Polymorphisms in Polycyclic Aromatic Hydrocarbon Metabolism and Conjugation Genes, Interactions with Smoking and Prostate Cancer Risk

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Abstract

The relationship between cigarette smoking and prostate cancer remains unclear. Any potential association may depend on the individuals' ability to metabolize and detoxify cigarette carcinogens—such as polycyclic aromatic hydrocarbons. To investigate this, we studied the association between prostate cancer and smoking, as well as the main and modifying effects of functional polymorphisms in genes that metabolize polycyclic aromatic hydrocarbons (*CYP1A1 Ile^{462}Val, microsomal epoxide hydrolase His^{139}Arg*) and detoxify reactive derivatives (*GSTM1 null deletion, GSTT1 null deletion, GSTP1 Ile^{105}Val and Ala^{114}Val*) using a family-based case-control design (439 prostate cancer cases and 479 brother controls). Within the entire study population, there were no main effects for smoking or any of the polymorphisms. However, the nondeleted *GSTM1* allele was inversely associated with prostate cancer [odds ratio (OR), 0.50; 95% confidence interval (95% CI), 0.26-0.94] among men with less aggressive disease (Gleason score < 7 and clinical tumor stage < T2c) and positively associated (OR, 1.68; 95% CI, 1.01-2.79) with prostate cancer in men with more aggressive disease (Gleason score ≥ 7 or clinical tumor stage ≥ T2c). We also found a statistically significant negative multiplicative interaction between the *GSTM1 nondeleted* allele and heavy smoking (> 20 pack-years) in the total study population (P = 0.01) and in Caucasians (P = 0.01). Among Caucasians, heavy smoking increased prostate cancer risk nearly 2-fold in those with the *GSTM1 null* genotype (OR, 1.73; 95% CI, 0.99-3.05) but this increased risk was not observed in heavy smokers who carried the *GSTM1 nondeleted* allele (OR, 0.95; 95% CI, 0.53-1.71). Our results highlight the importance of considering genetic modifiers of carcinogens when evaluating smoking in prostate cancer. (Cancer Epidemiol Biomarkers Prev 2006;15(4):756–61)

Introduction

Prostate cancer is the most commonly diagnosed nonskin cancer and the second leading cause of cancer death among men in the United States (1). Although the etiology of this disease remains largely unknown, it likely involves both environmental and genetic components (2). Cigarette smoking has been associated with prostate cancer in some (3, 4), but not all, studies (5). However, constituents of cigarette smoke, such as polycyclic aromatic hydrocarbons (PAH; ref. 6), require metabolic activation, evasion of detoxification processes, and subsequent binding to DNA to exert their carcinogenic action (7). Therefore, functional polymorphisms in genes involved in PAH metabolism and detoxification may modify the effect of smoking on prostate cancer.

To illustrate this, consider benzo(*a*)pyrene, a carcinogenic and abundant PAH in cigarette smoke (8). Benzo(*a*)pyrene may be initially metabolized to an epoxide [benzo(*a*)pyrene-7,8-epoxide] and subsequently metabolized from a dihydrodiol [benzo(*a*)pyrene-7,8-dihydrodiol] to a highly reactive diol epoxide [benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide] by CYP1A1 or CYP1B1 (9, 10). The *CYP1A1 Ile^{462}Val* and the *CYP1B1 Leu^{432}Val* polymorphisms have been implicated, albeit equivocally, in prostate cancer (11-14). We previously reported a weak association with the *CYP1B1 ^{432}Leu/Val* genotype compared with the *Leu/Leu* genotype among men with less aggressive disease [odds ratio (OR), 0.54; 95% confidence interval (95% CI), 0.28-1.05; P = 0.07; ref. 15]. Although both the *CYP1A1 ^{462}Val* (16) and *CYP1B1 ^{432}Val* (10) variant alleles result in higher enzymatic activity compared with their respective wild-type alleles, CYP1B1 seems to be highly expressed in the prostate (17, 18), especially in the peripheral zone (19) where most cancers arise (20), whereas CYP1A1 may only be induced by PAHs under androgen dependency (21).

Microsomal epoxide hydrolase (mEH) is required to hydrolyze the epoxide intermediate [benzo(*a*)pyrene-7,8-epoxide] to a dihydrodiol [benzo(*a*)pyrene-7,8-dihydrodiol]. *mEH* has two known functional polymorphisms, *Tyr^{113}His* and *His^{139}Arg*, with variant alleles that result in reduced and enhanced enzymatic activity, respectively (22, 23). Although there is evidence for mEH expression in the prostate (24, 25) and these mEH polymorphism have been implicated in other cancers (26, 27), reports in prostate cancer are lacking.

Before the electrophilic diol epoxide [benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide] can bind to DNA, creating a DNA adduct that may result in irreversible mutation, it may be detoxified by enzymes in the glutathione *S*-transferase (GST) family. Of the seven mammalian cystolic GST classes characterized (28), those that have shown substrate specificity for PAH metabolites and that are expressed in the human prostate include the μ (GSTM) and the π (GSTP) classes (18, 29-31). Although certain θ class (GSTT) isozymes are expressed in the

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prostate (32), their capacity to conjugate PAH derivatives has not been well studied. *GSTP1* has two polymorphisms, *Ile*^{105}*Val* and *Ala*^{114}*Val*, but *Ile*^{105}*Val* seems to have more influence on enzymatic activity because it is located near the hydrophobic binding site (33). Variants in *GSTM1* and *GSTT1* leading to complete loss of protein (*null deletion*) and the *GSTP1* ^{105}*Val* allele have been associated with increased prostate cancer risk in some studies (34-36) but decreased risk in others (37-39).

We hypothesize that the effect of smoking on prostate cancer risk is modified by functional variants in PAH metabolism and conjugation genes. To evaluate our hypothesis, we evaluated the association between prostate cancer and smoking, as well as the main and modifying effects of functional polymorphisms in genes that metabolize PAHs (*CYP1A1 Ile*^{462}*Val*, *mEH His*^{139}*Arg*) and detoxify reactive derivatives (*GSTM1 null deletion*, *GSTT1 null deletion*, *GSTP1 Ile*^{105}*Val* and *Ala*^{114}*Val*) using a family-based case-control study.

Materials and Methods

Study Population. The study design and population have been described elsewhere (40). Briefly, men with prostate cancer (n = 439) and their unaffected brothers (n = 479) were recruited from the major medical institutions in Cleveland, Ohio, and from the Henry Ford Health System in Detroit, Michigan. Of the 413 families participating in the study, ~90% were Caucasian, 9% African-American, and 1% were Asian or Latino. Institutional review board approval was obtained from all participating institutions. All study subjects provided informed consent.

The disease status of cases was confirmed by histology and their clinical characteristics were obtained from medical records. Prostate-specific antigen testing was conducted in unaffected sibling(s) and any of these men with a prostate-specific antigen >4 ng/mL were notified by one of the collaborating urologists and followed to confirm their disease-free status. All unaffected brothers were ≤8 years younger than their affected brother(s) and the median time between case diagnosis and recruitment into the study was 2 years.

Demographic information and smoking status were determined from a self-administered health and habits questionnaire. Subjects who reported smoking cigarettes regularly for a total of ≥6 months were considered smokers. We classified light and heavy smokers as subjects who smoked 1 to 20 pack-years and >20 pack-years, respectively.

Genotyping. Standard venipuncture was used to collect blood samples from all study participants in tubes with EDTA as an anticoagulant. Genomic DNA was extracted from buffy coats using the QIAmp DNA Blood kit (Qiagen, Inc., Valencia, CA). All purified DNA samples were diluted to a constant DNA concentration in 10 mmol/L Tris and 1 mmol/L EDTA buffer (pH 8).

The *CYP1A1 Ile*^{462}*Val* (rs1048943) and *mEPHx (mEH) His*^{139}*Arg* (rs2234922) polymorphisms were assayed using the Amplifluor SNPs Genotyping System (Chemicon, Temecula, CA) and analyzed on 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The primers were designed through the use of Amplifluor AssayArchitect Web-based software (Chemicon).

The *GSTP1 Ile*^{105}*Val* (rs947894) polymorphism was detected by an *Alw*26I restriction enzyme digest. The 421 bp PCR product was digested with *Alw*26I (MBI Fermentas, Hanover, MD) at 37°C for 2 hours. Wild-type alleles resulted in 309 and 112 bp fragments following restriction enzyme digestion. The variant alleles resulted in 250, 112, and 59 bp products after digestion.

The *GSTP1 Ala*^{114}*Val* (rs1799811) polymorphism was detected by an *Aci*II restriction enzyme digest. The 562 bp PCR product (41) was digested with *Aci*II (New England Biolabs, Beverly, MA) at 37°C for 2 hours. Wild-type alleles resulted in 365, 100, and 97 bp fragments following restriction enzyme digestion. The variant alleles resulted in 462 and 100 bp products after digestion.

The *GSTT1* and *GSTM1* polymorphisms, which both result in the presence (+, nondeleted) or absence (−, null deletion) of the enzymes, were detected by a PCR product coamplified with albumin within a multiplex PCR as a positive internal control (42). The 459, 350, and 219 bp PCR products of GSTT1, albumin, and GSTM1, respectively, were separated by electrophoresis and visualized by ethidium bromide staining.

To ensure quality control of all genotyping results, 5% of the samples were randomly selected and genotyped by a second investigator and 1% of the samples were sequenced using a 377 ABI automated sequencer.

Statistical Analysis. We first calculated genotype frequencies and tested for Hardy-Weinberg equilibrium within the major ethnic groups (i.e., Caucasian and African-American) among controls. We then used conditional logistic regression (with family as the matching variable) to estimate ORs and 95% CIs for the association between genotypes, smoking, and prostate cancer. To address the potential for additional familial correlation induced by matching on sibship, a robust covariance estimator (43) was used in the conditional logistic regression analysis. We also investigated modification of the effects by disease aggressiveness following Rebbeck et al. (44), where low aggressive disease was defined as having a Gleason score < 7 and a clinical tumor stage < T2c for all cases in the sibship and high aggressive disease was characterized as having a Gleason score ≥ 7 or a clinical tumor stage ≥ T2c for at least one case in the sibship. In addition, we examined the interaction between smoking and genetic factors using a conditional logistic regression model (with the robust covariance estimator described above) that included both main effect terms and term(s) for their multiplicative interaction(s). All results are adjusted for age (using age at diagnosis for cases and age at enrollment for controls). All P values are from two-sided tests. All analyses were undertaken with SAS (Version 8.2, SAS Institute, Inc., Cary, NC).

Results

Characteristics of the study population are provided in Table 1. The mean age of cases (61.5 years) was slightly younger than that of controls (62.8 years). Approximately 44% of the cases had a Gleason score of >7 and 13% had a clinical tumor stage of T2c or greater, resulting in about half of the cases having more aggressive disease (Gleason score ≥ 7 or clinical tumor stage ≥ T2c).

The percentage of men that reported smoking regularly for ≥6 months (ever smokers) was essentially the same among cases (63.3%) and controls (63.5%). In addition, the frequency of light (1-20 pack-years) and heavy (>20 pack-years) smoking was not materially different between cases and controls (Table 1). As anticipated, no main effect of smoking on prostate cancer risk was observed regardless of the variable form (continuous or categorical) in the total study population (Table 2), in Caucasians (Table 2), in Caucasians with less aggressive disease (Table 3), or in Caucasians with more aggressive disease (Table 3).

When looking at the candidate genes, all genetic variants were in Hardy-Weinberg equilibrium within ethnic groups. Ignoring the matching, there were no statistically significant allele frequency differences between cases and controls (Table 1). None of the PAH metabolizing or conjugating polymorphisms we investigated were associated with prostate

Table 1. Characteristics of the family-based case-control study population

	Cases (n = 439)	Controls (n = 479)
Mean age, y (SD)	61.5 (6.7)	62.8 (9.1)
Ethnicity, %		
Caucasian	397 (90%)	437 (91%)
African-American	38 (9%)	38 (8%)
Other	4 (1%)	4 (1%)
Gleason score ≥ 7	191 (44%)	—
Clinical tumor stage ≥ T2c	55 (13%)	—
Smoking, %		
Never smokers	161 (37%)	173 (37%)
Light smokers*	114 (27%)	125 (27%)
Heavy smokers*	154 (36%)	170 (36%)
Metabolizing genotypes, %		
CYP1A1 ^{462}*Ile/Ile*	404 (93%)	445 (93%)
CYP1A1 ^{462}*Ile/Val* †	29 (7%)	30 (6%)
CYP1A1 ^{462}*Val/Val*	3 (<1%)	4 (1%)
mEH ^{139}*His/His*	292 (66%)	317 (66%)
mEH ^{139}*His/Arg* †	121 (28%)	131 (28%)
mEH ^{139}*Arg/Arg*	25 (6%)	27 (6%)
Conjugating genotypes, %		
GSTP1 ^{105}*Ile/Ile*	175 (40%)	207 (43%)
GSTP1 ^{105}*Ile/Val* ‡	222 (50%)	214 (45%)
GSTP1 ^{105}*Val/Val*	42 (10%)	58 (12%)
GSTP1 ^{114}*Ala/Ala*	374 (85%)	410 (86%)
GSTP1 ^{114}*Ala/Val* ‡	64 (15%)	67 (14%)
GSTP1 ^{114}*Val/Val*	1 (<1%)	2 (<1%)
GSTM1 nondeleted§	222 (51%)	241 (50%)
GSTM1 null	216 (49%)	238 (50%)
GSTT1 nondeleted§	362 (83%)	398 (83%)
GSTT1 null	76 (17%)	81 (17%)

*Light smokers: ≤20 pack-years; heavy smokers: > 20 pack-years. Ten cases and 11 controls were missing smoking duration information.
†Base pair notation for *CYP1A1 Ile*^{462}*Val* is A4889G and for *mEH His*^{139}*Arg* is A9970G.
‡Base pair notation for *GSTP1* ^{105}*Ile/Val* and *Ala*^{114}Val polymorphisms are A1578G and C2293T, respectively.
§Includes subjects with at least one copy of the nondeleted (+) allele.

cancer risk in the total study population or in Caucasians only (Table 2). However, among men with less aggressive disease, there was an inverse association between carrying the *GSTM1 nondeleted* allele and prostate cancer (odds ratio, 0.50; 95% confidence interval, 0.26-0.94; P = 0.03; Table 3). Moreover, among men with more aggressive disease, the *nondeleted GSTM1* allele was associated with an increased risk of prostate cancer (OR, 1.68; 95% CI, 1.01-2.79; P = 0.05). Restricting these analyses to Caucasians only did not materially alter results (Table 3) but a weak inverse association was revealed in Caucasians with less aggressive disease who carried the *GSTT1 nondeleted* allele (OR, 0.46; 95% CI, 0.20-1.01; P = 0.06).

When we evaluated the potential modification of smoking by polymorphisms in the PAH metabolizing and conjugating genes, we observed a statistically significant negative multiplicative interaction between heavy smokers and the *nondeleted GSTM1* allele in the total study population (P = 0.01) and in Caucasians (P = 0.01). However, this interaction was not statistically significant in light smokers. In the total study population, the risk of prostate cancer was greater in heavy (OR, 1.56; 95% CI, 0.92-2.66) than in light (OR, 1.18; 95% CI, 0.66-2.09) smokers having the *GSTM1 null* genotype, but these effects were not statistically significant. Among Caucasians, heavy smoking increased prostate cancer risk nearly 2-fold in those with the *GSTM1 null* genotype (OR, 1.73; 95% CI, 0.99-3.05), but this increased risk was not observed in heavy smokers who carried the *GSTM1 nondeleted* allele (OR, 0.95; 95% CI, 0.53-1.71).

Because we observed different effects among men carrying the *GSTM1 nondeleted* allele in low and high aggressive disease groups, we also explored stratification by disease aggressiveness in the aforementioned interaction analyses. Among men with less aggressive disease, there was no statistically significant interaction between smoking and the *GSTM1* polymorphism (Table 4). However, among men with more aggressive disease, we found a statistically significant negative multiplicative interaction between the *GSTM1 nondeleted* allele and light (P = 0.02) and heavy (P = 0.009) smokers. Among Caucasians with more aggressive disease, the increased risk in light (OR, 2.07; 95% CI, 0.92-4.69) and heavy (OR, 2.10; 95% CI, 0.92-4.75) smokers carrying the *GSTM1 null* genotype was not statistically significant. However, an unexpected increased risk was observed in nonsmokers who carried the *GSTM1 nondeleted* allele (OR, 4.13; 95% CI, 1.75-9.73).

Discussion

We observed no association between smoking and prostate cancer risk when functional polymorphisms in PAH metabolism and conjugation genes were not considered. However, evaluation of gene × environment effects revealed a statistically significant negative multiplicative interaction between smoking and the *GSTM1 nondeleted* allele, which seemed to be driven by the heavy smokers. In particular, Caucasian heavy smokers with the *GSTM1 null* genotype had nearly a 2-fold increased prostate cancer risk, whereas those carrying the *GSTM1 nondeleted* allele did not have this risk. We also found that the association between the *GSTM1 nondeleted* allele and prostate cancer was influenced by disease aggressiveness in our study—men with less aggressive disease had a reduced risk whereas men with more aggressive disease had an increased risk. When stratifying the interaction analyses by disease severity, we found that the negative multiplicative interaction between smoking and the *GSTM1 nondeleted* allele was only statistically significant among men with more aggressive disease and that the increased risk observed with

Table 2. ORs for smoking, polymorphisms in PAH metabolism and conjugation genes, and prostate cancer

Variable	All subjects		Caucasians only	
	OR (95% CI)*	P	OR (95% CI)*	P
Smoking				
Never smoker	1.00 (Reference)	—	1.00 (Reference)	—
Light smoker†	0.86 (0.58-1.27)	0.45	0.98 (0.65-1.50)	0.94
Heavy smoker†	0.95 (0.67-1.36)	0.79	1.05 (0.72-1.52)	0.80
CYP1A1 Ile^{462}*Val*				
Ile/Ile	1.00 (Reference)	—	1.00 (Reference)	—
Ile/Val	1.14 (0.53-2.45)	0.74	1.07 (0.49-2.33)	0.87
Val/Val	0.90 (0.13-6.37)	0.91	0.85 (0.12-5.92)	0.89
mEH His^{139}*Arg*				
His/His	1.00 (Reference)	—	1.00 (Reference)	—
His/Arg	1.00 (0.66-1.52)	0.99	1.02 (0.66-1.57)	0.94
Arg/Arg	0.85 (0.37-1.96)	0.70	1.07 (0.40-2.88)	0.89
GSTP1 Ile^{105}*Val*				
Ile/Ile	1.00 (Reference)	—	1.00 (Reference)	—
Ile/Val	1.22 (0.84-1.77)	0.29	1.12 (0.76-1.65)	0.57
Val/Val	0.86 (0.45-1.64)	0.65	0.65 (0.34-1.25)	0.20
GSTP1 Ala^{114}*Val*				
Ala/Ala	1.00 (Reference)	—	1.00 (Reference)	—
Ala/Val	1.17 (0.71-1.92)	0.54	0.43 (0.02-11.41)	0.61
Val/Val	1.13 (0.68-1.88)	0.63	0.43 (0.02-10.90)	0.61
GSTM1 null deletion				
Null	1.00 (Reference)	—	1.00 (Reference)	—
Nondeleted ‡	1.00 (0.69-1.45)	0.99	1.09 (0.66-1.46)	0.94
GSTT1 null deletion				
Null	1.00 (Reference)	—	1.00 (Reference)	—
Nondeleted ‡	0.79 (0.47-1.31)	0.36	0.71 (0.41-1.22)	0.22

*All results are adjusted for age.
†Light smokers: 1 to 20 pack-years; heavy smokers: >20 pack-years.
‡Includes subjects with at least one copy of the nondeleted (+) allele.

the *GSTM1 nondeleted* allele in more aggressive disease became unexpectedly stronger in nonsmokers.

Although the heterogeneous risks we observed with the *GSTM1* polymorphism when stratifying by disease aggressiveness were not anticipated, they are not totally unfounded given the prior results of the *GSTM1* polymorphism in prostate cancer and the multiple functions of GSTs. Previously, the *GSTM1 nondeleted* allele has been associated with both increased (39) and decreased (36, 45) prostate cancer risk, whereas others have failed to find an association (34, 35, 37, 38). Furthermore, modification of the risk associated with the *GSTM1* polymorphism has been reported when variables of disease severity were considered (36) but the criteria used to evaluate aggressiveness has not been consistent (39, 45). Moreover, suggestions of a multiplicative interaction between smoking and the *GSTM1* polymorphism have been reported in prior population-based prostate cancer studies. Although Kelada et al. (46) did not find a statistically significant negative multiplicative interaction between the *GSTM1 nondeleted* allele and smoking ($P = 0.18$), their study had a smaller sample size than ours. Recently, Agalliu et al. (45) did not find a statistically significant multiplicative interaction between the *GSTM1* polymorphism and smoking ($P = 0.17$) but they did observe a significant linear increase in prostate cancer risk with increasing pack-years of smoking among men with the *GSTM1 null* genotype ($P_{trend} = 0.007$).

In terms of GST function, a conjugating (detoxifying) role provides biological support for the negative interaction we observed between smoking and the *GSTM1* nondeleted allele and the increased risk observed in heavy smokers with the GSTM1 null genotype. Although both GSTM1 and GSTP1 can conjugate reactive PAH metabolites generated from cigarette smoke, GSTM1 is more effective in prohibiting DNA adduct formation by the (+)-anti configuration of benzo(*a*)pyrene diol epoxide [(+)-anti-benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide], which is considered the ultimate bay-region PAH carcinogen (47). Moreover, although the *GSTM1 nondeleted* and *GSTP1* ^{105}*Val*/^{114}*Ala* genotypes have equal capacity to prevent DNA adduct formation by the ultimate fjord-region PAH carcinogen [(−)-anti-dibenzo(*a,l*)pyrene diol epoxide], the concentration of benzo(*a*)pyrene in cigarette smoke is 10 times greater than that of dibenzo(*a,l*)pyrene (8). GSTT1 has not been shown to catalyze the conjugation of PAH metabolites and may only play a role in conjugating smaller molecules, such as epoxides (28).

Although the predominant role of GSTs is generally believed to involve conjugation of reactive metabolites, many other functions are continuing to unfold (28). For example, GSTs can create more reactive and mutagenic derivatives from certain chemicals. GSTM1 can activate trichloroethylene (48), which is currently used worldwide as a metal degreaser and was previously applied in dry cleaning operations. Occupational cohorts exposed to trichloroethylene have an increased risk of renal cell cancer and individuals carrying the *GSTM1 nondeleted* allele have an even greater risk (49). There is also some evidence supporting a role for trichloroethylene in prostate cancer (50, 51), and, interestingly, trichloroethylene and other compounds GSTM1 is known to preferentially activate are not major constituents of cigarette smoke (8, 48). Therefore, perhaps, some other unmeasured exposure, such as trichloroethylene, is contributing to the increased risk we observed with the *GSTM1 nondeleted* allele among men with more aggressive disease, particularly in the nonsmokers.

Table 3. ORs for smoking and polymorphisms in PAH metabolism and conjugation genes stratified by disease aggressiveness and prostate cancer

Variable	Low aggressive*				High aggressive†			
	All subjects		Caucasians only		All subjects		Caucasians only	
	OR (95% CI)‡	*P*	OR (95% CI)‡	*P*	OR (95% CI)‡	*P*	OR (95% CI)‡	*P*
Smoking								
Never smokers	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
Light smokers§	0.87 (0.47-1.59)	0.64	0.93 (0.49-1.78)	0.83	0.82 (0.48-1.41)	0.48	1.03 (0.57-1.86)	0.91
Heavy smokers§	0.93 (0.55-1.57)	0.78	0.92 (0.53-1.60)	0.78	0.86 (0.51-1.44)	0.56	1.06 (0.61-1.82)	0.84
CYP1A1 Ile^{462}*Val*								
Ile/Ile	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
Ile/Val	1.57 (0.48-5.08)	0.45	1.58 (0.48-5.17)	0.45	0.73 (0.25-2.17)	0.57	0.58 (0.18-1.87)	0.36
Val/Val	3.04 (0.15-61.9)	0.47	3.07 (0.15-63.1)	0.47	—‖	—	—‖	—
mEH His^{139}*Arg*								
His/His	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
His/Arg	1.57 (0.80-3.10)	0.19	1.68 (0.83-3.42)	0.15	0.74 (0.41-1.32)	0.31	0.72 (0.39-1.34)	0.30
Arg/Arg	1.05 (0.28-4.05)	0.94	0.91 (0.21-4.01)	0.90	0.42 (0.14-1.31)	0.14	0.61 (0.16-2.37)	0.48
GSTP1 Ile^{105}*Val*								
Ile/Ile	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
Ile/Val	1.19 (0.66-2.16)	0.57	1.06 (0.56-2.10)	0.86	1.22 (0.74-1.99)	0.44	1.12 (0.67-1.87)	0.67
Val/Val	0.82 (0.30-2.23)	0.70	0.68 (0.24-1.88)	0.45	0.88 (0.36-2.18)	0.78	0.58 (0.23-1.46)	0.24
GSTP1 Ala^{114}*Val*								
Ala/Ala	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
Ala/Val	1.51 (0.72-3.17)	0.28	1.48 (0.69-3.17)	0.31	1.06 (0.52-2.18)	0.87	1.03 (0.49-2.15)	0.94
Val/Val	0.44 (0.01-15.3)	0.65	0.43 (0.01-15.4)	0.64	—‖	—	—‖	—
GSTM1 null deletion								
Null	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
Nondeleted¶	0.50 (0.26-0.94)	0.03	0.49 (0.25-0.96)	0.04	1.68 (1.01-2.79)	0.05	1.76 (1.00-3.11)	0.05
GSTT1 null deletion								
Null	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—	1.00 (Reference)	—
Nondeleted¶	0.58 (0.28-1.20)	0.14	0.46 (0.20-1.01)	0.06	0.83 (0.40-1.74)	0.62	0.79 (0.37-1.67)	0.53

*Includes cases with Gleason score <7 and a clinical tumor stage <T2c and their brothers.
†Includes cases with Gleason score ≥7 or a clinical tumor stage ≥T2c and their brothers.
‡All results are adjusted for age.
§Light smokers: 1 to 20 pack-years; heavy smokers: >20 pack-years.
‖No subject had the *CYP1A1* ^{462}*Val/Val* or *GSTP1* ^{114}*Val/Val* genotypes.
¶Includes subjects with at least one copy of the *nondeleted* (+) allele.

Table 4. ORs for smoking modified by the *GSTM1 null* deletion polymorphism and prostate cancer

	Never smokers		Light smokers*		Heavy smokers*		P_{int} †
	Cases/controls	OR (95% CI) ‡	Cases/controls	OR (95% CI) ‡	Cases/controls	OR (95% CI) ‡	
Total population							
GSTM1 null	74/95	1.0 (Reference)	63/69	1.18 (0.66-2.09)	76/67	1.56 (0.92-2.66)	
GSTM1 nondeleted	86/75	1.47 (0.85-2.56)	51/56	0.97 (0.55-1.74)	78/103	0.91 (0.53-1.58)	0.03
			IOR: 0.56 (0.25-1.26)		IOR: 0.40 (0.20-0.80)		
Caucasians only							
GSTM1 null	68/91	1.0 (Reference)	61/66	1.28 (0.71-2.31)	72/62	1.73 (0.99-3.05)	
GSTM1 nondeleted	72/68	1.43 (0.81-2.53)	46/45	1.14 (0.60-2.17)	71/95	0.95 (0.53-1.71)	0.04
			IOR: 0.62 (0.27-1.43)		IOR: 0.38 (0.18-0.80)		
Low aggressive§							
GSTM1 null	36/36	1.0 (Reference)	28/28	0.84 (0.32-2.20)	35/24	1.44 (0.64-3.25)	
GSTM1 nondeleted	34/38	0.50 (0.21-1.19)	18/24	0.42 (0.16-1.09)	30/49	0.36 (0.15-0.87)	0.29
			IOR: 1.01 (0.27-3.83)		IOR: 0.54 (0.17-1.45)		
Low aggressive§ (Caucasians only)							
GSTM1 null	34/34	1.0 (Reference)	27/28	0.74 (0.27-2.02)	33/23	1.35 (0.58-3.16)	
GSTM1 nondeleted	28/34	0.46 (0.19-1.15)	18/20	0.49 (0.18-1.34)	26/46	0.34 (0.13-0.86)	0.22
			IOR: 1.43 (0.36-5.69)		IOR: 0.54 (0.17-1.67)		
High aggressive‖							
GSTM1 null	35/54	1.0 (Reference)	32/35	1.63 (0.75-3.54)	37/41	1.60 (0.74-3.43)	
GSTM1 nondeleted	50/31	3.84 (1.67-8.79)	27/27	1.55 (0.67-3.57)	40/47	1.60 (0.72-3.55)	0.03
			IOR: 0.25 (0.07-0.83)		IOR: 0.26 (0.09-0.75)		
High aggressive‖ (Caucasians only)							
GSTM1 null	31/53	1.0 (Reference)	31/32	2.07 (0.92-4.69)	36/37	2.10 (0.92-4.75)	
GSTM1 nondeleted	42/28	4.13 (1.75-9.73)	23/20	2.00 (0.78-5.15)	37/43	1.97 (0.83-4.71)	0.02
			IOR: 0.23 (0.07-0.82)		IOR: 0.23 (0.08-0.70)		

Abbreviation: IOR, OR for interaction term.
*Light smokers are those who smoked ≤20 pack-years and heavy smokers are those who smoked >20 pack-years.
†P value for test of two interaction terms (GSTM1 × light smokers and GSTM1 × heavy smokers; $df = 2$).
‡All models are age adjusted.
§Includes cases with Gleason score <7 and clinical tumor stage <T2c and their brothers.
‖Includes cases with Gleason score ≥7 or clinical tumor stage ≥T2c and their brothers.

GSTs are also involved in the synthesis and transport of steroid hormones, which may play a role in prostate cancer aggressiveness. Enzymes in the GST α class are believed to influence testosterone synthesis (28); however, GSTM1 was the only isozyme that could bind testosterone in rat seminiferous tubular fluid (52). Although increasing serum-free testosterone levels have been associated with increased prostate cancer risk in a recent cohort study (53), the role of testosterone in disease aggressiveness is less clear and whether the *GSTM1 null deletion* polymorphism specifically affects testosterone levels in the human prostate requires further study.

Perhaps, a more compelling role for GSTs in aggressive prostate cancer may be their ability to negatively regulate inflammation- and apoptosis-associated proteins (28). In particular, GSTM1 has shown great affinity for the conjugation of the prostaglandin J2, which is required for inhibiting cell proliferation and regulating other signal transduction pathways, including response to inflammation (54). Moreover, inflammation is frequently present in prostate cancer tissue (55) and elevated levels of inflammatory markers have been associated with advanced disease (56). Therefore, the presence of the *GSTM1 nondeleted* allele may decrease levels of prostaglandin J2, which could, in turn, inhibit inflammatory response proteins and/or accelerate proliferation of initiated cells leading to more aggressive prostate cancer.

Taken together, the risk of more aggressive prostate cancer may be reduced among those carrying the *GSTM1 nondeleted* allele only in the presence of cigarette smoke because the GSTM1 enzyme is preferentially used for PAH metabolite detoxification, which may deplete the amount of GSTM1 available for conducting other functions that might contribute to disease progression such as (*a*) negative regulation of inflammation- and apoptosis-associated proteins; (*b*) modification of testosterone levels; and (*c*) activation of trichloroethylene or other chemicals with similar molecular structure.

Although the differing risks we observed with the *GSTM1* polymorphism according to disease severity may be attributed to statistical artifact, the even stronger effects associated with carrying the *GSTM1 nondeleted* allele among men with more aggressive disease when considering smoking status makes the results more difficult to dismiss. Unlike prior population-based studies, our family (sibling)–based study is not susceptible to population stratification, but this design could lead to other problems such as overmatching (57). However, because we undertook a matched analysis, overmatching on germ line variants should not lead to biased estimates of effect (57). We also used a robust covariance estimator (43) to address any additional familial correlation potentially induced by matching on sibship. However, future modeling of the complex mechanisms involved in PAH metabolism and conjugation, especially in the presence of mixtures like smoking, may be improved by the use of physiologically based toxicokinetic approaches (58) modified to include key toxicodynamic effects, such as PAH-DNA adducts.

In summary, when considering the joint effects of smoking and the *GSTM1* polymorphism, we observed that the *GSTM1 nondeleted* allele decreased the risk of prostate cancer in smokers, particularly Caucasian heavy smokers. These findings emphasize the importance of considering gene environment interactions when investigating the effects of smoking in prostate carcinogenesis.

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