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

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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 GSTs (2). Human GSTs consist of four distinct families of genes (α, μ, θ, and ω; 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 nearly all 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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2 To whom requests for reprints should be addressed, at Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115.

3 The abbreviations used are: GST, glutathione S-transferase; CI, confidence interval; OR, odds ratio; BMI, body mass index.
Polymorphisms and Colorectal Cancer

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.

Table I

<table>
<thead>
<tr>
<th></th>
<th>GSTM1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GSTT1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Both present</th>
<th>One null</th>
<th>Both null</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td>Present Null&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Present Null&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Both present</td>
<td>One null</td>
<td>Both null</td>
</tr>
<tr>
<td>Controls</td>
<td>104</td>
<td>117</td>
<td>169</td>
<td>51</td>
<td>75</td>
</tr>
<tr>
<td>OR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.0 (ref)</td>
<td>1.0 (0.7–1.5)</td>
<td>1.0 (ref)</td>
<td>0.7 (0.4–1.1)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Adjusted OR&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.0 (ref)</td>
<td>1.0 (0.7–1.5)</td>
<td>1.0 (ref)</td>
<td>0.8 (0.5–1.2)</td>
<td>1.0 (ref)</td>
</tr>
</tbody>
</table>

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

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
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 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 colorectal 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 cancer. 

### Table 2

<table>
<thead>
<tr>
<th>GSTM1</th>
<th>GSTT1</th>
<th>Both present</th>
<th>One null</th>
<th>Both null</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Null</td>
<td>Present</td>
<td>Null</td>
<td>Both present</td>
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<tr>
<td>Cases</td>
<td>43</td>
<td>36</td>
<td>63</td>
<td>17</td>
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<tr>
<td>Controls</td>
<td>104</td>
<td>117</td>
<td>169</td>
<td>51</td>
</tr>
<tr>
<td>OR</td>
<td>1.0 (ref)</td>
<td>0.7 (0.4–1.2)</td>
<td>1.0 (ref)</td>
<td>0.9 (0.5–1.9)</td>
</tr>
<tr>
<td>Adjusted OR</td>
<td>1.0 (ref)</td>
<td>0.7 (0.4–1.3)</td>
<td>1.0 (ref)</td>
<td>0.9 (0.5–1.7)</td>
</tr>
</tbody>
</table>

### Table 3

<table>
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<tr>
<th>GSTM1</th>
<th>GSTT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Null</td>
</tr>
<tr>
<td>Never smokers</td>
<td>Cases</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
</tr>
<tr>
<td>OR</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Past smokers</td>
<td>Cases</td>
</tr>
<tr>
<td>Controls</td>
<td>53</td>
</tr>
<tr>
<td>OR</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>Cases</td>
</tr>
<tr>
<td>Controls</td>
<td>11</td>
</tr>
<tr>
<td>OR</td>
<td>1.0 (ref)</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>GSTM1</th>
<th>GSTT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Null</td>
</tr>
<tr>
<td>Non smokers</td>
<td>Cases</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
</tr>
<tr>
<td>OR</td>
<td>1.0 (ref)</td>
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<tr>
<td>≤30 pack-years</td>
<td>Cases</td>
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<tr>
<td>Controls</td>
<td>19</td>
</tr>
<tr>
<td>OR</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>31–60 pack-years</td>
<td>Cases</td>
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<tr>
<td>Controls</td>
<td>19</td>
</tr>
<tr>
<td>OR</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>&gt;60 pack-years</td>
<td>Cases</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
</tr>
<tr>
<td>OR</td>
<td>1.0 (ref)</td>
</tr>
</tbody>
</table>

- *Genotype was missing for one person.
- †Genotype was missing for three people.
- ‡Homozygous null.
- §Unconditional logistic regression analysis, controlling for age and smoking status at baseline.
- ‡Ref. reference category.
- ‣Above model adjusted for BMI, physical activity, and alcohol use.
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 GSTTI null genotype and colorectal cancer (OR 1.9; 95% CI, 1.3–2.8), but no association with the combined GSTTI/GSTM1 null genotype. A positive association of the GSTTI 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 GSTTI 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 GSTTI null deletions on colorectal cancer risk was observed among smokers.

Our findings do not support an interaction of either GSTM1 or GSTTI 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 amine, such as 2-hydroxy-azo-3methyl-6-phenylindazol(4,5-b) pyridine (PhIP) and 2-hydroxy-azo-3methyl-imidazo(4,5-f) quinoline (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 colorectal 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 GSTTI 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 GSTTI (7, 8, 23) and 49–55% for GSTM1 (6, 7, 23, 28). Several earlier studies that observed positive associations for GSTM1 and GSTTI 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 GSTTI 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.

**Acknowledgments**

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**References**


ferases Ml and Tl, microsomal epoxide hydrolase, and cytochrome P450 enzymes.


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