonsmokers with *GSTM1*-null genotype, higher intake of ITCs significantly reduced the risk of lung cancer (OR, 0.54; 95% CI, 0.30–0.95), an effect not seen among those with detectable *GSTM1* (OR, 1.07; 95% CI, 0.50–2.29). Our results, in a Chinese female population, are consistent with the hypothesis that ITC is inversely related to the risk of lung cancer, and we show that among nonsmokers this effect may be primarily confined to *GST*-null individuals. Conjugation and elimination of ITCs is enhanced in *GST*-non-null relative to -null individuals, such that the *GST* metabolic genotype modifies the protective effect of ITCs on lung cancer development.

Introduction

Epidemiological evidence for the relationship between vegetable consumption and cancer risk is compelling, and it suggests an inverse association most marked for epithelial cancers of the respiratory and alimentary tracts (1). The relationship between *Brassica* vegetables and lung cancer, in particular, has been among the most consistently observed (2, 3), and this genus is distinguished by its high content of glucosinolates. These compounds are hydrolyzed to form indoles and ITCs,[3] which have anticarcinogenic properties (4, 5).

ITCs are among the most effective chemopreventive agents known. Their chemopreventive effect has been attributed to their ability to inhibit phase I enzymes that are responsible for the bioactivation of carcinogens and to induce phase II detoxification enzymes (6). Experimental studies in animals have demonstrated the efficacy of ITCs in inhibiting lung carcinogensis by known carcinogens, such as polycyclic aromatic hydrocarbons and NNK (5).

Human GSTs are phase II enzymes that play a major role in the detoxification of many reactive electrophilic compounds by conjugation with glutathione and also by noncovalent binding of many xenobiotics (7). GSTs can be classified into at least four genetically distinct groups (8) including GSTM1 and GSTT1. Polymorphisms in the *GSTM1* and *GSTT1* genes are caused by a complete deletion of the gene, which results in the loss of function (10, 11). Deficiency in GSTM1 and GSTT1 isoenzyme activity may predispose to the effects of electrophilic carcinogens and has been reported to be possibly associated with an increased susceptibility to lung cancer in some, but not all, studies (12). Induction of the GST class of enzymes is one of the important mechanisms by which ITCs inhibit carcinogenesis (13).

Interestingly, the GST family also encompasses key enzymes in the metabolism of ITCs in humans and demonstrate considerable substrate specificity for these compounds (14, 15). Conjugation of ITCs with glutathione is the first step leading to

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[3] The abbreviations used are: ITC, isothiocyanate; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; GST, glutathione *S*-transferase; OR, odds ratio; CI, confidence interval; ETS, environmental tobacco smoke.

the formation of the corresponding N-acetylcysteine conjugates (dithiocarbamates) and aids in the elimination of ITCs (15). Hence the GSTs promote the elimination not only of carcinogens, but also of ITCs themselves (16), and could thus decrease ITC chemopreventive effects. Modification of the ITC-mediated protective effect in lung cancer by *GSTM1* and *GSTT1* polymorphisms is biologically plausible and has been reported in two recent epidemiological studies, Refs. 17 and 18, among Shanghai Chinese men, and United States whites, respectively.

Lung cancer is currently the third most commonly diagnosed cancer among Singapore Chinese women and constitutes 9.8% of all cancers in this population (19). This population is unique in having an incidence of lung cancer comparable with many countries in the West despite a smoking prevalence of only 3% (20). It is also characterized by a high intake of cruciferous vegetables; the mean intake frequency being 363 times a year (and the average amount 42.5 g/day) among Chinese women. We previously demonstrated, in the same population, that individuals with *GSTT1*-non-null genotype had significantly higher levels of urinary ITCs when stratified by dietary intake of ITCs or cruciferae, suggesting that GSTT1 is a key enzyme in the conjugation and subsequent excretion of these compounds (21). In the present study, we determined the intake of ITCs obtained by dietary questionnaire from 420 Chinese women (233 lung cancer cases and 187 controls), and we used PCR-based methods to determine their G*STM1* and *GSTT1* genotypes. We examined the relationship between total ITC intake and lung cancer risk in Chinese women and the effect of *GSTM1* and *GSTT1* polymorphisms on this risk.

Subjects and Methods

Between April 1996 and September 1998, we conducted a case-control study on lung cancer and environmental exposures among Chinese women, details of which have been described elsewhere (22). Briefly, cases were incident lung cancers diagnosed at three of the major hospitals in Singapore. Controls were patients admitted to the same hospital as the cases, frequency-matched for age (within the same 10-year age group), with no history of cancer or any chronic respiratory condition. They were drawn from internal medicine, orthopedic, surgical/trauma, and eye wards. Between January 1997 and September 1998, all participants were asked to provide 6 ml of blood by venepuncture. A total of 233 patients with pathologically confirmed primary lung cancer and 187 age-matched controls consented and were thus included in the present study. They were similar to the larger study population in terms of age, country of birth, dialect group (indicating provincial origin in China) and smoking status.

Demographic information and data on smoking were obtained by standardized questionnaire administered in-person by a research nurse, who interviewed both cases and controls equally. For cases, interviews took place within 3 months of diagnosis of cancer. Interviewers were not blind to case or control status, but possible observer bias was monitored by tape-recording and review of a random sample of interviews. Subjects were classified as smokers if they had ever smoked at least one cigarette a day for 1 year or more. Ex-smokers were smokers who had stopped smoking for 1 year or more. Pathology specimens of all cases were reviewed and classified independently by two study pathologists; only pathologically confirmed cases with a diagnosis of squamous cell carcinoma, small cell carcinoma, adenocarcinoma, or large cell carcinoma were included.

Dietary Data. Forty-five food items including fruits and vegetables were specified in the questionnaire. Of the 20 vegetables listed in the questionnaire, 9 are members of the *Brassicaceae* family. They are bok choi (*Brassica chinensis*, also known as Chinese white cabbage), kai choi (*B. juncea* var. *rugosa*, also known as mustard cabbage or Chinese mustard), choi sum (*B. oleracea* var. *parachinensis*, also known as Chinese flowering cabbage), watercress (*Nasturtium officinale*), kai lan (*Brassica oleracea* var. *alboglabra*, also known as Chinese kale), head cabbage (*B. oleracea* var. *capitata*), wong nga pak (*B. pekinensis* var. *cylindrica*, also known as celery cabbage), broccoli (*B. oleracea* var. *italica*), and cauliflower (*B. oleracea* var. *botrytis*). Brussels sprouts and turnips are infrequently consumed in this population and were not included in the questionnaire. For each of these food items, the respondent was asked to indicate her average weekly serving frequency and usual serving size in the 3 years before hospital admission. Serving size was expressed as a multiple of a standard serving (standard serving = two rounded Chinese spoons of cooked vegetable). Total ITC contents in these nine cruciferous vegetables have been determined by high-performance liquid chromatography using samples obtained in Singapore (23). Estimated weekly intake of total ITCs was computed for each of the 420 study subjects via linkage of ITC contents in cruciferous vegetables with responses to the dietary questionnaire.

Identification of *GSTM1* and *GSTT1* Genotypes. At the time of interview, informed consent was obtained for the donation of 6 ml of blood for genotyping purposes. Isolation of genomic DNA from peripheral lymphocytes was carried out using a standard proteinase K-phenol-chloroform extraction procedure (24). A PCR method was used to detect the presence or absence of the *GSTM1* and *GSTT1* genes in genomic DNA samples. The absence of the *GSTM1*- or *GSTT1*-specific fragment indicated the corresponding null genotype.

The *GSTM1*-null genotype was determined by procedures described by Groppi *et al.* (25) with a slight modification. Briefly, two primers that hybridize within the fourth intron (1019: 5′-GAA GGT GGC CTC CTC CTT GG) and in the 3′ region of the fifth exon (526: 5′-AAT TCT GGA TTG TAG CAG AT) were used in the presence of another pair of primers (5′-ACA CAA CTG TGT TCA CTA GC-3′ and 5′-CTC AAA GAA CCT CTG GGT CC-3′) to amplify β-globin, included in the assay as a positive control for target DNA. A PCR reaction (amplification size: 165 bp for *GSTM1* presence; 299 bp for β-globin) was performed to detect the *GSTM1* deletion mutation at exon 5.

GSTT1-null genotype was determined using a similar modification of a PCR approach described previously (11), with the addition of primers for a β-globin control fragment (299 bp). The primers used to amplify the target DNA were: 5′-TTC CTT ACT GGT CCT CAC ATC TC (468–491) and 5′-TCA CCG GAT CAT GGC CAG CA (703–723). The presence of at least one *GSTT1* allele was identified by a 480-bp PCR product.

The presence or absence of the *GSTM1* and *GSTT1* genes was analyzed by ethidium bromide 1.6% agarose gel electrophoresis. All stages of the analysis were carried out blind to the patient's disease status.

Statistical Analysis. ORs and their corresponding 95% CIs for the association between lung cancer and estimated ITC intake were computed for all subjects, ever-smokers and lifetime nonsmokers. Logistic regression analysis was used to obtain age- and smoking-adjusted ORs stratified by smoking status. Intensity and duration of smoking was accounted for in the analyses

Table 1 Distribution of selected variables among Chinese female lung cancer patients and controls [*n* (%)]

Characteristics	Cases (n = 233)	Controls (n = 187)
Age in years (mean ± SD)	65.5 ± 12.8	63.6 ± 12.0
Dialect group		
Hokkien	91 (39.1)	83 (44.4)
Teochew	58 (24.9)	37 (19.8)
Cantonese	54 (23.2)	34 (18.2)
Hainanese	18 (7.7)	7 (3.7)
Hakka	6 (2.6)	23 (12.3)
Other	6 (2.6)	3 (1.6)
Country of birth		
Singapore	131 (56.2)	124 (66.3)
Malaysia	20 (8.6)	25 (13.4)
China	77 (33.0)	33 (17.6)
Other	5 (2.1)	5 (2.7)
Smoking status		
Nonsmokers	137 (58.8)	169 (90.4)
Current and ex-smokers	96 (41.2)	18 (9.6)
For ever-smokers		
Years of smoking (mean ± SD)	43.3 ± 17.7	39.3 ± 17.5
Number of cigarettes smoked per day (mean ± SD)	13.7 ± 14.6	10.8 ± 13.3
GSTM1-null	146 (62.7)	119 (63.6)
GSTT1-null	132 (56.7)	102 (54.5)
GSTM1- and -T1-null	82 (35.2)	66 (35.3)

by including the number of years of smoking and the number of cigarettes smoked per day as continuous variables in the regression model. All calculations were performed using the SPSSWIN v10.0 statistical package (SPSS, Chicago, IL).

Results

The distribution of characteristics of the study population, which comprises 233 lung cancer patients and 187 controls is given in Table 1. Of the 420 individuals, 306 (72.9%) were lifetime nonsmokers, and 114 (26.9%) were either current or ex-smokers. The proportion of current and ex-smokers among the cases was 41.2% (96 women), and that among the controls was 9.7% (18 women). In general, cases tended to be marginally older and there was a slight overrepresentation of Cantonese women among cases (23.2%) compared with controls (18.2%). Among the study population, cases were more likely to be foreign-born, particularly migrants from China.

The proportion of cases and controls with the *GSTM1*, *GSTT1*, and combined null genotypes was similar (Table 1). Frequencies among controls are similar to previous estimates reported for the Singapore population (26). Neither *GSTM1*, *-T1*, nor the combined null genotype was associated with lung cancer risk in this study population. The adjusted ORs (95% CIs) for the *GSTM1*-null *versus* the non-null genotypes were 1.50 (0.51–4.40) and 0.90 (0.56–1.43) for ever-smokers and lifetime nonsmokers, respectively. For *GSTT1*-null genotypes, the corresponding ORs were 1.95 (0.68–5.58) and 0.97 (0.62–1.53), and for the combined null genotype, they were 1.86 (0.56–6.23) and 0.95 (0.60–1.53). We did not find any significant association between *GST* genotype and lung cancer when the population was stratified by histological type.

Among the 420 study subjects, the distribution of estimated weekly intake level of ITCs was unimodal and markedly skewed to the right, with a range of 0.0–449.0 μmol and a median of 53.0 μmol. There was a 9.8-fold difference between the 90th and the 10th percentiles in the distribution. Table 2 shows the effect of weekly intake of ITCs on lung cancer risk. For subjects who reported an intake above the median value for controls, the risk of lung cancer was reduced. For all women, the age- and smoking-adjusted OR was 0.63 (95% CI, 0.41–0.95). The protection afforded by higher ITC intake was more marked among ever-smokers (OR, 0.31; 95% CI, 0.10–0.96) than among lifetime nonsmokers (OR, 0.70; 95% CI 0.45–1.11). Additional adjustment for place of birth did not materially affect the estimates.

Table 2 Distribution of weekly intake level of ITCs among Chinese female lung cancer patients and controls [*n* (%)]

	Cases (n = 233)	Controls (n = 187)	OR (95% CI)[a]	OR (95% CI)[b]
All subjects				
≤53.0 μmol	132 (56.7)	78 (41.7)		
>53.0 μmol	101 (43.3)	109 (58.3)	0.56 (0.38–0.83)	0.63 (0.41–0.95)
Nonsmokers				
≤53.0 μmol	70 (51.1)	72 (42.6)		
>53.0 μmol	67 (48.9)	97 (57.4)	0.70 (0.45–1.11)	
Current and ex-smokers				
≤53.0 μmol	62 (64.4)	6 (33.3)		
>53.0 μmol	34 (35.4)	12 (66.7)	0.31 (0.10–0.92)	0.31 (0.10–0.96)

[a] Adjusted for age (in completed years).
[b] Further adjusted for smoking. Smoking-related regression covariates were smoking at recruitment (yes/no), years of smoking, and number of cigarettes smoked per day.

Table 3 shows the effect of *GSTM1* and *-T1* genotypes on ITC-associated risk. Because of the small number of controls who were ever-smokers (n = 18), we present data stratified by *GSTM1* and *-T1* genotype for all subjects and for lifetime nonsmokers only. Among all subjects, high ITC intake conferred a 40–50% reduction in risk that was statistically significant among those with the null genotype for *GSTT1*, *-M1*, or both combined. The effect of high ITC intake was less clear among those with the non-null genotypes. Among nonsmokers, the same was true for the *-M1* genotype; persons with high ITC intake and null for this genotype had a significant reduction in risk (age-adjusted OR, 0.54; 95% CI, 0.30–0.95), whereas persons with the non-null genotype did not (OR, 1.07; 95% CI, 0.50–2.29; P for interaction, 0.13). The pattern was consistent with the *-T1* and combined genotypes in this subgroup as well. Overall, the magnitude of the inverse association was largest (adjusted OR, 0.47 in all subjects) among those who were both *GSTM1*- and *GSTT1*-null. In all cases, the multiplicative interaction terms for the difference in OR between null and non-null genotypes were not statistically significant.

Discussion

In summary, we describe an inverse association of dietary ITCs on lung cancer risk among Singapore Chinese women, which is modified by *GSTM1* and *-T1* genotypes. Those with the null genotype for either or both enzymes experienced a significant reduction in risk with higher intake of ITCs, but the effect was smaller and not statistically significant if either or both genes were present. We also report, for the first time, a modifying effect of the *GSTM1* genotype on the effect of ITCs in lifetime nonsmokers.

Overall, our data demonstrate a significant association between dietary ITC intake and lung cancer risk. The stronger effect in smokers is not surprising, and it is consistent with the evidence that ITCs are known to reduce lung carcinogenesis by

Table 3 Weekly intake level of ITC in relation to risk of lung cancer according to *GSTM1* and *GSTT1* genotypes [*n* (%)]

	All subjects ($n = 420$)		Lifetime nonsmokers ($n = 306$)	
	Cases/Controls	OR (95% CI)[a]	Cases/Controls	OR (95% CI)[b]
GSTM1-null				
≤53.0 μmol	90 (61.6)/51 (42.9)	1.00	49 (57.0)/46 (42.6)	1.00
>53.0 μmol	56 (38.4)/68 (57.1)	0.55 (0.33–0.93)	36 (42.4)/63 (57.8)	0.54 (0.30–0.95)
GSTM1 detected				
≤53.0 μmol	42 (48.3)/27 (39.7)	1.00	21 (40.4)/26 (43.3)	1.00
>53.0 μmol	45 (51.7)/41 (60.3)	0.78 (0.39–1.59)	31 (59.6)/34 (56.7)	1.07 (0.50–2.29)
GSTT1-null				
≤53.0 μmol	79 (59.8)/42 (41.2)	1.00	41 (54.7)/40 (42.6)	1.00
>53.0 μmol	53 (40.2)/60 (58.8)	0.54 (0.31–0.95)	34 (45.3)/54 (57.4)	0.62 (0.33–1.13)
GSTT1 detected				
≤53.0 μmol	53 (52.0)/36 (42.4)	1.00	29 (46.8)/32 (43.2)	1.00
>53.0 μmol	48 (47.5)/49 (57.6)	0.75 (0.40–1.40)	33 (53.2)/43 (57.3)	0.82 (0.41–1.62)
GSTM1-null and *GSTT1*-null				
≤53.0 μmol	50 (61.0)/26 (39.4)	1.00	27 (56.3)/24 (39.3)	1.00
>53.0 μmol	32 (39.0)/40 (60.6)	0.47 (0.23–0.95)	21 (43.8)/37 (60.7)	0.50 (0.23–1.08)
GSTM1 or *GSTT1* detected				
≤53.0 μmol	82 (54.3)/52 (43.0)	1.00	43 (48.3)/48 (44.9)	1.00
>53.0 μmol	69 (45.7)/69 (57.0)	0.69 (0.41–1.17)	46 (51.7)/60 (55.6)	0.83 (0.47–1.46)

[a] Adjusted for age (in years), years of smoking, number of cigarettes smoked per day, and smoking at recruitment (yes/no).
[b] Adjusted for age (in years).

tobacco-related carcinogens. Polycyclic aromatic hydrocarbons such as benzo(*a*)pyrene and NNK, a tobacco-specific nitrosamine, require metabolic activation. Agents such as ITC, which decrease formation of the electrophilic DNA binding intermediates, reduce DNA damage and thereby inhibit carcinogenesis. Mechanistic studies have shown that this chemopreventive activity is attributable to the inhibition of phase I enzymes and the induction of phase II enzymes (6). Specifically, phenethyl ITC has been shown to inhibit NNK-induced lung tumorigenesis in animal studies (27, 28), and the consumption of watercress by smoking volunteers led to increased urinary excretion of NNK metabolites (5).

The most thoroughly studied examples of ITC inhibition of carcinogenesis are in relation to tobacco-related carcinogens (29), and the evidence linking ITC to lung cancer risk among nonsmokers is less consistent than for smokers. However, the experimental evidence does point to the capability of ITCs to inhibit carcinogenesis in a wide range of target organs and against a variety of chemical carcinogens (29). Among the epidemiological studies of *Brassica* vegetable intake and lung cancer that have examined risk among nonsmokers or within smoking strata, there has been no clear evidence of an inverse relationship among nonsmokers (2, 30, 31). Compared with most of these studies, the current study population has a relatively high intake of cruciferae (21). We show that in the subgroup of nonsmokers who are null for *GSTM1*, high intake of ITC reduces risk by nearly 50%. The effect is unlikely to be caused by ETS exposure. Among the nonsmokers in our study population, 127 (41.5%) reported ever being exposed to ETS at home on a daily basis. When we examined *GSTM1*-null individuals stratified by this variable, the association with ITC was not confined to, or stronger among, those who had been exposed to ETS daily (age-adjusted OR, 0.65; 95% CI, 0.27–1.59) than among those had never been exposed or were infrequently exposed to ETS (OR, 0.47; 95% CI, 0.21–1.01). Adjusting for the intake of total fruit and vegetable intake (number of standard servings weekly) also did not materially affect the estimates (OR, 0.52; 95% CI, 0.29–0.95), indicating that the inverse association between ITC and lung cancer risk among *GSTM1*-null nonsmokers is not likely to be merely a surrogate for the effects of other nutrients in fruit and vegetables.

Apart from its effects on carcinogen metabolism, ITCs have been shown to induce apoptosis and influence protein kinase activities (32, 33), suggesting that they may play a role in various stages of the carcinogenic process. The mechanisms by which ITCs exert their effect in nonsmokers deserve additional study and may provide useful clues to the etiology of lung cancer in these women.

A key finding in this report is the interaction between *GST* genotype and the reduction in risk of lung cancer by ITC intake. Two other recent studies (17, 18) have described a similar observation. London *et al.* (17) showed that, among Chinese men in Shanghai, individuals with detectable urinary ITCs had a significantly reduced risk of lung cancer, and that this effect was primarily confined to individuals with *GSTM1*- or -*T1*- (or both) null genotypes. Similarly, in a United States population, Spitz *et al.* (18) found that a combination of low ITC intake and *GSTM1*- and -*T1*-null genotypes conferred the highest risk of lung cancer among smokers. Our study extends these findings to a population of Chinese women, of whom a large proportion are nonsmokers, and the results are consistent with the theoretical framework that elimination of ITCs by GST results in an attenuation of their protective effect.

One of the strengths of the current study is the inclusion of only pathologically confirmed, incident lung cancer cases, whereas its limitations include those inherent in a retrospective study. The use of hospital controls may have introduced bias if controls suffered from conditions systematically related to higher or lower cruciferous vegetable intake. We drew controls from a wide variety of disciplines to minimize the likelihood of such bias, and we also note that the distribution of ITC intake among controls is not dependent on *GST* genotype and does not explain the effect modification observed. We have attempted to measure ITC intake based on a variety of cruciferous vegetables commonly eaten locally, and we have based our estimates of ITC intake on actual quantification of ITC content in each of these nine vegetables.

In conclusion, our results provide additional evidence that

ITCs from cruciferous vegetable consumption protect against lung cancer, and we extend these findings to a Chinese population with a high proportion of lifetime nonsmokers. In addition, ITC intake and *GSTM1* and *GSTT1* polymorphisms interact in the etiology of lung cancer such that persons with the null genotype experience a greater reduction in risk because these compounds are less rapidly metabolized and eliminated from the body.

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