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Glutathione *S*-transferase (*GST*) gene polymorphisms, cigarette smoking and colorectal cancer risk among Chinese in Singapore

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Cigarette smoking is a risk factor for colorectal cancer. Putative colorectal procarcinogens in tobacco smoke include polycyclic aromatic hydrocarbons and heterocyclic aromatic amines that are known substrates of glutathione *S*-transferases (GSTs). This study examined the influence of functional *GST* gene polymorphisms on the smoking–colorectal cancer association in a population known to be minimally exposed to dietary sources of these procarcinogens. Incident cases of colorectal cancer ($n = 480$) and matched controls ($n = 1167$) were selected from the Singapore Chinese Health Study, a population-based prospective cohort of 63 257 men and women who have been followed since 1993. We determined the deletion polymorphisms of *GSTM1* and *GSTT1* and the functional polymorphism at codon 105 of *GSTP1* for each subject. A three level composite *GST* index was used to examine if *GST* profile affected a smoker's risk of developing colorectal cancer. While there was no statistically significant association between cigarette smoking and colorectal cancer risk among subjects absent of any at-risk *GST* genotypes, smokers possessing two to three at-risk *GST* genotypes exhibited a statistically significant increased risk of colorectal cancer compared with non-smokers ($P = 0.0002$). In this latter stratum, heavy smokers exhibited a >5-fold increased risk relative to never-smokers (odds ratio, 5.43; 95% confidence interval, 2.22–13.23). Subjects with one at-risk *GST* genotype displayed a statistically significant but weaker association with smoking. These findings suggest that *GST* gene polymorphisms influence interindividual susceptibility to smoking-associated colorectal cancer. Our data indicate an important role for GST enzymes in the detoxification of colorectal carcinogens in tobacco smoke.

Introduction

There is much experimental evidence to suggest that chemical carcinogens such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAAs) are implicated in colorectal carcinogenesis (1). These compounds are present in tobacco smoke and also found in meat cooked on an open flame or at a high temperature (2–4). Metabolic activation of these compounds can lead to formation of highly reactive mutagens that readily react with DNA bases (1). Alternatively, these compounds undergo detoxification through conjugation reactions with the phase II enzymes such as uridine diphosphate-glucuronosyltransferases or glutathione *S*-transferases (GSTs) to form stable polar products that are readily eliminated (5,6).

In humans, the GST enzymes can be divided into five main classes: alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT) and zeta (GSTZ) (7,8). Among them, GSTM1, GSTT1 and GSTP1 are detoxification enzymes that have been known to metabolize a wide range of carcinogens from tobacco smoke and diet, including HAAs and PAHs (9). These GSTs are polymorphic enzymes with interindividual variations in enzymatic level and activity. The homozygous deletion genotypes of *GSTM1* and *GSTT1* result in an absence of GSTM1 and GSTT1 expression and they are relatively more common in Caucasian than Asian populations (10). A transition of adenine (A) to guanine (G) at nucleotide 313 in exon 5 of the *GSTP1* gene results in a change from isoleucine (Ile) to valine (Val) at position 104 in the amino acid sequence of the corresponding protein. This polymorphism is proximal to the hydrophobic-binding site for electrophiles, and the valine-containing homozygous variant, *GSTP1 BB* and the heterozygous Ile–Val variant, *GSTP1 AB*, have been shown to possess decreased specific activity and affinity for electrophilic compounds (11).

Previous work in the Singapore Chinese Health Study has demonstrated an increased risk of colorectal cancer associated with cigarette smoking (12). Furthermore, it has been shown that the diet of Southern Chinese, including the Singapore Chinese, is low in PAH and HAA (13–15). Hence, the present study could meaningfully examine the interaction effects of *GST* genotypes on cigarette smoking–colorectal cancer risk association in the absence of significance dietary contribution of these procarcinogens.

Abbreviations: CI, confidence interval; GST, glutathione S-transferase; HAA, heterocyclic aromatic amine; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon.

Materials and methods

Study subjects

This case–control study was nested within the Singapore Chinese Health Study, a population-based cohort of 63 257 Chinese women and men, aged 45–74 years and residents of government housing estates (where 86% of the entire Singapore population resided) at enrollment, which occurred between April 1993 and December 1998 (16). Our cohort subjects were drawn from the two major dialect groups of Chinese in Singapore, the Hokkiens and the Cantonese, who originated from two contiguous prefectures in Southern China. The Institutional Review Boards at the National University of Singapore and the University of Minnesota had approved this study.

At recruitment, subjects were interviewed in-person using a structured questionnaire that asked for information including demographics, use of tobacco, menstrual (including menopausal status) and reproductive (including use of hormone replacement therapy) histories (women only), medical history as well as a dietary component assessing current intake patterns. Respondents were asked to choose from predefined frequency and portion size categories for each of the 165 listed food/beverage items that he/she consumed during the past 12 months. We used the Singapore Food Composition Table to estimate average daily intake of roughly 100 nutrient and non-nutrient compounds for each study subject (16).

For cigarette smoking, the study population was divided into never, former and current smokers based on their choice of three possible responses to the following question, 'Have you ever smoked at least one cigarette a day for 1 year or longer'. Subjects who answered 'no' were classified as 'never-smokers', those who answered 'yes, but I quit smoking' were classified as 'former smokers' and those who answered 'yes, and I currently smoke' were classified as 'current smokers'. Ever smokers (former and current) were then asked about age at smoking initiation (four categories: <15, 15–19, 20–29 and ≥ 30); number of cigarettes smoked per day (six categories: ≤ 6, 7–12, 13–22, 23–32, 33–42 and ≥ 43) and duration of smoking (four categories: <10, 10–19, 20–39, ≥ 40).

Between April 1994 and December 1999, we attempted to collect blood and single-void urine specimens from a random 3% sample of cohort enrollees. Details of the biospecimen collection, processing and storage procedures have been described (17). If the subject refused to donate blood, he/she was asked to donate buccal cells. From the 1194 subjects who gave biospecimens, we excluded 27 subjects who had a history of colorectal cancer at recruitment ($n = 5$) or developed first colorectal cancer ($n = 22$) by 31 December 2005 and the remaining 1167 subjects constituted the control group for the present study.

We identified incident colorectal cancer cases through the population-based cancer registry in Singapore (18). As of 31 December 2005, 1005 cases of colorectal cancer had occurred among the cohort participants. All cases were

further verified by manual checking of pathological and medical records. We attempted to collect blood/buccal cell and urine samples from all incident colorectal cancer cases. Blood or buccal specimens were available on 480 (47.8%) incident colorectal cancer cases. Compared with colorectal cancer patients who did not donate a blood or buccal sample, those who donated had a similar mean age at cancer diagnosis (67.9 versus 66.4 years). Male patients were more likely to donate biospecimen than female patients (51.7 versus 43.1%). Patients who did not donate blood or buccal samples were less educated (37.1% had no formal education) than those who did (27.3% had no formal education). Although colon cancer constituted 57% among the cases who donated biospecimens, 66% were colon cancer among cases who did not donate. Those who did not donate biospecimens were also more likely to have advanced disease (59%) compared with those who donated (49%). Otherwise, there was no significance difference between the two groups in the percentage of biopecimen availability by level of body mass index, cigarette smoking, alcohol drinking, dialect group or history of diabetes mellitus.

GSTM1, T1 and P1 genotyping

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*, *GSTT1* and *GSTP1* was performed using the fluorogenic 5′-nuclease assay (TaqMan Assay) (19). The TaqMan assays were performed using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The oligonucleotide primers for amplification of the polymorphic region of *GSTP1* were GC070 for (5′-CCTGGTGGACATGGTGAATG-3′) and GC070 rev (5′-TGCTCACAC-CATAGTTGGTGTAGATGA-3′). In addition, the fluorogenic MGB oligonucleotide probes used to detect each of the alleles were GC070F (5′-TGCAAATACGTCTCCCT-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°C for 10 min followed by 50 cycles of 95°C for 25 s and 60°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′-GTGCAAACACCTCCTGGAGAT-3′) and GC003rev (5′-AGTCCTTGGCCTTCAGAATGA-3′), GC004for (5′-CT TGGAGGAACTCCCTGAAAAG-3′) and GC004rev (5′-TGGAACCTCCA-TAACACGTGA-3′), GC005for (5′-CGATTTTCTTTTTAGGGCAGTAGC-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′-ATGCTGCCCATCCCTGCCC-3′), GC004FAM (5′-AAGCGGCCATGGT TTGCAGG-3′) and GC005FAM (5′-CGCCTGAGCCAGAGATTTCCCA-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°C for 10 min followed by 50 cycles of 95°C for 25 s and 60°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 ΔRn 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

Data were analyzed by standard methods for unmatched case–control studies (20). Unconditional logistic regression models were used to examine the associations between the different genotypes and risk of colorectal cancer and their possible interaction with smoking status. The associations were measured by odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) and *P* values (two sided). All ORs were adjusted for age (year) at recruitment, year of recruitment, gender, dialect group (Cantonese, Hokkien), level of education (no formal schooling, primary school, secondary school and higher), body mass index (<20, 20 to <24, 24 to <28 and 28+ kg/m^2), frequency of alcohol consumption (non-drinker, monthly drinker, weekly drinker, daily drinker), familial history of colorectal cancer (yes, no), weekly physical activity (yes, no), history of diabetes mellitus (yes, no) and dietary intake of isothiocyanates (quartiles). Heavy smokers were defined as those who started to smoke before age 15 years and smoked, on average, ≥13–22 cigarettes/day (see Materials and Methods—Study subjects for more details); all other smokers were labeled as light smokers. Statistical analysis was carried out using the SAS software version 9.2 (SAS Institute, Cary, NC).

Results

Of 480 incident cases of colorectal cancer in this study, 275 (57%) had cancers of the colon and the remaining 205 (43%) had either rectal or rectosigmoid cancers. The mean age of cases at the time of diagnosis was 66.4 (SD 8.2) years, with a range of 47–85 years. The median time interval between the baseline interview and cancer diagnosis was 5.6 years (range: 1 month to 12 years). The association between smoking and colorectal cancer in this nested case–control study was similar to what we have previously reported in the entire cohort (12). Current smokers had a 37% increased risk of colorectal cancer (95% CI, 1.00–1.88). There was a dose-dependent increased risk with smoking duration and intensity and an almost 3-fold increased risk of colorectal cancer among the heavy smokers compared with never-smokers (OR, 2.95; 95% CI, 1.72–5.06). Consistent with our previous findings (12), the smoking–cancer risk association was much stronger for rectal cancer than for colon cancer (Table I).

The prevalence of the *GSTT1* and *GSTM1* null genotypes among controls were 40.8 and 45.1%, respectively, and that of the *GSTP1 AB* and *BB* genotypes were 29.6 and 4.4%, respectively, which were similar to our previous results (21). There was no association between either the *GSTM1* or the *GSTT1* polymorphism and colorectal cancer risk. A reduced risk of colorectal cancer was seen among individuals possessing low-activity *AB* or *BB* genotypes relative to the *AA* genotype of *GSTP1*, but the risk reduction was not statistically significant (OR, 0.80; 95% CI, 0.62–1.02). Overall, there was no association between the number of null/low activity genotypes and colorectal cancer risk (Table II).

Given the potential overlap in enzyme function and activity of the GST genes studied, we created a composite *GST* profile to examine their combined effect modification on the association between smoking and colorectal cancer risk (Table III). Among subjects who did not possess any *GST* null or low activity genotypes, there was no statistical evidence of an increased risk of colorectal cancer in heavy smokers versus never-smokers (OR: 1.34; 95% CI: 0.38–4.76). On the other hand, among subjects with at least two GST null/low-activity genotypes, there was a highly statistically significant increased risk of colorectal cancer for heavy smokers (OR, 5.43; 95% CI, 2.22–13.23 relative to never-smokers). The OR of colorectal cancer risk for heavy smokers relative to never-smokers among those possessing one GST null/low-activity genotype was 2.43 (95% CI, 1.01–5.86). This gradient of risk associated with cigarette smoking dependent on the number of at-risk *GST* genotypes was similarly present when data were analyzed separately for cancers of the colon and rectum (Table III).

Discussion

Using a case–control study nested in a population-based cohort study in Singapore, we have evaluated the modifying effects of *GST* genotypes on the tobacco smoking–colorectal cancer risk association. This is the first study that shows a gene–dose-dependent amplification of the tobacco smoking–colorectal cancer risk association across subjects with increasingly less favorable *GST* profile. Our findings support the hypothesis that the GST enzymes are involved in the detoxification of colorectal carcinogens in cigarette smoke.

Since GST enzymes play a role in the detoxification of PAH and HAA present in tobacco smoke, many groups have investigated whether polymorphic variations in *GST* genes modify the association between tobacco smoking and colorectal cancer risk. All studies to date have been conducted in western populations, and the majority of them reported no effect modification of *GST* genetic variation on the smoking–colorectal cancer association (22–27). However, these studies all possess two major methodological limitations that render their findings difficult to interpret, for the following reasons.

Table I. Cigarette smoking and colorectal cancer risk

	Colorectal cancer			Colon cancer		Rectal cancer	
	Controls	Cases	OR (95% CI)	Cases	OR (95% CI)	Cases	OR (95% CI)
Smoking status							
Never	845	273	1.00	175	1.00	98	1.00
Former	129	87	1.21 (0.85–1.72)	46	1.08 (0.70–1.67)	41	1.38 (0.86–2.22)
Current	193	120	1.37 (1.00–1.88)	54	1.01 (0.68–1.51)	66	1.88 (1.25–2.84)
Cigarettes/day							
Never	845	273	1.00	175	1.00	98	1.00
<13	134	69	1.07 (0.75–1.52)	33	0.85 (0.54–1.34)	36	1.37 (0.86–2.18)
13+	188	138	1.51 (1.10–2.08)	67	1.20 (0.81–1.78)	71	1.94 (1.27–2.95)
P for trend			0.014		0.443		0.002
Age at starting							
Never	845	273	1.00	175	1.00	98	1.00
15+	278	152	1.08 (0.80–1.44)	79	0.92 (0.64–1.32)	73	1.29 (0.87–1.91)
<15	44	55	2.80 (1.77–4.43)	21	1.83 (1.01–3.31)	34	4.23 (2.44–7.32)
P for trend			0.0004		0.255		<0.0001
Smoking index[a]							
None	845	273	1.00	175	1.00	98	1.00
Light	292	167	1.16 (0.87–1.54)	83	0.94 (0.66–1.34)	84	1.45 (0.99–2.13)
Heavy	30	40	2.95 (1.72–5.06)	17	2.18 (1.11–4.29)	23	4.12 (2.15–7.88)
P for trend			0.002		0.246		<0.0001

All ORs were adjusted for age, gender, dialect group, year of recruitment, level of education, body mass index, history of diabetes, family history of colorectal cancer, alcohol consumption, physical exercise and dietary intake of isothiocyanates.
[a]Light smokers were those who started to smoke cigarettes at or after 15 years of age or smoked ≤12 cigarettes/day. Heavy smokers were those who started to smoke cigarettes before 15 years of age and smoked at least 13 cigarettes/day.

Table II. GST polymorphism in relation to risk of colorectal cancer

	Controls	All CRC		Colon		Rectal	
		Cases	OR (95% CI)	Cases	OR (95% CI)	Cases	OR (95% CI)
GSTT1							
Present	691	294	1.00	174	1.00	120	1.00
Null	476	186	0.92 (0.73–1.16)	101	0.83 (0.62–1.10)	85	1.06 (0.77–1.46)
GSTM1							
Present	641	246	1.00	136	1.00	110	1.00
Null	526	234	1.12 (0.89–1.41)	139	1.23 (0.93–1.61)	95	1.03 (0.75–1.41)
GSTP1							
AA	771	343	1.00	194	1.00	149	1.00
AB	345	122	0.82 (0.63–1.06)	72	0.86 (0.63–1.18)	50	0.76 (0.53–1.09)
BB	51	15	0.65 (0.35–1.21)	9	0.74 (0.35–1.58)	6	0.55 (0.22–1.37)
AB/BB	396	137	0.80 (0.62–1.02)	81	0.85 (0.63–1.14)	56	0.73 (0.52–1.04)
No. of 'null or low activity' GST genotypes[a]							
0	263	108	1.00	63	1.00	45	1.00
1	483	209	1.02 (0.76–1.36)	119	0.98 (0.68–1.40)	90	1.06 (0.70–1.61)
2	348	141	0.95 (0.69–1.31)	77	0.89 (0.60–1.31)	64	1.06 (0.68–1.65)
3	73	22	0.76 (0.44–1.32)	16	0.95 (0.50–1.78)	6	0.48 (0.19–1.23)
P for trend			0.410		0.601		0.498

All ORs were adjusted for age, gender, dialect group, year of recruitment, level of education, body mass index, history of diabetes, family history of colorectal cancer, alcohol consumption, smoking index (none, light and heavy smokers), dietary intake of isothiocyanates and physical exercise.
[a]Null or low activity genotypes were *GSTM1 null*, *GSTT1 null* and *GSTP1 AB/BB* genotypes.

It has been shown that the Western diet contains high HAA and PAHs that are also found in tobacco smoke (28–31). Thus, in the published literature examining *GST* genotypes' influence on the association between cigarette smoking and colorectal cancer risk, both cigarette smoking and diet are major contributors of carcinogenic PAHs and HAAs. Unfortunately, none of the previously published studies assessed dietary PAHs/HAA intake in study subjects in order to take into account this additional source of exposure while evaluating the interactive effect of *GST* genotypes and cigarette smoking on colorectal cancer risk. On the other hand, dietary PAH/HAA is known to be low in a typical southern Chinese diet, and specifically, for this study population (13,15). In other words, cigarette smoking is the sole major contributor of carcinogenic PAH/HAA in our study population. The current study, therefore, overcomes a major methodological limitation of prior studies that affect the validity of their findings. Our findings also strengthen evidence for the role of PAHs and HAAs in colorectal carcinogenesis. Since these compounds can be found in cigarette smoke as well as in meats cooked at high temperature, the latter being common in Western diet, the dual increase in prevalence of smoking and westernization of diet may explain the rise in incidence of colorectal cancer in many Asian populations.

Another reason for the failure of other studies to demonstrate a modifying effect of *GST* polymorphisms on the smoking–colorectal cancer risk association was suboptimal classification of study subjects by their *GST* profile. *GSTM1*, *GSTT1* and *GSTP1* genetic polymorphisms have been widely investigated in colorectal cancer risk

Table III. GST genotype and smoking-related colorectal cancer risk

	Controls	Colorectal cancer Cases	Colon cancer OR (95% CI)	Rectal cancer Cases	OR (95% CI)	Cases	OR (95% CI)
With zero *GST* 'null or low activity' genotype[a]							
Never smokers	181	63	1.00	44	1.00	19	1.00
Light smokers	75	37	0.82 (0.43–1.55)	15	0.35 (0.15–0.84)	22	2.00 (0.81–4.90)
Heavy smokers	7	8	1.34 (0.38–4.76)	4	0.68 (0.14–3.22)	4	3.23 (0.57–18.11)
P for trend			0.916		0.087		0.085
With one *GST* 'null or low activity' genotype[a]							
Never smokers	350	126	1.00	81	1.00	45	1.00
Light smokers	120	69	1.09 (0.70–1.68)	32	0.86 (0.50–1.49)	37	1.37 (0.77–2.44)
Heavy smokers	13	14	2.43 (1.01–5.86)	6	2.05 (0.66–6.33)	8	3.01 (1.05–8.62)
P for trend			0.143		0.732		0.052
With two to three *GST* 'null or low activity' genotypes[a]							
Never smokers	314	84	1.00	50	1.00	34	1.00
Light smokers	97	61	1.69 (1.03–2.77)	36	1.92 (1.04–3.54)	25	1.39 (0.71–2.72)
Heavy smokers	10	18	5.43 (2.22–13.23)	7	4.25 (1.36–13.30)	11	6.04 (2.14–17.04)
P for trend			0.0002		0.005		0.003

All ORs were adjusted for age, gender, dialect group, year of recruitment, level of education, body mass index, history of diabetes, family history of colorectal cancer, alcohol consumption, dietary intake of isothiocyanates and physical exercise.
[a]Null or low activity genotypes were *GSTM1 null*, *GSTT1 null* and *GSTP1 AB/BB* genotype.

because of their high expression in the intestinal tract and their role in detoxification of food- and tobacco-derived carcinogens. It is possible that deficiency in one class of GST enzymes due to genetic polymorphism is compensated by the presence of other classes of GST enzymes. However, none of the prior studies on *GST* genotype, cigarette smoking and colorectal cancer risk incorporated all three *GST* genes in their study design. Since the three *GST* genes share common substrates, studies that investigated only one or two of the *GST* genes could have misclassified subjects according to their overall status of GSTs, leading to false null associations. Indeed, several studies examining either *GSTM1* and *GSTT1* genotypes (22,25–27) or only *GSTM1* genotype (23,24) failed to demonstrate an interaction effect with tobacco smoking on colorectal cancer risk (25). In contrast, the present study determined all three *GST* genotypes and employed a composite *GST*–genotype index. The double selection of *GSTM1* and *GSTT1* null genotypes, as well as the frequency of the *GSTP1 BB* genotype with less catalytic activity, has been documented to be higher among Chinese compared with Caucasians and other ethnic groups (32). Our findings therefore suggest that Chinese may be more susceptible to smoking-induced colorectal carcinogenesis compared with other ethnic groups. Our current report of a stronger association between smoking and risk of rectal cancer compared with that of colon cancer is consistent with our previous publication on the association between smoking and colorectal cancer risk using data from the entire cohort (12). The results in the current study show that among genetically susceptible subjects, the association between smoking and colon cancer risk is enhanced.

The current study has several strengths. Singapore is a small city–state where there is good access to specialized medical care. The nationwide cancer registry has been in place since 1968 and has been shown to be comprehensive in its recording of cancer cases (33). Thus, colorectal cancer case ascertainment can be assumed to be complete. Our study subjects originated from two contiguous regions in South China, leading to a high degree of genetic homogeneity. All dietary and lifestyle factors which were included as covariates in the statistical models were assessed prior to cancer diagnosis and thus can be presumed to be free of recall bias. A limitation of the study is potential bias in case selection since cases in this study had less advanced disease compared with those who did not donate biospecimens for this study. In our interest to examine the associations by subsite, we have stratified the cases into colon and rectal cancers. Unfortunately, the small case numbers in some cells resulted in rather imprecise estimates. Another limitation is the use of relatively old methods in the genotyping. Recently, Moore *et al.* (34) demonstrated that more sensitive genotype methods quantifying the number of *GSTM1* and *GSTT1* alleles may more accurately measure the phenotypic differences between genotypes. Their results suggest that older genotyping methods, such as the ones employed in this study, could result in non-differential misclassification. In this case, our risk estimates could be underestimated. Although we have included three *GST* genotypes in this study, other work has reported positive smoking interactions in colorectal cancer with phase I metabolism enzymes (35–37), which are not investigated in the present study. We have also not included genetic polymorphisms in the genes coding for GSTA and GSTZ enzymes. However, these two classes of enzymes are less widely investigated in colorectal cancer risk because they are not known to have high expression in the intestinal tract or to play a major role in detoxification of food- and tobacco-derived carcinogens.

In conclusion, our study shows that the *GSTM1/GSTT1/GSTP1* genotypic profile of a cigarette smoker affects his/her risk of developing colorectal cancer due to exposure from colorectal procarcinogens present in tobacco smoke. Our data, therefore, also indicate an important role for GST enzymes in the detoxification of colorectal carcinogens in tobacco smoke.

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