The *Glutathione S-Transferase-μ* and *-θ* Genotypes in the Etiology of Prostate Cancer: Genotype-Environment Interactions with Smoking[1]

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Abstract

It has been reported that individuals who express *GSTT1*, the gene coding for the *θ* class of the glutathione *S*-transferases (GSTs), have an elevated risk of prostate cancer (CaP). This result is supported by studies that show glutathione conjugation of some xenobiotics by the GSTs can produce mutagenic intermediates. However, the potential role of environmental factors in modifying the risk of CaP conferred by *GSTT1* is not known. We investigated whether there was an interaction between smoking and the non-deleted genotypes of the *μ* (*GSTM1*) and *θ* (*GSTT1*) *GST* genes using a clinic-based study of 276 CaP cases and 499 controls. We observed no main effect of smoking (odds ratio, 0.95; confidence interval, 0.69–1.29) or *GSTM1* (odds ratio, 1.00; confidence interval, 0.73–1.36) with CaP, but did observe a statistically significant main effect of *GSTT1* with CaP (odds ratio, 1.61; confidence interval, 1.14–2.28) as reported previously. No interaction between smoking and *GSTM1* was observed. A significant increase in the probability of having CaP was observed in men who were both smokers and carried a non-deleted *GSTT1* genotype compared with men who had neither or only one of these risk factors ($P = 0.049$). Approximately 30.9% of CaP cases in this study could be attributed to the smoking×*GSTT1* interaction. Whereas the mechanism of this interaction is not known, it is plausible that the metabolism of carcinogenic intermediates or the response to chronic inflammation associated with smoking may be modulated by the *GSTT1* genotype and may modify CaP risk.

Introduction

The *μ* and *θ* classes of the *GST*s[3] have been implicated in the etiology of cancer at several sites (1–5). To date, most studies have focused on homozygous deletions in these genes as risk factors for chemical carcinogenesis; homozygous deletions exist at *GSTM1* in ~40–60% of the Caucasian population in the United States and at *GSTT1* in ~20–30% of the Caucasian population in the United States (1). Homozygous deletions at *GSTM1* have been associated with cancers of the lung (6), colorectum (7), and stomach (8). Less is known about cancer risk attributable to homozygous deletions at the *GSTT1* locus, although increased breast and larynx cancer risk have been associated with *GSTT1* deletion (9, 10). A recent case-control study showed that individuals carrying the non-deleted *GSTT1* allele, either as heterozygotes or homozygotes, were at increased risk of developing prostate cancer (OR, 1.83; 95% CI, 1.19–2.80; Ref. 2). That sample contained many of the same individuals who are studied in the present paper. This finding was consistent with the hypothesis that GST-*θ* catalyzes the bioactivation of certain xenobiotics to genotoxic metabolites (11–14) such as dichloromethane and other halogenated alkanes.

Results from previous studies evaluating smoking as a risk factor for prostate cancer have been equivocal (15, 16). However, few studies have evaluated interactions between exposures (*e.g.*, smoking or occupational exposures) and genes encoding carcinogen metabolism enzymes in prostate cancer etiology. We hypothesized that interactions between smoking and the non-deleted *GSTM1* or *GSTT1* genotype may be associated with increased risk of prostate cancer. We investigated this hypothesis using a case-control study of 276 men (ages 41–80 years) that developed prostate cancer between 1994 and 1998, and 499 controls identified during the same time period from clinics of HUP.

Materials and Methods

Study Subjects. A sample of incident cases was identified through Urological Oncology Clinics at HUP between 1994 and 1998. All study subjects provided informed consent for participation in this research under a protocol approved by the Committee for Studies Involving Human Subjects at the University of Pennsylvania. Men were excluded from the study if they reported having exposure to finasteride (Proscar), were diagnosed with prostate cancer more than 12 months before joining the study, or had a prior diagnosis of cancer at any site.

The controls studied here were men attending HUP general medicine clinics. These clinics see a patient population that is demographically similar to those seen in the Urological Oncology Clinics at HUP. These men were ascertained con-

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[3] The abbreviations used are: GST, glutathione *S*-transferase; HUP, Hospital of the University of Pennsylvania; CaP, prostate cancer; AR, attributable risk.

Table 1 Descriptive statistics of the case-control study population

	Cases	Controls	Total	Comparison[a]
	$n = 276$	$n = 499$	775	
Age				
Mean	61.2	60.2		$T = -1.67$; $P = 0.095$
SD	7.04	9.32		
Range	41–80	41–80		
Race				
Caucasian	240	411	651 (84.0%)	Fisher's exact test, $P = 0.211$
African-American	29	74	103 (13.3%)	
Other	14	7	21 (2.7%)	
Smoking				
No. (%)	159 (57.6)	291 (58.3)	450 (58.1)	$\chi^2 = 0.04$; df = 1, $P = 0.848$
Genotype				
GSTM1-0 (%)	120 (46.7)	195 (46.7)	315 (46.7)	$\chi^2 = 0.00$; df = 1, $P = 0.992$
GSTM1-1 (%)	137 (53.3)	223 (53.4)	360 (53.3)	
GSTT1-0 (%)	60 (23.4)	155 (33.1)	215 (29.7)	$\chi^2 = 7.17$; df = 1, $P = 0.007$
GSTT1-1 (%)	196 (76.6)	314 (70.0)	510 (70.3)	

[a] df, degrees of freedom.

currently with the CaP cases (*i.e.,* between September 1994 and April 1998). Controls were excluded from this study if they ever had an abnormal prostate-specific antigen test (*i.e.,* ≥4 ng/dl), if they had ever had an abnormal digital rectal examination, if they had a previous cancer diagnosis, or if they reported having had exposure to finasteride (Proscar) at the time of study ascertainment. Prostate-specific antigen values were available from medical records and/or self-report from all controls. Information was not available about non-cancer diagnoses or exposures to medications used to treat these conditions. Controls were frequency-matched to cases on the basis of age and race. However, because the frequency matching was approximate, analyses adjusted for age and race were also undertaken to account for residual variation attributable to these factors. The mean age in the cases was 61.2 years *versus* 60.2 years in the controls. The vast majority of cases and controls were Caucasian (84.0%), 13.3% were African-American, and about 3% of the study populations were of Asian or other racial category.

Data Collection. Medical history and prostate cancer diagnostic information were obtained by using a standardized questionnaire and a review of medical records. With regard to smoking history, subjects were asked if they had ever smoked cigarettes for a period of 1 year or longer. Those who replied in the affirmative were considered smokers in the present analyses.

Samples of genomic DNA were obtained by self-collection by each study subject using sterile cheek swabs. The samples were processed and analyzed using the protocol described previously by Rebbeck *et al.* (2). A small number of genotypes were not determined for either *GSTM1* or *GSTT1* because of genotype assay failures. The following genotype categories were used in this study: (*a*) homozygous deletion genotypes at *GSTM1*, denoted "*GSTM1–0*," were compared with genotypes for which at least one non-deleted allele was present, denoted "*GSTM1–1*"; (*b*) the same definition was used to distinguish between the deleted and non-deleted *GSTT1* genotypes ("*GSTT1–0* " and "*GSTT1–1*," respectively).

Analytical Methods. Genotype-disease associations, smoking-disease associations, and genotype-smoking interactions were analyzed using logistic regression models. A likelihood ratio test was used to evaluate whether genotype-smoking interactions significantly improved the prediction of CaP risk by comparing a reduced model, which included the main effects of smoking and genotype as predictors, with a complete model, which included those terms plus an interaction term. The likelihood ratio test statistic is approximately distributed as a χ^2 with degrees of freedom equal to the difference in the number of model parameters between the reduced and complete models. We also investigated whether the interaction between genotype and smoking was age-dependent using the Cochran-Armitage test for trend (17) and the Breslow-Day test of homogeneity (18).

To estimate the potential public health implications of an interaction, we calculated the *AR* of the interaction using the following equation (19):

$$AF_{ge} = [P_{ge} \times (R_{ge} - R_e - R_g + 1)]/R_{ge}$$

where AF_{ge} is the attributable fraction of cases because of the interaction, P_{ge} is the proportion of cases with both the risk-conferring genotype and exposure to the environment of interest (*i.e.,* smoking), R_{ge} is the relative risk of CaP comparing those who were exposed to both smoking and genotype compared with those who were never smokers and who did not have the risk-conferring genotype, R_e is the relative risk attributable to the exposure (*i.e.,* smoking) compared with those who were never smokers, and R_g is the relative risk attributable to the risk-conferring genotype compared with those who did not carry this genotype.

Results

Descriptive. Table 1 presents a description of the sample population. Because of incomplete genotype data on some study subjects, the total number of subjects with *GSTM1* genotype data were 675 (257 cases, 418 controls), and the total number of subjects with *GSTT1* genotype data were 725 (256 cases, 469 controls). Case and controls ranged in age from 41–80 years. There were no significant differences in the age and smoking distribution between the groups with only *GSTM1* genotype data and the total sample set. Approximately 53% of both cases and controls were *GSTM1–1*. *GSTT1–1* made up 76.6% of cases, whereas 70.0% of controls were *GSTT1–1*.

Among 257 cases and 418 controls for whom *GSTM1* genotype data were available, 146 (56.8%) and 244 (58.4%) smoked for 1 year or more, respectively. One hundred and forty-seven (57.4%) of the 256 cases with *GSTT1* genotype

Table 2 Logistic modeling results

Full sample	OR	95% CI	Wald test *P*	Model χ^2	*P*
A. Logistic modeling results with *GSTM1*					
Reduced model (2 df[a])				0.16	0.923
Smoking	0.94	0.69–1.29	0.690		
GSTM1	1.00	0.73–1.37	0.997		
Complete model (3 df)				1.92	0.590
Smoking	1.18	0.74–1.86	0.490		
GSTM1	1.28	0.79–2.06	0.315		
Smoking × *GSTM1*	0.65	0.35–1.23	0.185		
Likelihood ratio test comparing complete and reduced models				1.76	0.189
B. Logistic modeling results with *GSTT1*					
Reduced model (2 df)				7.53	0.023
Smoking	0.98	0.72–1.33	0.878		
GSTT1	1.61	1.14–2.28	0.007		
Complete model (3 df)				11.39	0.010
Smoking	0.58	0.31–1.06	0.076		
GSTT1	1.06	0.62–1.81	0.833		
Smoking × *GSTT1*	2.03	1.00–4.12	0.049		
Likelihood ratio test comparing complete and reduced models				3.86	0.049

[a] df, degrees of freedom.

smoked for 1 year or longer, as compared with 275 of the 469 controls (58.6%).

Analysis of Smoking and Genotype. The results of the analyses evaluating the effect of smoking and genotype are presented in Table 2, *A* and *B*. Ever having smoked for 1 year or more was not associated with the probability of having CaP (OR, 0.95; 95% CI, 0.69–1.29), nor was the *GSTM1* genotype (OR, 1.00; 95% CI, 0.73–1.36). Overall, the model containing no interaction was not statistically significant ($P = 0.923$). Mutually adjusting for smoking and for *GSTM1* genotype did not substantially alter the observed ORs associated with smoking or with the *GSTM1* genotype (Table 2*A*). A model containing a smoking×*GSTM1* interaction was also not statistically significant ($P = 0.590$). Using nonsmokers with the *GSTM1–0* genotype as the reference category, the ORs for CaP were: (*a*) OR, 0.94 (95% CI, 0.69–1.29) in *GSTM1–0* smokers; (*b*) OR, 1.00 (95% CI, 0.73–1.37) in *GSTM1–1* nonsmokers; and (*c*) OR, 0.94 (95% CI, 0.49–1.61) in smokers with *GSTM1–1*.

The *GSTT1* genotype was a significant predictor of CaP with an OR of 1.61 (95% CI, 1.14–2.28; Wald $P = 0.007$). More importantly, the addition of the smoking×*GSTT1* interaction term to form the complete model was also statistically significant (Wald $P = 0.049$), as was the likelihood ratio test comparing the reduced and complete models ($\Delta\chi^2 = 3.86$; $P < 0.05$). Using nonsmokers with the *GSTT1–0* genotype as the reference category, the ORs for CaP were OR, 0.98 (95% CI, 0.72–1.33) in *GSTT1–0* smokers; OR, 1.61 (95% CI, 1.14–2.28) in *GSTT1–1* nonsmokers; and OR, 1.57 (95% CI, 1.09–2.22) in smokers with *GSTT1–1*.

Because race is known to be strongly associated with CaP risk, we undertook a subset analysis of Caucasians to evaluate our initial results in a more racially homogeneous population ($n = 607$; 222 cases; 385 controls). The same logistic regression models were built, and results were obtained that were very similar to those described above. In fact, the magnitude of the smoking×*GSTT1* interaction term was greater (OR, 2.21; 95% CI, 1.03–4.76) and slightly more significant (Wald $P = 0.043$) in the Caucasian-only group (results not shown).

To further examine the interaction between *GSTT1* and smoking across age groups, we stratified our sample by age decades and evaluated the differences in relative frequency of the *GSTT1–1* genotype among smokers and nonsmokers in cases and controls. ORs and 95% CIs were also calculated and are presented concurrently with the genotype frequencies in Figs. 1 and 2. In nonsmokers (Fig. 1), the relative frequency of *GSTT1–1* genotype among cases and controls was fairly similar across the age groups. An exception was in the first age group in which 100% of the cases had the *GSTT1–1* genotype, although the sample size for cases in this age group was small ($n = 11$). In comparison, among smokers, the relative frequency of the *GSTT1–1* genotype among cases was consistently greater than among the controls across the four age groups of smokers, indicating the increased likelihood of cases who smoked to have the *GSTT1–1* genotype. These same patterns of *GSTT1–1* genotype frequency and ORs were observed when the sample was limited to Caucasians only.

Because there appeared to be a slight increase in *GSTT1–1* genotype frequency across age groups in cases who smoked, we used the Cochran-Armitage test for trend to evaluate the pattern of *GSTT1–1* genotype frequencies. In smokers, the increase in *GSTT1–1* genotype frequency in controls across the four age groups was significant ($P = 0.040$), but the increase in *GSTT1–1* genotype frequency in cases was not ($P = 0.820$). The test of the trend of *GSTT1–1* genotype frequency in nonsmokers was not significant for cases or controls ($P = 0.200$ and $P = 0.987$, respectively). Tests of homogeneity (Breslow and Day; Ref. 18) of the ORs across the age groups in smokers and nonsmokers were not statistically significant ($P = 0.306$ for smokers; $P = 0.225$ for nonsmokers). We conclude that there was no effect of age on the interaction.

Using the *AR* equation in the "Materials and Methods" section (Khoury *et al.*, Ref. 19) to estimate the *AR* of disease because of the smoking×*GSTT1* interaction, we estimate P_{ge} to be 44.92%, R_{ge} to be 2.03, R_e to be 0.58, and R_g to be 1.06 (taken from Table 2*B*). Substituting these estimates into the AR equation yields a value of 30.9%.

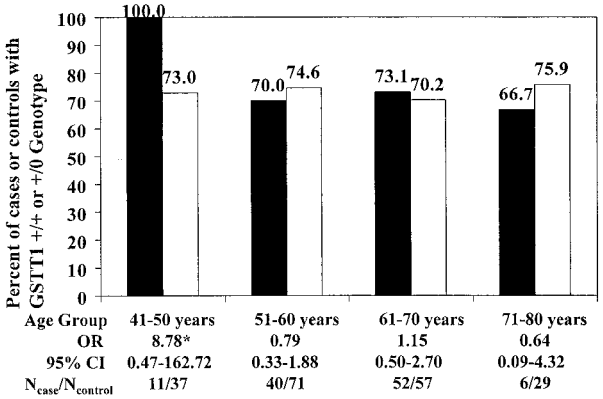

Fig. 1. Percent of nonsmoking cases or controls with at least one non-deleted *GSTT1* allele by age decade. *, 0.5 was substituted into one cell of the two by two table where $n_{ij} = 0$. ■, cases; □, controls.

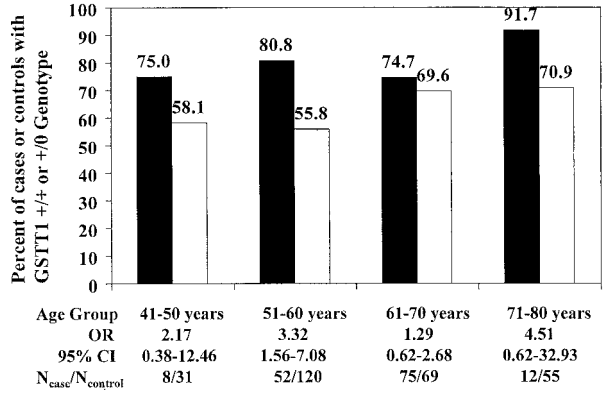

Fig. 2. Percent of smoking cases or controls with at least one non-deleted *GSTT1* allele by age decade. ■, cases; □, controls.

Discussion

We report a significant interaction between *GSTT1* and smoking that elevates the risk of CaP among carriers of the non-deleted *GSTT1* genotype. No interaction exists between *GSTM1* and smoking. Additionally, the *GSTT1*×smoking interaction could explain ~27.2% of the CaP cases in our study. Age did not have an appreciable effect on the interaction between smoking and *GSTT1*, although the sample size available for the test of trend in some age groups was small.

In several studies, genetic risk of environmentally induced disease has been observed to be higher in individuals who lack genes coding for carcinogen metabolism enzymes or express less active forms of these enzymes (20–22). This has been observed with the GSTs (3–8, 10). For example, persons lacking *GSTM1* are at higher risk of developing lung cancer (5). In our case, we have observed the opposite; that is, individuals possessing the non-deleted *GSTT1* genotype (*GSTT1–1*) were found to be at higher risk of prostate cancer, and this risk is heightened by an interaction with smoking.

The mechanisms by which such an interaction between *GSTT1* and smoking elevate the risk of prostate cancer are unknown at this point. However, two plausible mechanisms can be proposed to explain the observed relationship. First, some metabolic intermediates in the GST-θ pathway of glutathione conjugation are mutagenic (11–14). At least three studies have shown that GST-θ-dependent glutathione conjugation of dichloromethane yields formaldehyde, which is capable of producing DNA-protein crosslinks or formaldehyde-RNA adducts in the human and mouse liver (12–14). Byproducts of tobacco smoke include methyl chloride (23), which is a substrate of GST-θ detected at appreciable levels in cigarette smoke. Methyl chloride undergoes a biotransformation similar to that of dichloromethane (24). Conjugation of methyl chloride by GST-θ produces S-methylglutathione and subsequently S-methylcysteine after cleavage by transpeptidases. Methylthiopyruvic acid is then formed by a transamination reaction, and then is decarboxylated to methylthioacetic acid, with methanethiol being the next metabolite. Experimental evidence from Garnier *et al.* (25) and Chellman *et al.* (26) suggests that this may be a toxifying rather than a detoxifying pathway, because the oxidation of methanethiol produces formaldehyde and hydrogen sulfide (24), both of which are toxic metabolites (27). Formaldehyde can form DNA-protein crosslinks or formaldehyde-RNA adducts, as seen during the metabolism of dichloromethane, and hydrogen sulfide is an inhibitor of cytochrome oxidase (28). Given that GST-θ is highly expressed in the prostate (29) and that systemic circulation of methyl chloride could perfuse the prostate, the production of mutagenic intermediates in the prostate itself may be a mechanism promoting tumorigenesis. The pharmacokinetic issues of whether or not methyl chloride reaches the prostate and in what concentration are important, unstudied issues that would need to be addressed to substantiate this etiological model.

Alternatively, it is plausible that smoking directly or indirectly induces inflammation of the prostate. Chronic inflammation has been linked to carcinogenesis in several organs (30–32). Systemic depletion of antioxidants by chronic exposure to tobacco combustion products as well as lung tissue injury by constituents of tobacco smoke can result in inflammation and lead to a state of oxidative stress (33–36). The subsequent release of inflammatory cytokines can then promote inflammation. Endogenous substrates of GST-θ have not yet been identified, but it is known that the GSTs, in general, participate in leukotriene synthesis and are mediators of the inflammatory response. The conversion of Leukotriene A4 to Leukotriene C4 is catalyzed by the GSTs (37). Because GST-θ is highly expressed in the prostate, it is possible that upon receiving the signal of inflammatory cytokines, a more vigorous or more chronic inflammation response is mounted in the prostate gland of individuals with the *GSTT1–1* genotype *versus* the *GSTT1–0* genotype individual. Inflammatory cells release reactive oxygen and nitrogen species, which can bind and alter DNA (38–39). The prostate may already be vulnerable because of a preexisting condition of oxidative stress as well. There is evidence which suggests that inflammation of the prostate may be linked to the development of neoplasia (40–43), lending credence to this model. Additionally, inflammatory cells such as neutrophils have been shown to activate procarcinogens, *e.g.*, constituents of tobacco smoke, aromatic amines, and polycyclic aromatic hydrocarbons, to DNA-damaging species by oxidant-dependent mechanisms, providing another mechanism by which inflammation of the prostate could promote carcinogenesis (44). Finally, other lines of evidence from different types of studies also suggest that oxidative stress may be etiologically important and may be responsible for inducing carcinogenesis or disease progression in the prostate. One study has shown that dietary antioxidants inhibit prostate tumor cell proliferation in mice (45), and similar studies have led to a large-scale clinical trial that is testing the hypothesis that dietary antioxidants can prevent CaP disease progression (46).

There are a number of limitations of our study. One limitation is the way in which smoking status was assessed. Study participants were asked in a yes/no format whether they had ever smoked cigarettes for a period of 1 year or more. This question does not discern between individuals who may have vastly different smoking histories in terms of type of cigarette smoked (*i.e.*, filtered *versus* nonfiltered cigarettes), amount smoked, or type of exposure (*e.g.*, cigarette *versus* pipe and cigar smoking). Hence, we were unable to stratify our analyses by smoking exposure type or level. Had such stratification been feasible, it would have been possible to assess a potential dose-response relationship, perhaps revealing greater magnitudes of interaction between smoking and *GSTT1*. Additionally, diet and occupational exposures are confounders that may have influenced our results. Both have been implicated in CaP development by several studies (47–50) and were not simultaneously addressed in this study. Finally, we detected an effect of age on genotype frequencies at *GSTT1* in smokers but not in nonsmokers. The observation of a trend toward decreasing *GSTT1–0* frequency with age in smokers could reflect the fact that smokers with this genotype were selectively removed from the population because of other illnesses. Therefore, the observation that prostate cancer may be associated with the presence of *GSTT1* genotype could be attributable to the fact that *GSTT1–0* controls were in deficit in the population because of the effects of this genotype on the risk of other smoking-related diseases. However, our sample of smokers in each age group was relatively small, and may have not been adequate to evaluate trends of genotype by age. Therefore, any potential inferences about age-specific effects by genotype are limited in the present sample.

Additional research is needed to confirm these findings, preferably using more detailed assessments of smoking exposures. Future directions for this area of research should also aim to further assess interactions between *GSTT1* and other environmental exposures. Occupational exposure to other substrates of GST-θ might also prove to be important in prostate cancer etiology. The mechanism(s) of these interactions also needs to be addressed to help support or diminish the strength of the hypotheses raised by the present results. Biomarkers of exposure could be used in molecular epidemiological studies to help elucidate the mechanism. The use of a prostate cell line or an animal model of prostate cancer in which human *GSTT1* is expressed might also provide pathophysiological data to help differentiate between the potential inflammation-induced and bioactivation pathways of prostatic carcinogenesis.

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