Glutathione *S*-Transferase *GSTM1* AND *GSTT1* Polymorphisms and Colorectal Cancer Risk: A Prospective Study[1]

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Abstract

Glutathione *S*-transferase (GST) *M1* and *T1* genes encode GST enzymes, and are polymorphic in humans. These enzymes catalyze conjugation with glutathione, which is an important step in the detoxification of certain carcinogens. Several case-control studies have found associations of the homozygous null deletions in *GSTM1* and *GSTT1* with increasing the risk of colorectal and lung cancer. We prospectively examined the associations of the *GSTM1* and *GSTT1* polymorphisms with colorectal cancer risk in a nested case-control study (212 cases of colorectal cancer and 221 controls) within the Physicians' Health Study. Among controls, the prevalence of the *GSTM1* homozygous null genotype was 53% and for *GSTT1* homozygous null genotype, 23%. We found no increase in the risk of colorectal cancer for either *GSTM1* null [odds ratio (OR) = 1.0; 95% confidence interval (CI), 0.7–1.5] or *GSTT1* null (OR = 0.8; 95% CI, 0.5–1.2) genotypes. No differences were seen by site of colon cancer (proximal *versus* distal) or by age (≤60 years *versus* >60 years). Current cigarette smokers with *GSTM1* null genotype were not at an increased risk of colon cancer (OR = 1.2; 95% CI, 0.3–4.2) compared with current smokers without the null genotype; for the *GSTT1* null genotype this OR was 1.1 = 95% CI (0.3–4.7). This lack of association persisted when we examined pack-years of smoking and age at starting smoking. Our results do not support an association of *GSTM1* or *GSTT1* polymorphisms with colorectal cancer or an interaction with cigarette smoking.

Introduction

Carcinogens undergo bioactivation and detoxification via metabolic pathways involving Phase I (activation) and Phase II (conjugation) enzymes (1). Conjugation with glutathione is an important step in the detoxification of many electrophilic compounds, including polycyclic aromatic hydrocarbons, and this reaction is catalyzed by GST[3] (2). Human GSTs consist of four distinct families of genes (α, μ, π, and θ; Ref. 3). Both *GSTM1*, a member of the μ class gene family, and *GSTT1*, a member of the θ class gene family, are polymorphic in humans (4, 5). *GSTM1* is absent in 35–60% of individuals (6–9), and *GSTT1* is absent in 10–65% of the populations studied (6–8). The absence of *GSTM1* and *GSTT1* enzyme activity correlates with homozygosity for deletions in the respective genes (4, 5), and studies have shown higher levels of DNA damage in persons with the *GSTM1* and *T1* null genotypes. (10–12)

Differences in carcinogen metabolism may explain differences in cancer susceptibility, especially as most cancers are thought to be influenced by environmental factors (13). Dietary factors such as red meat intake, low folate intake, alcohol consumption, and early onset of cigarette smoking have been implicated as risk factors for colorectal cancer (14–16), and their effects may be modulated by between-person differences in their metabolism. Gene-environment interactions have been the focus of a number of recent studies of the occurrence of colorectal cancer and colon adenomas. For instance, in some studies the adverse effect of meat intake has been limited to subjects with polymorphisms in the *N-Acetyltransferase 2* gene, which makes them rapid acetylators (17–19).

The *GSTM1* polymorphism has been studied in relation to cancer of the lung (2, 7, 20), stomach (6, 21), colon (6–8, 21), and bladder (22–26), as well as inflammatory bowel disease (27). In a recent meta-analysis of 12 case-control studies of *GSTM1* and lung cancer, the risk for the *GSTM1* null deletion and lung cancer was significantly elevated (OR = 1.4; 95% CI, 1.23–1.61; Ref. 20) Of the studies examining the role of *GSTM1* null genotype and bladder cancer risk, the majority have been positive with relative risks of approximately 1.7 (22–26). It has been estimated that up to 25% of bladder cancers may be attributable to the *GSTM1* null genotype (24). Previous studies of the association of *GSTM1* polymorphisms and colorectal cancer have been conflicting. Recent case-control studies have found no overall association between *GSTM1* and colorectal cancer (6–8); however, an earlier study (28) did observe a positive association that was stronger for proximal cancers. The homozygous null *GSTT1* genotype was positively associated with colorectal cancer in three studies (7, 8, 21), but no association was found by Katoh *et al.* (6).

We assessed the association between the *GSTM1* and *GSTT1* polymorphisms and colorectal cancer in a large prospective study of American men, with particular emphasis on the potential interaction of genotype with smoking history.

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[3] The abbreviations used are: GST, glutathione *S*-transferase; CI, confidence interval; OR, odds ratio; BMI, body mass index.

Table 1 ORs and 95% CI for colorectal cancer risk by *GSTM1*, *GSTT1* and combined *GSTM1/T1* genotypes

	GSTM1[a]		*GSTT1*[b]		*GSTM1/T1*		
	Present	Null[c]	Present	Null[c]	Both present	One null	Both null
Cases	97	114	173	36	83	101	24
Controls	104	117	169	51	75	122	23
OR[d]	1.0 (ref)[e]	1.0 (0.7–1.5)	1.0 (ref)	0.7 (0.4–1.1)	1.0 (ref)	0.7 (0.5–1.2)	1.0 (0.5–1.9)
Adjusted OR[f]	1.0 (ref)	1.0 (0.7–1.5)	1.0 (ref)	0.8 (0.5–1.2)	1.0 (ref)	0.7 (0.5–1.1)	1.0 (0.5–2.0)

[a] Genotype was missing for one person.
[b] Genotype was missing for four people.
[c] Homozygous null genotype.
[d] Conditional logistic regression analysis.
[e] ref, reference category.
[f] Above model adjusted for BMI, physical activity, and alcohol use.

Materials and Methods

This study is a nested case-control study within the prospective Physicians' Health Study, a randomized, double-blind, placebo-controlled 2 × 2 factorial trial of low-dose aspirin and β-carotene. The original participation rates were as follows: (*a*) questionnaires were sent to 261,248 physicians; (*b*) questionnaires were returned from 112,528 participants; (*c*) of 33,223 participants in the run-in phase, 22,701 were randomized (29). The 22,701 healthy American male physicians at baseline in 1982 were ages 40–84 years; about 98% are Caucasian. Exclusion criteria included history of myocardial infarction, stroke or transient ischemic attack, cancers other than nonmelanoma skin cancer, peptic ulcer or gout, current renal or liver disease, and current use of vitamin A or β-carotene supplements. Information on smoking history, alcohol intake, diet and physical activity, as well as disease diagnoses was obtained through biennial-mailed questionnaires. Frequency of meat intake (defined as intake of beef, pork, or lamb as a main dish, sandwich, or mixed dish) was assessed by questionnaire (30).

Blood samples were collected from the men at baseline, and specimens were received for 14,916 (68%) of the randomized physicians. The nested case-control design has been described previously (31). Two hundred twelve cases of colorectal cancer were ascertained between 1982 and 1996 and were matched to 221 controls, based on year of birth and also smoking history (past, present, or never, at baseline) as the primary analyses of interest in the blood study related to levels of carotene and plasma nutrients, which are influenced by cigarette smoking. This number includes 10 cases that were included at a later stage, and each matched to two controls.

GSTM1 and *GSTT1* null genotypes were determined by PCR analysis, as described previously (4, 32), from genomic DNA isolated from blood. Positive and negative controls were included with each analysis. Genotype was not able to be determined for one control for *GSTM1* and four controls for *GSTT1*.

Conditional logistic regression analysis (33) was used to obtain age- and smoking-adjusted ORs and 95% CIs for the associations with colorectal cancer. We compared null genotypes for *GSTM1* and *GSTT1* genotypes with the remaining category, which includes individuals with one or two copies of the gene. Where numbers permitted, we controlled for BMI, physical activity, and alcohol intake by including indicator variables in the conditional and unconditional logistic models. To assess the effects of genotype for strata of smoking and colorectal cancer sub-site, we used unconditional logistic regression models, controlling for the matching factors age and smoking history, because stratifying conditional models resulted in splitting of pairs across strata and wide CIs. We compared conditional models with unconditional models that used only the complete-matched pairs in each strata and obtained essentially the same ORs. Addition of an interaction term between age and smoking in the unconditional models did not alter the results. Interaction with smoking was assessed by the log likelihood test, comparing the likelihood of the model without interaction terms with the model with interaction terms included (34). Duration of smoking and age at initiation of smoking were derived from the baseline and 1986 questionnaires. Information on pack-years of smoking was calculated using data on the average number of cigarettes smoked/day, from the 1987 questionnaire, as well as baseline data on number of cigarettes smoked at enrollment.

Results

The mean age of cases at baseline was 59.8 years (range, 41–83) and of controls was 59.8 years (range, 41–82), reflecting the matching. At baseline, 9% of controls were current cigarette smokers, 55% past smokers, and 36% never smokers. Among past or current smokers, the mean duration of smoking was 24.6 years (SD, 12.9 years). Prevalence of the *GSTM1* null genotype and *GSTT1* null genotype among controls was 53% and 23%, respectively. The prevalence of the combined *GSTM1* and *GSTT1* null genotype was 11%. There was no overall association of either *GSTM1* or *GSTT1* null genotypes with colon cancer; the multivariate OR for *GSTM1* was 1.0 (95% CI, 0.7–1.5) and for *GSTT1*, 0.8 (95% CI, 0.5–1.2). When compared with men who had no homozygous deletion in either *GSTM1* or *GSTT1*, men with homozygous deletions in both genes did not have an increased risk of colon cancer (OR = 1.0; 95% CI, 0.5–2.0; Table 1). Results are similar for younger (age <60 years) and older (age ≥60 years) men. The OR for the null *GSTM1* genotype was 0.9 (95% CI, 0.6–1.6) for men 60 years of age or older and 1.2 (95% CI, 0.7–2.0) for men under 60 years of age. The OR for the null *GSTT1* genotype was 0.9 (95% CI, 0.5–1.7) for men 60 years of age or older and 0.5 (95% CI, 0.2–1.0) for men under 60 years of age. No effect modification by age was seen for the combined *GSTM1/T1* null genotype and colorectal cancer risk.

We examined the associations of genotypes with the site of colon cancer, proximal *versus* distal (Table 2). Of the 212 colorectal cancers, 80 were proximal, 92 were distal, 29 were rectal, and 11 were of unknown site. The rectal cancers and cancers of unknown site were excluded from the stratified analyses by site due to their small numbers. Overall, there were no differences seen by site of colon cancer for either genotype (Table 2).

We examined the interaction of both *GSTM1* and *GSTT1*

Table 2 ORs and 95% CI for colon cancer risk by genotype and colon cancer site

	GSTM1[a]		*GSTT1*[b]		*GSTM1/T1*		
	Present	Null[c]	Present	Null[c]	Both present	One null	Both null
Proximal cancer							
Cases	43	36	63	17	37	31	11
Controls	104	117	169	51	75	122	23
OR[d]	1.0 (ref)[e]	0.7 (0.4–1.2)	1.0 (ref)	0.9 (0.5–1.9)	1.0 (ref)	0.5 (0.3–1.0)	0.9 (0.4–2.1)
Adjusted OR[f]	1.0 (ref)	0.7 (0.4–1.3)	1.0 (ref)	0.9 (0.5–1.7)	1.0 (ref)	0.5 (0.3–0.9)	1.0 (0.4–2.4)
Distal cancer							
Cases	38	54	76	13	30	52	7
Controls	104	117	169	51	75	122	23
OR[d]	1.0 (ref)	1.2 (0.8–2.0)	1.0 (ref)	0.5 (0.2–1.1)	1.0 (ref)	1.1 (0.6–1.8)	0.8 (0.3–2.0)
Adjusted OR[f]	1.0 (ref)	1.4 (0.8–2.3)	1.0 (ref)	0.6 (0.3–1.2)	1.0 (ref)	1.1 (0.6–2.0)	0.9 (0.4–2.5)

[a] Genotype was missing for one person.
[b] Genotype was missing for four people.
[c] Homozygous null.
[d] Unconditional logistic regression analysis, controlling for age and smoking status at baseline.
[e] ref, reference category.
[f] Above model adjusted for BMI, physical activity, and alcohol use.

Table 3 ORs and 95% CI for colorectal risk by *GSTT1* and *GSTM1* genotypes and smoking status at baseline[a]

		GSTM1[b]		*GSTT1*[c]	
		Present	Null[d]	Present	Null[d]
Never smokers	Cases	36	41	61	16
	Controls	40	40	60	19
	OR[e]	1.0 (ref)[f]	1.1 (0.6–2.1)	1.0 (ref)	0.8 (0.4–1.8)
Past smokers	Cases	52	63	98	15
	Controls	53	67	93	27
	OR	1.0 (ref)	1.0 (0.6–1.6)	1.0 (ref)	0.5 (0.3–1.1)
Current smokers	Cases	9	10	14	5
	Controls	11	10	16	5
	OR	1.0 (ref)	1.2 (0.3–4.2)	1.0 (ref)	1.1 (0.3–4.7)

[a] Test for smoking-genotype interactions: $P = 0.91$ for *GSTM1*; $P = 0.59$ for *GSTT1*.
[b] Genotype was missing for one person.
[c] Genotype was missing for three people.
[d] Homozygous null.
[e] Unconditional logistic regression analysis, controlling for age.
[f] ref, reference category.

Table 4 ORs and 95% CI for colorectal risk by *GSTT1* and *GSTM1* genotypes and pack-years of smoking at baseline[a]

		GSTM1[b]		*GSTT1*[c]	
		Present	Null[d]	Present	Null[d]
Non smokers	Cases	36	41	61	16
	Controls	40	40	60	19
	OR[e]	1.0 (ref)[f]	1.1 (0.6–2.1)	1.0 (ref)	0.8 (0.4–1.8)
≤30 pack-years[g]	Cases	23	18	32	8
	Controls	19	21	30	10
	OR	1.0 (ref)	0.7 (0.3–1.7)	1.0 (ref)	0.7 (0.2–2.2)
31–60 pack-years	Cases	15	23	31	6
	Controls	19	24	33	10
	OR	1.0 (ref)	1.2 (0.5–2.8)	1.0 (ref)	0.7 (0.2–2.1)
>60 pack-years	Cases	15	20	30	5
	Controls	20	17	32	5
	OR	1.0 (ref)	1.6 (0.6–4.0)	1.0 (ref)	1.0 (0.3–3.9)

[a] Test for smoking-genotype interactions: $P = 0.71$ for *GSTM1*; $P = 0.84$ for *GSTT1*.
[b] Genotype was missing for one person.
[c] Genotype was missing for three people.
[d] Homozygous null.
[e] Unconditional logistic regression analysis, controlling for age and smoking status at baseline.
[f] ref, reference category.
[g] Information on pack-years of smoking was missing for 41 people.

with cigarette smoking. Table 3 shows the stratification of genotype by smoking status at baseline. Current or past smokers who had the *GSTM1* null genotype were not at an increased risk of colon cancer (OR 1.2; 95% CI 0.3–4.2 and OR 1.0; 95% CI 0.6–1.6, respectively). Similarly, there was no increase in risk for current or past smokers with the *GSTT1* null genotype (Table 3). Information on pack-years of smoking was derived from information from the baseline and 1986 questionnaires on smoking history, age at start of smoking, and average intensity of smoking. There was no significant increase in risk with increasing pack-years for either *GSTM1* or *GSTT1* null genotypes (Table 4). The OR for men who smoked >60 pack-years and were *GSTM1* null compared with men who were not *GSTM1* null and never smoked was 1.6 (95% CI, 0.6–4.0). For the *GSTT1* null genotype, this OR was 1.0 (95% CI, 0.3–3.9). There was no significant trend in risk of colon cancer by number of cigarettes smoked/day or for age at starting smoking for either genotype (data not shown).

The OR for men who had the *GSTM1* homozygous null genotype and consumed more than one serving of red meat/day was 0.8 (95% CI, 0.4–2.0), compared with men who did not have the null genotype and consumed <0.5 servings of red meat/day; for the *GSTT1* null genotype, this OR was 0.4 (95% CI, 0.1–1.4).

Discussion

We observed no association of either *GSTM1* or *GSTT1* null polymorphisms with colorectal cancer. This lack of association persisted after adjustment for age, smoking, BMI, physical activity, and alcohol use. No association was seen with the combined *GSTM1/GSTT1* null genotype, either for all colorectal cancers or for proximal or distal cancers. In addition, we observed no effect modification by age or cigarette smoking for either class *M1* or *T1* null genotypes alone or in combination.

Several studies have examined the association between *GSTM1* and *GSTT1* and colon cancer risk, however, these results have been conflicting. Zhong *et al.* (28) found a significant excess of the *GSTM1* deletion among 196 cases of colorectal cancer (56% among cases *versus* 42% among 225 controls) and noted a stronger association for proximal colon

cancers. There was no association between the *GSTM1* genotype and colorectal cancer in several case-control studies (6–8), although Katoh *et al.* (6) observed an association limited to distal colorectal cancers. Deakin *et al.* (7) observed a positive association between the *GSTT1* null genotype and colorectal cancer (OR 1.9; 95% CI, 1.3–2.8), but no association with the combined *GSTT1*/*GSTM1* null genotype. A positive association of the *GSTT1* phenotype was observed in another small study for combined gastric and colon adenocarcinoma (21) and in a larger study of 132 cases in which the association for the *GSTT1* null genotype was present only among people older than 70 years (8). However, a recent study did not find an association among 103 colorectal cancers and 126 controls, although the authors did observe a significant positive association for gastric cancer, which was stronger among smokers (6).

GSTs play an important role in the detoxification of smoking-related carcinogens, in particular polycyclic aromatic hydrocarbons (35), and thus differences in GST activity are hypothesized to be important in the etiology of smoking-related malignancies. Cigarette smoking has not been consistently associated with colorectal cancer risk. Although a few studies have suggested an association between cigarette smoking and colorectal cancer (15, 36, 37), other studies have not supported these findings (38–41). An association between colon cancer and early age at starting smoking has been reported, suggesting that smoking may be relevant in the initiation of colon cancer (15). Studies have examined interactions with the *GSTM1* homozygous null genotype, smoking, and lung cancer (10), with a meta-analysis showing an elevated risk of 1.4 (95% CI, 1.23–1.61); however, in the only other study (6) that has looked at the interactions between *GSTM1* or *T1* polymorphisms and smoking in relation to colorectal cancer, no influence of the *GSTM1* or *GSTT1* null deletions on colorectal cancer risk was observed among smokers.

Our findings do not support an interaction of either *GSTM1* or *GSTT1* with cigarette smoking, in relation to colon cancer. Because there may be an early effect of smoking in the etiology of colon cancer, we also examined the effect of early age at starting smoking and found no increase in the risk of colon cancer for either *GSTM1* or *T1* null genotypes within each smoking category. In this nested case-control study, there was no main effect of current smoking due to the matching on smoking status at baseline in the design of the study; this also somewhat limited our ability to examine other smoking characteristics, such as age of starting smoking, as independent predictors of colorectal cancer risk. The matching does not compromise our ability to examine whether associations with genotype were modified by smoking characteristics.

Heterocyclic amines, such as 2-hydroxy-amino-3methyl-6-phenylimidazo[4,5-b] pyridine (PhiP) and 2-hydroxy-amino-3methyl-imidazo[4,5-f] quinolone (IQ), are produced in high temperature cooking of protein-rich foods and are potential substrates for GST, although isoenzyme specificity has not been identified (2). Red meat intake has been associated with colon cancer in several epidemiological studies (17, 18, 42, 43), and it has been hypothesized that the heterocyclic amines produced in the cooking of red meat may be the relevant harmful agents. Studies have observed associations between N-Acetyl transferase polymorphisms and colon cancer among people with high red meat intake, although the data are equivocal (17–19). We, therefore, assessed the association of *GSTM1* and *GSTT1* and colorectal cancer among men who consumed red meat frequently and found no evidence of an interaction when comparing men who ate >1.0 servings/day of red meat with those who ate <0.5 servings/day. However, it should be noted that we did not have information on methods used to cook the meat, which contribute substantially to heterocyclic amine production.

There are several limitations to our study. Although larger than some previous studies, our power for subgroup analyses was limited, as evidenced by the wide CIs in the stratified analyses. The generalizability of our study is limited to white males, as potential gene-environment interactions may be modified by other genes which differ in prevalence among ethnic groups. Although breaking the matching and controlling for matching factors in the unconditional analysis could potentially result in bias, careful comparison of unconditional and conditional models yielded very similar ORs. Strengths of the study include the prospective nature of the study (which reduces the likelihood of recall bias), the ability to control for other colon cancer risk factors, and the use of incident colorectal cancer cases, which reduces the possibility that these polymorphisms might influence survival rather than occurrence of the disease.

The prevalence of the null genotypes in our control group was similar to that seen in other studies, which ranged between 15–21% for *GSTT1* (7, 8, 23) and 49–55% for *GSTM1* (6, 7, 23, 28). Several earlier studies that observed positive associations for *GSTM1* and *GSTT1* and colorectal cancer had small sample sizes, and it is possible that these associations were chance findings. However, we cannot exclude the possibility that misclassification of smoking status attenuated our results, and this might explain the lack of interactions with long-term or early onset smoking.

Our results do not support the hypothesis that null deletions in *GSTM1* and *GSTT1* are independently associated with a substantial increase in risk of colorectal cancer; however, the metabolism of carcinogens is complex, and interactions with other genes and other exposures may also be important.

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