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• *COLORECTAL CANCER* •

Vegetable/fruit, smoking, glutathione S-transferase polymorphisms and risk for colorectal cancer in Taiwan

Chih-Ching Yeh, Ling-Ling Hsieh, Reiping Tang, Chung Rong Chang-Chieh, Fung-Chang Sung

Chih-Ching Yeh, Institute of Environmental Health, National Taiwan University, Taipei 100, Taiwan, China
Ling-Ling Hsieh, Chung Rong Chang-Chieh, Department of Public Health, Chang Gung University, Tao-Yuan 333, Taiwan, China
Reiping Tang, Colorectal Section, Chang Gung Memorial Hospital, Taipei 244, Taiwan, China
Fung-Chang Sung, Institute of Environmental Health, China Medical University, Taichung 404, Taiwan, China
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Correspondence to: Dr. Fung-Chang Sung, Institute of Environment Health, China Medical University, 91 Hsueh-Shih Road, Taichung 404, Taiwan, China. fcsung@mail.cmu.edu.tw
Telephone: +886-4-2205-4070 Fax: +886-4-2201-9901
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Abstract

AIM: To investigate the colorectal cancer risk associated with polymorphic GSTM1, GSTT1 and GSTP1 and the effect of diet and smoking.

METHODS: With consents, genotypes of the genes were determined using PCR methods for 727 cases and 736 sex and age-matched healthy controls recruited at a medical center in the Northern Taiwan. Nurses who were blind to the study hypothesis conducted interviews with study participants for the information of socio-demographic variables, diet and smoking.

RESULTS: There was no significant association between GSTM1 genotypes and the disease. Men, not women, with GSTT1 null genotype were at significant risk of colorectal cancer, but limited to rectal tumor, and in men aged 60 years and less. The corresponding association with the GSTP1 with G allele compared to GSTP1 A/A genotype was at borderline significance. Compared to men with GSTT1 present and GSTP1 A/A combined, men with both GSTT1 null and GSTP1 with G allele genotypes were at significant risk (odds ratio (OR) = 1.91, 95% confidence interval (CI) = 1.21-3.02), also limited to the rectal tumor and younger men. The beneficial effects of vegetable/fruit intake on colorectal cancer were much higher for men with GSTT1 present (OR = 0.32, 95%CI = 0.20-0.50) or GSTP1 A/A genotypes (OR = 0.40, 95%CI = 0.25-0.64). These effects remained significant for women. But, the greatest protective effect from vegetable/fruit intake for women was observed in those with GSTT1 null or GSTP1 with G allele genotypes. In addition, non-smoking men benefitted significantly from combined effect of higher vegetable/fruit intake and GSTT1 present or GSTP1 A/A genotypes with OR = 0.17 and 0.21 respectively.

CONCLUSION: This study suggests that the GSTT1 gene can modulate the colorectal cancer risk and vegetable/fruit-related colorectal cancer risk, particularly in men of no smoking history.

Key words: Colorectal cancer; Glutathione S-transferase; Polymorphisms; Vegetables; Smoking

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INTRODUCTION

Human glutathione S-transferase (GST) consists of four main classes, alpha (A), mu (M), pi (P) and theta (T)[1], involving in the detoxification of many electrophilic compounds by conjugation reaction with glutathione[2]. Most GST substrates are thought as xenobiotics or products of oxidative stress, including polycyclic aromatic hydrocarbons present in the diet or from tobacco smoke[2]. The GST enzymes also conjugate isothiocyanates, potent enzyme inducers, detoxifying mutagens[3] to glutathione and divert them from the enzyme induction pathway to excretion[4,5]. Functional polymorphisms known in most studies are GSTM1, GSTT1 and GSTP1[6-8]. The absence of GSTM1 and GSTT1 enzyme activity correlates with homozygosity for deletions in these genes, termed the null genotype[6,7]. For GSTP1, a polymorphic Ile105Val (resulting from an A to G substitution at base 1 578) has been found to modify the enzyme's activity and affinity for electrophilic substrates[8]. Studies have shown that higher levels of DNA damage are associated with the GSTM1/T1 null genotype and GSTP1 with G allele[9,10].

Several studies have examined the relationship between the GSTM1 null genotype and susceptibility to colorectal cancer[11-15]. The first study by Zhong *et al*[16] found significantly increased colon cancer risk associated with the GSTM1 null genotype, particular for cancers of the proximal colon. A significant risk for distal colorectal cancer was observed later in a Japanese study[17]. Sgambato *et al*[15] also reported strongest association between GSTM1 null genotype and colon cancer and with a younger age (<60 years) of

onset. Other studies failed to find similar associations[11-14]. Several studies also examined whether the GSTT1 null genotype exhibition conferred susceptibility to colorectal cancer[11-15]. Only the largest study[18], with 252 cases and 577 controls, demonstrated an increased risk for the GSTT1 null genotype due to excess GSTT1 deletion for cases with left colon and rectal cancer. Five studies examined the GSTP1 A/G polymorphism for colorectal cancer. None reported a significant association[11,12,19-21].

Differences in carcinogen metabolism might explain the differences in cancer susceptibility especially as most cancers are influenced by environmental factors[22]. Etiological studies have attributed more than 85% of colorectal cancer to environmental factors[23,24], particularly dietary factors[25]. These dietary effects might be modulated by genetic polymorphisms of the metabolic genes[26]. For instance, Lin *et al*[27] found that the protective effect of broccoli for colorectal adenoma was observed only among subjects with the GSTM1 null genotype. Recent GST polymorphism studies have focused on gene-environment interactions in the occurrence of colorectal cancer[14,28-30]. Slattery *et al*[30] reported that the beneficial effect of cruciferous vegetable intake was stronger for subjects aged less than 55 years old, with GSTM1 null genotype than with GSTM1 present genotype.

Three studies linking these GST polymorphisms with colorectal cancer for Chinese subjects[31-33] were limited to small sample sizes or no control for potential confounders. In addition, the incidence of colorectal cancer increased 2.6 folds in the past decade in Taiwan and dietary behavior has changed by increasing meat consumption. The present hospital-based case-control study examined the role of polymorphisms of GSTM1, GSTT1 and GSTP1 genes in colorectal cancer and their combined effects with environmental factors such as meat consumption, cigarette smoking and vegetable/fruit intake.

MATERIALS AND METHODS

Subjects

Detailed descriptions for the study participants have been published previously[34]. In brief, the colorectal adenocarcinoma cancer cases (n = 776) were newly diagnosed and histologically confirmed at the Chang Gung Memorial Hospital between January 1995 and January 1999. Patients with familial adenomatous polyposis (n = 11), hereditary nonpolyposis colorectal cancer (n = 18), or inflammatory bowel disease and other malignancies (n = 20) were excluded from the data analysis. Seven hundred and twenty-seven cases (94%) were included (56.4% men) in this study. During the same period, seven hundred and forty-seven subjects were recruited as controls from the Physical Check-Up Department for comprehensive health examinations including colonoscopies matched by same age and sex. After excluding individuals diagnosed with other colorectal diseases (n = 5) and with a history of other cancers (n = 6), 736 controls (98%) were included (55.6% men) in this study.

Questionnaire

Professional nurses employed were trained to conduct interviews with all participants in the hospital prior to surgical operations for cases and colonoscopies for the controls. These nurses were blind to the study hypothesis regarding diet, lifestyle and colorectal cancer. All interviews were administered uniformly in the wards. The standardized interview was conducted with informed consent using a structured questionnaire covering socio-demographic characteristics, lifestyle factors (including physical activities, cigarette and alcohol use and coffee intake), dietary consumption and medical history. The reliability (standardized Cronbach's alpha) of this questionnaire was 0.92 based on the lifestyle and dietary variables. This interview took approximately 20 min to complete. Information on usual dietary intake 5 years preceding the date of diagnosis for the cases and the date of selection for the controls was collected. The intake frequency was categorized into six levels ranging from never, less than once a month, 1-3 times a month, once a week, 2-3 times a week to almost everyday.

Cigarette and alcohol use and coffee intake were evaluated in both the amount and duration. Total cigarette smoking and alcohol drinking were estimated in pack-years and bottle-years using daily pack and bottle (600 mL) consumption multiplied by the number of years respectively. Because less than 10% of the subjects in our study had a coffee intake habit (more than one cup of coffee per month), coffee intake was estimated by status ("Yes" or "No") rather than total consumption.

Genotyping

WBC DNA was isolated from 10 mL whole blood using standard procedures with sodium dodecyl sulfate (SDS)-proteinase K-RNase digestion and phenol-chloroform extraction. The extracted DNA was dissolved in Tris-EDTA buffer to a concentration of 500 ng/μL. The polymorphisms were analyzed by polymerase chain reaction (PCR) assays for GSTM1 and GSTT1 or combined with restriction fragment length polymorphism (RFLP) assays for GSTP1 Ile105Val. All of the PCR reactions were performed in a 25 μL final volume containing 200 ng of each primer, 100 ng genomic DNA, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs and 1.0 unit of Taq DNA Polymerase in the buffer provided by the manufacturer. Amplification was performed in a Programmable Thermal Controller (MJ Research, Waltham, MA) for the PCR reaction.

The multiple PCR used to detect the presence or absence of the GSTM1 and GSTT1 genes was determined using the following primers: GSTT1 (5'-TTCCTTACTGGTCC-TCACATCTC-3' and 5'-CACCGGATCATGGCCAGCA-3'), GSTM1 (5'-TGCCCTACTTGATTGATGGG-3' and 5'-CTGGATTGTAGCAGATCATGC-3') and the internal control, β-globin (5'-CACAACTGTGTTCACTAGC-3' and 5'-CAACTTCATCCACGTTCACC-3'). The PCR program was a 5-min denaturing step at 94 ℃ followed by 30 cycles of 30 s at 94 ℃, 30 s at 59 ℃ and 60 s at 72 ℃. Individuals without intact GSTT1 or GSTM1 genes showed no amplification of the 480 bp GSTT1 fragment or the 273 bp GSTM1 fragment and a positive internal control[35]. The GSTP1 A/G (Ile105Val) polymorphism was determined using the following primers: sense, 5'-CCTCTCCCTTTCCT-CTGTTC-3'; antisense, 5'-CAGGTGAGGGGGACATCT-

3'. PCR program was a 5-min denaturing step at 95 ℃ followed by 30 cycles of 30 s at 94 ℃, 30 s at 55 ℃ and 30 s at 72 ℃. The 176 bp PCR product was digested with Alw26I (New England BioLabs, Beverly, MA): the Val allele was cut into 91 and 85 bp fragments (Ile allele not digested)[36].

Statistical analysis

Food items were coded into three major groups: staples (rice, noodles and instant noodles), meat (red meat and white meat), and vegetables and fruits. Viscera fish and shrimp were considered separate food items in the data analysis. The consumption of each food item was scored from 1 for "almost ate every day" to 6 for "never ate". The group score was the total sum of scores from the intake frequency for the individual food items. Because of etiological differences between colon cancer and rectal cancer and between men and women[37], all analyses in this study were estimated separately by sex and cancer site. Most risk factors were dichotomized into "Low" and "High" according to the median of the controls in the data analysis. Only 4% of the women were smokers and only 5.1% of women were alcohol users. The risks for these two factors were therefore calculated only for men.

Some unmatched cases occurred when we were unable to interview an eligible control or controls who were eliminated by the exclusion criteria. Unmatched controls also occurred when cases with hereditary syndromes or other malignancies were excluded. To make the use of information on unmatched subjects, and because we had to break the matches due to stratification by sex and tumor site, unconditional logistic regression was used to control for the matching factor (age as continuous variable) to identify potential risk factors. The odds ratio (OR) and the 95% confidence interval (CI) were measured. A stepwise selection procedure was applied to determine the covariates included in the final multivariate models beginning with all dietary and non-dietary variables. Multivariate unconditional logistic regressions were also used to examine the association between the GSTM1, GSTT1 and GSTP1 polymorphisms and risk for colorectal cancer controlling for age and selected covariates. We conducted interaction analyses on the basis of a multiplicative scale. The likelihood ratio test was used to evaluate the interaction between GST genes and cigarette smoking, meat consumption and vegetable/fruit intake on the risk for colorectal cancer. Analysis was also conducted separately to observe the differences between age groups at diagnosis. All analyses were performed using the SAS statistical package (version 8.2 for Windows; SAS Institute, Inc., Cary, NC) and all statistical tests were two-sided.

RESULTS

Overall, 410 men and 317 women with eligible colorectal cancer cases and 409 male and 327 female controls were included in this analysis. These four groups of subjects had a similar average age of 60 years (Table 1). The stepwise selection procedure produced six potential covariates, including age, physical activity, coffee use, cigarette smoking, drinking, and meat consumption associated with the disease for men. The three potential risk covariates for women included age, consumption of staples and meat. Vegetable/fruit and fish/shrimp intakes had protective effect for both men and women. The frequencies of the polymorphisms for GSTM1, GSTT1 and GSTP1 genes were also presented in Table 1. Only the GSTT1 null genotype was moderately

Table 1 Selected colorectal cancer case and control distribution by sex in Taiwan

Variable	Men			Women		
	Cases *n* = 410 (%)	Controls *n* = 409 (%)	P^1	Cases *n* = 317 (%)	Controls *n* = 327 (%)	P^1
Age (mean±SD, yr)	60.5±12.2	60.6±12.7		60.2±13.6	60.7±13.4	
Colon/rectum, *n*	185/225			167/150		
Physical activity	53 (14.1)	95 (23.2)	<0.01	NI[2]		
Coffee	63 (16.5)	36 (8.8)	<0.01	NI[2]		
Smoking			0.18	NA[3]		
Never	122 (31.3)	150 (36.7)				
Ex-smoker	91 (23.3)	98 (24.0)				
Current	177 (45.4)	161 (39.4)				
Alcohol drinking			0.01	NA[3]		
Never	186 (48.1)	205 (50.1)				
Ex-drinker	17 (4.4)	37 (9.1)				
Current	184 (47.6)	167 (40.8)				
High staple[4]	NI[2]			156 (49.2)	112 (34.3)	<0.01
High meat[4]	294 (71.7)	244 (59.7)	<0.01	203 (64.0)	151 (46.2)	<0.01
High vegetable/fruit[4]	168 (44.7)	266 (65.0)	<0.01	147 (50.2)	197 (60.2)	0.01
Fish/shrimp (almost everyday)	174 (46.3)	256 (62.6)	<0.01	125 (42.8)	167 (51.1)	0.04
GSTM1 null	213 (52.2)	215 (52.7)	0.89	189 (60.0)	195 (60.0)	1.00
GSTT1 null	216 (52.9)	189 (46.3)	0.06	180 (57.1)	171 (52.6)	0.25
GSTP1			0.78			0.39
A/A	277 (67.9)	287 (70.2)		224 (71.3)	226 (69.5)	
A/G	117 (28.7)	109 (26.7)		84 (26.8)	87 (26.8)	
G/G	14 (3.4)	13 (3.2)		6 (1.9)	12 (3.7)	

[1]χ^2; [2]Not included as covariates in the multiple logistic regression model; [3]Not available for analysis; [4]The cut-point of score using the median of the score distribution among controls for high consumption of staple, meat and vegetable/fruit were <10, <5 and <3 respectively.

higher in cases than in controls (0.55 *vs* 0.49, $P = 0.06$). Because of the low frequency of the GSTP1 G/G genotype, we combined heterozygous (GSTP1 A/G) and homozygous (GSTP1 G/G) genotypes, as GSTP1 with G allele, to estimate the cancer risk associated with the G allele.

The ORs of the polymorphisms of GSTM1, GSTT1 and GSTP1 for the colorectal cancer risks in men were shown in Table 2. After controlling for covariates, the risk for colorectal cancer was statistically significantly increased by GSTT1 null genotype (OR = 1.45, 95%CI = 1.07-1.97), compared to GSTT1 present genotype. A moderately increased risk for colorectal cancer was also observed in men with GSTP1 with G allele than those with A/A genotype (OR = 1.36, 95%CI = 0.98-1.89). Further stratified analysis by tumor site and age at diagnosis showed that, male individuals with the GSTT1 null genotype were at significant risk for rectal cancer with OR = 1.55 (95%CI = 1.08-2.23) and those diagnosed before 60 years old with OR = 2.03 (95%CI = 1.29-3.21) respectively. An increased risk for GSTP1 with G allele was also observed in these subgroups. However, the polymorphism of GSTM1 gene was not associated with colorectal cancer even in the stratified analysis.

The associations between these polymorphisms of GST and the colorectal cancer risks in women were shown in Table 3. No significant difference in proportions of GSTM1

Table 2 Odds ratio (OR) and 95% confidence interval (CI) of the polymorphisms of GSTM1, GSTT1 and GSTP1 for colorectal cancer by age at diagnosis and tumor site in men

	GSTM1		GSTT1		GSTP1	
	Present	Null	Present	Null	A/A	With G[2]
Total population						
Cases/Controls	195/193	213/215	192/219	216/189	277/287	131/122
OR (95%CI)[1]	1.0	0.93 (0.68-1.26)	1.0	1.45 (1.07-1.97)	1.0	1.36 (0.98-1.89)
Tumor site						
Colon						
Cases/Controls	82/193	101/215	89/219	94/189	127/287	56/122
OR (95%CI)[1]	1.0	1.00 (0.67-1.48)	1.0	1.24 (0.83-1.83)	1.0	1.15 (0.75-1.76)
Rectum						
Cases/Controls	113/193	112/215	103/219	122/189	150/287	75/122
OR (95%CI)[1]	1.0	0.87 (0.61-1.25)	1.0	1.55 (1.08-2.23)	1.0	1.48 (1.00-2.18)
Age at diagnosis						
≤60 yr						
Cases/Controls	95/85	92/100	86/106	101/79	124/129	63/56
OR (95%CI)[1]	1.0	0.86 (0.53-1.30)	1.0	2.03 (1.29-3.21)	1.0	1.45 (0.89-2.37)
>60 yr						
Cases/Controls	100/108	121/115	106/113	115/110	153/158	68/66
OR (95%CI)[1]	1.0	1.03 (0.67-1.58)	1.0	1.12 (0.73-1.71)	1.0	1.34 (0.84-2.12)

[1]ORs and 95%CIs were estimated from multivariate unconditional logistic regressions controlling for age, physical activity, coffee, cigarette, alcohol, meat, vegetable/fruit and fish/shrimp; [2] Combined genotype of A/G and G/G.

Table 3 Odds ratio (OR) and 95% confidence interval (CI) of the polymorphisms of GSTM1, GSTT1 and GSTP1 for colorectal cancer by age at diagnosis and tumor site in women

	GSTM1		GSTT1		GSTP1	
	Present	Null	Present	Null	A/A	With G[2]
Total population						
Cases/Controls	126/130	189/195	135/154	180/171	224/226	90/99
OR (95%CI)[1]	1.0	0.99 (0.71-1.38)	1.0	1.18 (0.85-1.64)	1.0	1.00 (0.70-1.43)
Tumor site						
Colon						
Cases/Controls	61/130	105/195	72/154	94/171	116/226	49/99
OR (95%CI)[1]	1.0	1.19 (0.78-1.81)	1.0	1.23 (0.82-1.84)	1.0	1.04 (0.67-1.60)
Rectum						
Cases/Controls	65/130	84/195	63/154	86/171	108/226	41/99
OR (95%CI)[1]	1.0	0.84 (0.56-1.27)	1.0	1.20 (0.79-1.81)	1.0	0.93 (0.60-1.46)
Age at diagnosis						
≤60 yr						
Cases/Controls	52/52	84/82	50/55	86/79	99/89	37/45
OR (95%CI)[1]	1.0	1.08 (0.63-1.85)	1.0	1.20 (0.71-2.05)	1.0	0.84 (0.48-1.47)
>60 yr						
Cases/Controls	74/78	105/113	85/99	94/92	125/137	53/54
OR (95%CI)[1]	1.0	0.95 (0.61-1.46)	1.0	1.15 (0.75-1.76)	1.0	1.13 (0.71-1.80)

[1]ORs and 95%CIs were estimated from multivariate unconditional logistic regressions controlling for age, sex, physical activity, coffee, cigarette, alcohol, meat, vegetable/fruit and fish/shrimp; [2] Combined genotype of A/G and G/G.

null, GSTT1 null and GSTP1 with G allele genotypes were observed between cases and controls for all female subjects and their subgroups by anatomical site and diagnosed age.

Men with more risk genotypes of GSTT1 and GSTP1 had higher risk than those without any risk genotypes (trend test $P<0.01$) (Table 4). Compared to men with both GSTT1 present and GSTP1 A/A genotypes, those with either or both GSTT1 null and GSTP1 with G allele genotypes had 1.4-fold (OR = 1.42, 95%CI = 1.01-1.99) and 1.9-fold (OR = 1.91, 95%CI = 1.21-3.02) risk of colorectal cancer respectively. A stronger risk of rectal cancer was seen for men with both GSTT1 null and GSTP1 with G allele genotypes (OR = 2.20, 95%CI = 1.28-3.78). The corresponding effect on colorectal cancer for men aged less than 60 years at diagnoses was even greater (OR = 3.05, 95%CI = 1.51-6.12). However, these trends were not observed for women (data not shown).

Table 5 demonstrated a multiplicative synergistic effect for men and an antagonistic effect for women between the GST genes (GSTT1 and GSTP1) and vegetable/fruit intake on the colorectal cancer risk. The protective effect of high vegetable/fruit consumption was enhanced for men with GSTT1 present genotype (OR = 0.32, 95%CI = 0.21-0.50), compared to men with GSTT1 null genotype and low vegetable/fruit consumption. Similarly, the protective effect for men was strongly associated with GSTP1 A/A genotype and high vegetable/fruit consumption (OR = 0.40, 95%CI = 0.25-0.64). However, the beneficial effect of high vegetable/fruit intake was attenuated for women with the protective GSTT1 present or GSTP1 A/A genotypes. The strongest protective effect from vegetable/fruit intake was observed among women having GSTT1 null or GSTP1 with G allele genotypes. The stratification analysis also showed that the multiplicative protective effect was also significant in the rectum and for younger men (data not shown).

The combined effects of GSTT1 present or GSTP1 A/A genotypes, and vegetable/fruit intake on colorectal cancer varied with smoking status (Table 6). Since very few women were smokers (<5%); this stratification analysis by smoking status was calculated only for men. The protective effects of high vegetable/fruit consumption enhanced by the GSTT1 present or GSTP1 A/A genotypes were consistent and the association was robust, particularly for non-smokers. The ORs for these joint effects were 0.17 (95% CI = 0.07-0.42) for GSTT1 and 0.21 (95% CI = 0.09-0.52) for GSTP1. There was no significant interaction between meat intake and GST genes to the risk of colorectal cancer (data not shown).

DISCUSSION

This is the first hospital-based case-control study with a relatively large sample simultaneously examining the association

Table 4 Combined genotype of GSTT1 and GSTP1 on colorectal cancer risk by tumor site and age in men

	GSTT1 / GSTP1					
	Present / (A/A)		Present / with G + null / (A/A)		Null / with G	
	Cases/Controls	OR (95%CI)[1]	Cases/Controls	OR (95%CI)[1]	Cases/Controls	OR (95%CI)[1]
Total population[2]	132/157	1.00	205/191	1.42 (1.01-1.99)	71/60	1.91 (1.21-3.02)
Tumor site						
Colon	64/157	1.00	88/191	1.16 (0.75-1.79)	31/60	1.42 (0.79-2.55)
Rectum[2]	68/157	1.00	117/191	1.65 (1.09-2.50)	40/60	2.20 (1.28-3.78)
Age at diagnosis						
≤60 yr[2]	57/73	1.00	96/89	1.73 (1.04-2.89)	34/23	3.05 (1.51-6.12)
>60 yr	75/84	1.00	109/102	1.25 (0.78-2.02)	37/37	1.41 (0.75-2.64)

[1]Adjusted for age, physical activity, coffee, cigarette, alcohol, meat, vegetable/fruit and fish/shrimp; [2]Trend test $P<0.01$.

Table 5 Combined effects of GST genotypes and vegetable/fruit consumption on colorectal cancer risk

Genotype	Vegetable/fruit consumption	Men			Women		
		Cases	Controls	OR (95%CI)[1]	Cases	Controls	OR (95%CI)[2]
GSTT1							
Null	Low	106	69	1.00	90	62	1.00
Present	Low	101	74	0.87 (0.55-1.36)	54	66	0.60 (0.36-0.98)
Null	High	110	120	0.57 (0.37-0.88)	90	109	0.47 (0.30-0.75)
Present	High	91	145	0.32 (0.20-0.50)	81	88	0.53 (0.33-0.85)
P for interaction				0.21			0.06
GSTP1							
With G	Low	60	49	1.00	44	35	1.00
A/A	Low	147	94	1.09 (0.67-1.76)	99	93	0.84 (0.49-1.45)
With G	High	71	73	0.73 (0.43-1.25)	46	64	0.51 (0.28-0.94)
A/A	High	130	193	0.40 (0.25-0.64)	125	133	0.58 (0.34-0.99)
P for interaction				0.03			0.42

[1]Adjusted for age, physical activity, coffee, cigarette, alcohol, meat, vegetable/fruit and fish/shrimp; [2]Adjusted for age, staple, meat, vegetable/fruit and fish/shrimp.

Table 6 Smoking specific risk for colorectal cancer for the combined effects of GST genotypes and vegetable/fruit consumption in men

Genotype	Vegetable/fruit consumption	Non-smokers			Smokers		
		Cases	Controls	OR (95% CI)[1]	Cases	Controls	OR (95% CI)[1]
GSTT1							
Null	Low	23	13	1.00	83	56	1.00
Present	Low	28	16	0.85 (0.33-2.21)	73	58	0.85 (0.51-1.41)
Null	High	45	55	0.41 (0.18-0.96)	59	65	0.66 (0.39-1.11)
Present	High	25	65	0.17 (0.07-0.42)	52	80	0.43 (0.25-0.73)
P for interaction				0.23			0.49
GSTP1							
With G	Low	19	10	1.00	41	39	1.00
A/A	Low	32	19	0.80 (0.30-2.15)	115	75	1.21 (0.69-2.12)
With G	High	25	33	0.41 (0.15-1.10)	40	40	0.96 (0.49-1.88)
A/A	High	45	88	0.21 (0.09-0.52)	71	105	0.55 (0.31-0.98)
P for interaction				0.48			0.07

[1]Adjusted for age, physical activity, coffee, alcohol, meat and fish/shrimp.

between GSTM1, GSTT1 and GSTP1 polymorphisms and colorectal cancer risk for a Chinese population in Taiwan. The results revealed an elevated risk for colorectal cancer for men with GSTT1 null and GSTP1 with G allele genotypes, particularly for rectal cancer and diagnosed before 60 years old. We also found a very significant protective effect for men with GSTT1 present or GSTP1 A/A genotypes and higher consumption of vegetable/fruit. This combined effect reduces the risk for colorectal cancer to 0.40 or less, even for smokers, and further to about 0.20 for non-smokers, suggesting that these metabolic genes can modulate the risk of vegetable/fruit related colorectal cancer.

There is conflicting evidence concerning the role of GST polymorphisms in colorectal cancer susceptibility. It might be associated with the ethnic differences in allele frequency for these polymorphisms[11]. Carcinogen exposures might vary among populations with the pathogenesis for colorectal cancer differing by tumor site. Moreover, inadequate study design such as non-random sampling, limited sample size and little attempt to adjust for potential confounders should also be considered.

It seems unlikely that the GSTM1 null genotype could predispose an individual to colorectal cancer because GSTM1 is only expressed at low levels in the colon[38]. Conversely, GSTT1 and GSTP1 seem more likely susceptibility gene candidates because they are the predominant GST isoenzymes in colorectal tissue[38,39] and are involved in the inactivation of heterocyclic amine[40]. One previous publication on the GSTP1 A/G (Ile105Val) polymorphism identified that subjects having GSTP1 with G allele are at an elevated risk compared to those with the A/A genotype (OR = 1.77, 95%CI = 1.03-3.06)[36]. However, one of the Alpha class isoenzymes, GSTA1, abundant in the human liver not colon, can also catalyze the detoxification of *N*-acetoxy-PhIP[40,41] and increases the risk of colorectal cancer[41]. Association between the polymorphism of GSTA1 and the risk of colorectal cancer for our subjects needed to be clarified in our future study.

Our results support that GSTT1 and GSTP1 might have a role in colorectal cancer susceptibility. The significant risk of the GSTT1 null genotype for colorectal cancer in our study might be due to the higher prevalence of GSTT1 null (49%) than in Caucasians (15-27%)[11]. Although the frequency of the GSTP1 G allele in our controls (17%) was lower than that in Caucasians (23-38%)[42], the large sample size enabled us to find a moderate relationship between the GSTP1 with G allele and an increased risk of colorectal cancer of men.

Consistent with other studies[37,43], this study also shows that risk factors for colorectal cancer is different between men and women in Taiwan. The GSTT1 null and GSTP1 with G allele genotypes were associated with increased risk of colorectal cancer, particularly in younger men and rectal cancer. The 2-fold increased risk of the GSTT1 null genotype relative to the GSTT1 present genotype for colorectal cancer in younger men is in line with other studies[15,19,44]. Studies in human suggest that the detoxification potential of the GST enzymes decreases with age[45]. It is possible that individuals with the null genotype develop tumors at a younger age than individuals that do express this enzyme. This study showed a slightly stronger genetic effect for the rectum over the colon. This was consistent with findings from previous studies[17,18]. The risks associated with diet tend to be the strongest in the distal colon for men[46]. Carcinogenesis within the distal colon has been associated with bulky-adduct-forming (BAF) agents[47]. The GST polymorphisms of reduced detoxification genotype might thus more likely predispose the rectum rather than the colon to cancer.

In studies on the combined effects of GST polymorphisms and vegetable/fruit consumption on colorectal lesions, three studies indicated that the high isothiocyanates content in cruciferous vegetables enhances the cancer preventive effect for humans with the GSTM1 or T1 null genotypes[27,28,30]. Potentially slower excretion of isothiocyanates induces GST. Other studies have reported that people with lower levels of enzyme activity (i.e., those with GSTM1 null genotype) obtain less protection from consuming cruciferous vegetables[48,49]. Our data showed that the greatest protective effect from higher vegetable/fruit consumption was observed for men with GSTT1 present or GSTP1 A/A genotypes, but for women with GSTT1 null or GSTP1 with G allele genotypes. In addition, Lampe *et al*[50] reported that brassica vegetables increased GSTA and GST activity for the GSTM1 null individuals in a randomized clinical tria. It is warranted to evaluate the gene-gene interactions of the polymorphic GST genes and their interactions with vegetables in further studies.

Because cruciferous vegetables not only induce phase I activating enzyme (e.g., cytochrome P4501A2 (CYP1A2)) and phase II inactivating enzyme (e.g., GST) expression and isothiocyanates themselves are a GST substrate[51], the delicate balance between phase I, phase II enzymes and their regulators is undoubtedly an important determinant for cancer risk. Although cigarette smoking seems not to interact with GST genes to affect the colorectal cancer risk[17,20,21,52], smoking can modify the association between GSTM1, cruciferous vegetables and colon cancer[30]. Because metabolizing enzymes can detoxify carcinogens in cigarette smoke, cigarette smoking might play a role in the balance of these enzymes and their regulators. Smoking might modify this effect by reducing the benefit for approximately 30% for the study subjects. It has been proven that among frequent consumers of cruciferous vegetables, smokers with the GSTM1 null genotype have CYP1A2 activity 2-fold over non-smokers with the GSTM1 present genotype[49]. Subjects with elevated CYP1A2 activity might be predisposed to colorectal cancer by activating heterocyclic amines and other procarcinogens[53].

Because GSTT1 and GSTP1 are expressed at higher level in colorectal tissue and were associated with colorectal cancer risk in men, we postulate that people with the genotype for higher detoxified enzyme activity and higher vegetable/fruit consumption exhibit multiplicative synergistic beneficial effect for reduced colorectal cancer risk. This protective effect is extended for non-smokers without enzyme activation or carcinogens exposure. On the other hand, if the detoxifying enzymes were not related to colorectal cancer risk, higher vegetable/fruit consumption could exert their beneficial effect without being excreted by metabolizing enzymes.

This study was limited by obtaining dietary information retrospectively after diagnosis. We cannot exclude the effect of early preclinical disease on the dietary habits in these colorectal cancer cases. This effect might be negligible because the difference in eating habit changes in the previous 10 years among cases and controls was not significant ($P = 0.12$). In addition, the similar interview settings provided reassurance against potential information bias. Other limitations arise from the questionnaire, which included only the diet frequency for major food groups. We could not assess the impact of potential carcinogens derived from processed food or the effects of specific macronutrient or micronutrient.

The strengths of our study are the inclusion of newly diagnosed and histologically confirmed adenocarcinoma colorectal cancer cases and using controls that received colonoscopies. The possibility of misclassification is minimal. This large sample size allowed stratified data analysis. The observed environmental effects in the study population agreed with other published studies[54] supporting the validity of our results.

Our study results suggest that the GSTT1 null genotype and the GSTP1 with G allele are potential risk alleles for colorectal cancer for the Chinese male population, but of beneficial effect for women with higher vegetable/fruit intake. The beneficial effect of vegetable/fruit on colorectal cancer is enhanced with GSTT1 present and GSTP1 A/A genotypes for both men and women, in particular for non-smokers. Studies with specific vegetable types, related metabolic enzymes and dietary regulators in different age groups and tumor sites would help to clarify the association.

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