Carcinogenesis vol.26 no.2 pp.395–401, 2005
doi:10.1093/carcin/bgh326

GSTM1, *GSTT1* and *GSTP1* polymorphisms, environmental tobacco smoke exposure and risk of lung cancer among never smokers: a population-based study

A.S.Wenzlaff[1,*], M.L.Cote[1], C.H.Bock[1], S.J.Land[1–3] and A.G.Schwartz[1]

[1]Population Studies and Prevention Program, [2]Center for Molecular Medicine and Genetics and [3]Molecular Biology and Human Genetics Program, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, USA

[*]To whom correspondence should be addressed
Email: wenzlaff@med.wayne.edu

Glutathione *S*-transferases detoxify polycyclic aromatic hydrocarbons found in tobacco smoke by glutathione conjugation. Polymorphisms within the *GSTM1*, *GSTT1* and *GSTP1* genes, coding for enzymes with deficient or reduced activity, have been studied as potential modifiers of lung cancer risk. It is hypothesized that risk associated with potential susceptibility gene polymorphisms might be most evident at low levels of exposure. Never smokers developing lung cancer represent a highly susceptible subset of the population, exposed to tobacco carcinogens only through environmental tobacco smoke. This population-based case-control study examines the association between *GSTM1*, *GSTT1* and *GSTP1* genotypes and lung cancer in one of the largest samples of never smokers to date. Cases ($n = 166$) were identified through the metropolitan Detroit Surveillance, Epidemiology and End Results (SEER) program and age- and race-matched population-based controls ($n = 181$) were identified using random digit dialing. Overall, there was no significant association between single or combinations of genotypes at *GSTM1*, *GSTT1* or *GSTP1* and lung cancer risk after adjustment for age, race, sex and household ETS exposure in years. However, in never smokers exposed to 20 or more years of household ETS, carrying the *GSTM1* null genotype was associated with a 2.3-fold increase in risk [95% confidence interval (CI) 1.05–5.13]. Individuals in this high ETS exposure category carrying the *GSTM1* null and the *GSTP1* Val allele were at over 4-fold increased risk of developing lung cancer (OR = 4.56, 95% CI: 1.21–17.21). These findings suggest that in the presence of ETS, the *GSTM1* genotype both alone and in combination with the *GSTP1* genotype alters the risk of developing lung cancer among never smokers.

Introduction

In 2004, over 173 000 new lung cancer diagnoses and 160 000 lung cancer deaths are expected in the US (1). While it is well recognized that 80–90% of all lung cancer is attributable to cigarette smoking, only 15% of all smokers develop this disease and ~10% of all diagnoses are among never smokers (2). This suggests that there is great individual variation in susceptibility to lung carcinogens. Never smokers developing lung cancer represent an understudied population even though the estimated number of deaths from this disease in never smokers alone is approximately equal to the number of deaths from ovarian cancer annually (1). Most never smokers with lung cancer have been exposed to tobacco smoke through environmental tobacco smoke (ETS). Developing lung cancer despite this lower level of exposure suggests that never smokers represent a particularly susceptible subset of the general population.

It is hypothesized that a large proportion of lung cancer susceptibility is determined by the balance between an individual's capacity to activate and detoxify carcinogens in tobacco smoke. Glutathione *S*-transferases (GSTs), which comprise one superfamily of phase II detoxification enzymes, detoxify polycyclic aromatic hydrocarbons found in tobacco smoke by conjugating them with glutathione (3). Polymorphisms exist within the *GSTM1*, *GSTT1* and *GSTP1* genes, the focus of this analysis. Both the *GSTM1* and the *GSTT1* null variants result in the lack of enzyme production. GSTP1 is the most abundant isoform in the lung. One polymorphism in *GSTP1*, an Ile to Val conversion at codon 105, has been reported to result in an enzyme with reduced activity (4). It is possible that deficient or reduced activity of these enzymes might result in an increased susceptibility to cancer.

While a number of studies have evaluated lung cancer risk associated with *GST* polymorphisms in smokers, studies of never smokers have been limited in size and in number. Studies of *GSTM1* have shown increased lung cancer risk associated with having the null variant both in non-smokers (5) and in heavy smokers (6). A recent meta-analysis estimates increased lung cancer risk associated with the *GSTM1* null genotype is moderate [odds ratio (OR) = 1.17, 95% confidence interval (CI): 1.07–1.27) (7)]. Individual studies of *GSTT1* and *GSTP1* variants have been conducted predominantly in smoking populations and have yielded conflicting results (5,6,8–10). These two polymorphisms are most often associated with lung cancer risk only in conjunction with at least one additional *GST* variant allele (11,12).

The objective of this study is to jointly evaluate the association between *GSTM1*, *GSTT1* and *GSTP1* polymorphisms and lung cancer risk in never smokers with low levels of exposure using a population-based case-control approach. Another group suspected to be susceptible at low levels of exposure includes those diagnosed at an early age. One of the studies included in the analysis is focused specifically on individuals under the age of 50, so the analysis is enriched for those thought to have a heightened risk potentially mediated through genetic variation in metabolic activity.

Abbreviations: CI, confidence interval; ETS, environmental tobacco smoke; ITC, isothiocyanates; OR, odds ratio.

Materials and methods

Study subjects

Lifetime never smokers were identified through two case-control studies. The first study included individuals newly diagnosed between 1984 and 1987, age 40–84 years (Study 1) (13) and the second (ongoing) study includes lung cancer cases under age 50 years, newly diagnosed on or after September 15, 1990 (Study 2). Study 2 was conducted in three phases, with phase I limited to those under age 40, phase II included those under age 45 and the current phase III, which includes individuals under the age of 50. In both Study 1 and Study 2, cases were identified through the population-based Metropolitan Detroit Cancer Surveillance System (MDCSS), a participant in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program. Population-based controls were identified through random digit dialing. A greater proportion of controls than cases were lifetime never smokers, so controls were randomly selected from within each study to match never smoking cases on race, sex, 5-year age group and county of residence. Whenever possible, controls were frequency matched to cases in a 1:1 ratio in Study 1 and 2:1 in Study 2. Lifetime never smokers in both studies were defined as individuals smoking fewer than 100 cigarettes in their lifetimes.

The overall interview response rate was 66.1% for cases. It was not possible to calculate a response rate for controls because eligibility could not be determined in households refusing to answer the eligibility screening questionnaire. Of those individuals answering the telephone and providing a household census to determine eligibility, 93.0% participated in the study.

Data and biosample collection

All local institutional and review boards approved this study. Informed consent was obtained from each subject prior to study participation. Trained interviewers conducted telephone interviews to collect demographic information, smoking history, health history and lifetime estimates of ETS exposure. If the study subject was unable to participate due to illness or death, an attempt was made to obtain proxy data by interviewing someone familiar with the subject's history. Demographic information included date and place of birth, residence at time of diagnosis, marital status, race and number of years of education completed. Medical history included physician diagnoses of asthma, emphysema, allergies, pneumonia, bronchitis, chronic obstructive pulmonary disease, tuberculosis and cancer. Year of first diagnosis of each illness was also requested, when applicable. Participants were asked about their ETS exposure at home and the workplace, as well as hours and years of exposure in each location. Family history of lung cancer was coded as yes or no based on detailed first-degree family history information collected. Lung cancer diagnosis dates and histology information for cases were obtained through the MDCSS.

Biological samples were collected from cases and controls through blood, buccal swabs and tissue blocks. Samples were available for 166 never smoking cases and 181 selected never smoking controls. Case biospecimens consisted of 17 blood, five buccal and 144 tissue block samples. Normal tissue could be extracted from 54 of the blocks, 58 were determined to be mixed and 32 were mostly tumor. Among the controls, 95 blood and 86 buccal samples were available for analysis. A small number of these cases and controls could not be genotyped at varying polymorphisms due to poor sample quality.

Genotyping

DNA was isolated from whole blood with the Genomic DNA Purification System (Gentra Systems, Minneapolis, MN), buccal swabs with the BuccalAmp™ DNA Extraction Kit (Epicentre Technologies, Madison, WI), or paraffin-embedded tissue with the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturers' protocols. When multiple tissues were obtained from a study participant, DNA extracted from blood was used preferentially, followed by DNA extracted from buccal swabs and then DNA extracted from normal tissue in paraffin blocks. To evaluate whether there might be allelic loss or somatic mutations in the GSTs in the tumor tissue we compared genotype results for normal versus mixed and tumor tissue blocks. No differences in genotype distribution were observed by tissue type (normal only versus mixed/tumor). χ^2 *P*-values comparing allele frequencies for *GSTM1*, *GSTT1* and *GSTP1* among normal versus mixed and tumor tissue were 0.52, 0.39 and 0.27, respectively.

DNA isolated from buccal cells or paraffin-embedded tissue was pre-amplified using a nested PCR strategy. Outer amplification was carried out in a 25 μl reaction containing 2.5 mmol/l $MgCl_2$, 0.5 μmol/l of the gene-specific primers, 1.25 U AmpliTaq Gold polymerase, and 200 μmol/l of dATP, dCTP, dGTP and dTTP. The outer amplification mixture was denatured at 95°C for 10 min and amplification was achieved by 15 cycles of 94°C for 30 s, 63°C for 30 s and 72°C for 1 min, followed by a final extension at 72°C for 10 min. The outer amplification was performed on a Mastercycler® Gradient thermocycler (Eppendorf, Westbury, NY). Gene-specific allelic discrimination (as described below) was determined directly from amplification of DNA from whole blood or pre-amplified DNA from buccal or paraffin-embedded tissue. Five percent of the products were randomly sequenced and 10% of genotypes were carried out in duplicate. Table I lists the sequences of the primers and probes used in genotyping *GSTM1*, *GSTT1* and *GSTP1* polymorphisms. Beta-globin was used as an amplification control for the null mutations of *GSTM1* and *GSTT1*. No template controls and sequenced controls were used for all assays.

Table I. Primer and probe sequences used in *GSTM1*, *GSTT1* and *GSTP1* genotyping

Primer name	Sequence
GSTM1-194F	5′-GGA GAA GAT TCG TGT GGA CA-3′
GSTM1-273R-2	5′-CTG GAT TGT AGC AGA TCA TAC-3′
GSTM1-OF	5′-ATG CTG AGA TTG AGT CTG TGT TTT GT-3′
GSTM1-OR	5′-AAA CTC TGT CAG ATG CAG CTC ACT-3′
GSTP1-IF	5′-CCC TGG TGG ACA TGG TGA A-3′
GSTP1-IR	5′-CAA CCC TGG TGC AGA TGC T-3′
GSTP1-OF	5′-GGT TGG CCC ATC CCC A-3′
GSTP1-OR	5′-CTT TCT TTG TTC AGC CCC CA-3′
GSTT1-IF	5′-TTC CTT ACT GGT CCT CAC ATC TC-3′
GSTT1-IR	5′-TCA CCG GAT CAT GGC CAG CA-3′
GSTT1-OF	5′-TTG GAT GTG ACC CTG CAG TT-3′
GSTT1-OR	5′-TGC AAG GGT GAG GTT TCC C-3′
HBB-354F	5′-GTG CAC CTG ACT CCT GAG GAG A-3′
HBB-455R	5′-CCT TGA TAC CAA CCT GCC CAG-3′
HBB-OF	5′- TTG GCC AAT CTA CTC CCA GGA-3′
Probe name	Sequence
GSTM1-215T	5′-6FAM-TTT GGA GAA CCA GAC CAT GGA CAA C-TAMRA-3′
GSTP1-A	5′-6FAM-CCG CTG CAA ATA CAT CTC CCT CAT CTA-TAMRA-3′
GSTP1-G	5′-VIC-CGC TGC AAA TAC GTC TCC CTC ATC TA-TAMRA-3′
HBB-402T	5′-JOE-AAG GTG AAC GTG GAT GAA GTT GGT GG-TAMRA-3′

The *GSTM1* null mutation was assayed using quantitative PCR methods as described previously (14). Briefly, 25 ng of DNA extracted from whole blood or 2 μl of pre-amplification reaction was mixed with forward (GSTM1-194F) and reverse (GSTM1-273-2) primer and the product was detected with a fluorescent probe (GSTM1-215T) at an annealing temperature of 55°C.

The *GSTT1* null mutation was determined as described previously (15). Briefly, following amplification with an annealing temperature of 58°C, the PCR products were electrophoresed through 3% NuSieve 3:1 agarose (FMC) and visualized with ethidium bromide.

Primers for amplification of the region of the *GSTP1* gene containing the functional polymorphism and fluorescent probes for allelic discrimination were designed using Primer Express software (Applied Biosystems). Standard TaqMan conditions were applied in an ABI PRISM 7900 Sequence Detection System (Applied Biosystems) for amplification and detection of both alleles. Briefly, 25 ng of DNA derived from whole blood or 2 μl of pre-amplification reaction was added to 1× Universal PCR mix (Applied Biosystems) containing forward (GSTP1-IF) and reverse (GSTP1-IR) primers and *GSTP1* A^{313} allele (GSTP1-A) and *GSTP1* G^{313} allele (GSTP1-G) probes at an annealing temperature of 64°C.

Statistical analysis

Allele frequencies for each polymorphism were calculated for cases and controls, stratifying by race. For *GSTM1* and *GSTT1*, our genotyping methods did not distinguish between homozygous non-null and heterozygous individuals. Therefore, Hardy–Weinberg equilibrium tests of genotype distribution in controls, by race, were conducted only for *GSTP1*. The effect of each *GST* variant and each combination of *GST* variants on lung cancer risk was tested in unconditional logistic regression models, adjusting for age at diagnosis (cases)/interview (controls), race (Caucasian/African American), sex and years exposed to household ETS. To increase the study power when testing gene–gene interactions, *GSTP1* genotypes were collapsed so that individuals with at least one valine allele (Ile/Val or Val/Val) were compared with individuals carrying the Ile/Ile genotype. Inclusion of ETS at work, education, history of congestive lung diseases and family history of lung cancer did not alter results in the multivariate models and therefore were not included in the final models.

ORs and 95% CIs for each genotype were calculated from coefficients in the final models. Analyses were repeated after stratification by histology, ever/never exposure to household ETS, and years of household ETS exposure. The Cochran–Armitage test was used to assess possible linear trends in stratified data and power calculations were conducted (16). All statistical analyses were performed using SAS version 8.02.

Results

Demographics for cases and controls are shown in Table II. The mean age was 62.4 years for the cases and 54.9 years for the controls ($P < 0.001$). This age difference was due to the oversampling of non-smoking controls from Study 2, where the participants were younger. Among those reporting ETS exposure, the mean number of years of household ETS exposure was greater in cases (28.2 years) than controls (23.8 years) ($P = 0.02$). There were no significant differences in sex or race between cases and controls ($P = 0.19$ and $P = 0.57$, respectively). Cases were less likely to have finished high school than controls and had significantly lower education levels overall. In this group of lifetime never smokers, adenocarcinoma was the most prominent histological type (54.2%), with squamous cell carcinoma as the second most common histologic type (15.7%).

Table II. Select characteristics of cases and controls

Characteristic	Cases ($n = 166$)		Controls ($n = 181$)		*P*-value
Mean age (SD)	62.4 (13.9)		54.9 (13.3)		<0.0001
Mean ETS years[a] (SD)	28.2 (15.8)		23.8 (14.0)		0.02
	n	%	*n*	%	
Gender					
Male	70	42.2	89	49.2	0.19
Female	96	57.8	92	50.8	
Race					
Caucasian	135	81.3	151	83.4	0.57
African American	31	18.7	30	16.6	
Education					
Less than high school	45	27.1	24	13.3	0.03
H.S. graduate or GED	46	27.7	55	30.4	
At least some college	66	39.8	102	56.3	
Do not know	9	5.4	0	0.0	
Histology					
Squamous cell carcinoma	26	15.7			
Small cell carcinoma	11	6.6			
Adenocarcinoma	90	54.2			
Large cell carcinoma	12	7.2			
Other/unknown type	27	16.3			

[a]Household ETS exposure among exposed individuals only.

Genotype data were available for *GSTM1* for 160 cases and 177 controls, for *GSTT1* for 153 cases and 175 controls, and for *GSTP1* for 141 cases and 180 controls. Allele frequencies for these polymorphisms are reported in Table III. The *GSTP1* genotype distribution for the total sample of controls was in Hardy–Weinberg equilibrium (χ^2 *P*-value = 0.35), as were the distributions in controls by race (χ^2 *P*-value = 0.64 in Caucasians and χ^2 *P*-value = 0.17 in African Americans). Racial differences in allele frequencies in the controls were seen only for *GSTM1*, with African Americans having a lower frequency of the null allele (27%) than Caucasians (52%) ($P = 0.01$).

There was no significant association between genotype at *GSTM1* and lung cancer risk after adjustment for age at diagnosis (cases)/interview (controls), sex, household ETS exposure in years and race where appropriate (Table IV). The allele frequency distribution for *GSTM1* varied significantly by race. While there were no observed differences in risk for the *GSTM1* null genotype among Caucasians, there was a non-significant 2-fold increase in risk in African Americans (OR = 1.92, 95% CI: 0.49–7.45).

Neither the *GSTT1* null variant nor the *GSTP1* Val allele was found to be significantly associated with lung cancer risk among never smokers, a finding that was similar across racial groups (Table IV). While none of the findings were statistically significant, ORs estimating the association between *GSTT1* null variant and lung cancer risk were all <1. Evaluation of combinations of *GST* genotypes (Table IV) also revealed no significant association between genotypes and the development of lung cancer among never smokers.

In this group of never smokers, exposure to tobacco carcinogens was in the form of ETS. Risk of lung cancer increased with increasing quartile of household ETS exposure, however, the trend was not statistically significant (Cochran–Armitage two-sided *P*-value = 0.08) (data not shown). Stratifying by ever/never household ETS exposure demonstrated increased risks for *GSTM1* null individuals with at least some ETS exposure. Furthermore, the interaction between *GSTM1* null and quartile of household ETS exposure

Table III. Distribution of *GST* genotypes in a population of lifetime never smokers stratified by race

Characteristic	Total sample			Caucasians			African Americans		
	Cases *n* (%)	Controls *n* (%)	*P*-value	Cases *n* (%)	Controls *n* (%)	*P*-value	Cases *n* (%)	Controls *n* (%)	*P*-value
GSTM1[a]									
Present	82 (51)	92 (52)	0.89	62 (47)	70 (48)	0.96	20 (69)	22 (73)	0.71
Null	78 (49)	85 (48)		69 (53)	77 (52)		9 (31)	8 (27)	
GSTT1[b]									
Present	125 (82)	138 (79)	0.52	100 (81)	115 (79)	0.81	25 (86)	23 (77)	0.35
Null	28 (18)	37 (21)		24 (19)	30 (21)		4 (14)	7 (23)	
GSTP1[c]									
Ile/Ile	47 (33)	68 (38)	0.64	39 (35)	61 (40)	0.65	8 (27)	7 (24)	0.69
Ile/Val	73 (52)	90 (50)		58 (52)	72 (48)		15 (52)	18 (62)	
Val/Val	21 (15)	22 (12)		15 (13)	18 (12)		6 (21)	4 (14)	

[a]Results missing for four Caucasian cases, two African American cases, four Caucasian controls and zero African American controls.
[b]Results missing for 11 Caucasian cases, two African American cases, six Caucasian controls and zero African American controls.
[c]Results missing for 23 Caucasian cases, two African American cases, zero Caucasian controls and one African American control.

Table IV. OR and associated CI for lifetime never smokers by race

	Caucasians[a]		African Americans[b]		Total OR[d] (95% CI)
	Cases/controls	OR[c] (95% CI)	Cases/controls	OR[c] (95% CI)	
GSTM1	59/70	1.00 (ref.)	19/21	1.00 (ref)	1.00 (ref.)
GSTM1 null	66/76	1.03 (0.62–1.71)	8/8	1.92 (0.49–7.45)	1.11 (0.70–1.78)
GSTT1	96/114	1.00 (ref.)	23/22	1.00 (ref)	1.00 (ref.)
GSTT1 null	22/30	0.81 (0.43–1.54)	3/7	0.40 (0.08–2.01)	0.74 (0.41–1.33)
GSTP1 Ile/Ile	37/61	1.00 (ref.)	7/7	1.00 (ref)	1.00 (ref.)
GSTP1 Ile/Val	54/71	1.37 (0.78–2.41)	14/17	0.83 (0.20–3.40)	1.28 (0.76–2.14)
GSTP1 Val/Val	15/18	1.45 (0.64–3.30)	5/4	1.59 (0.24–10.37)	1.51 (0.72–3.17)
GSTP1 Ile/Val or Val/Val	69/89	1.39 (0.81–2.37)	19/21	0.95 (0.24–3.72)	1.32 (0.81–2.17)
Joint effects[e]					
GSTM1 and *GSTT1*					
0	48/56	1.00 (ref.)	16/14	1.00 (ref)	1.00 (ref.)
1	55/67	0.95 (0.54–1.66)	8/15	0.59 (0.16–2.15)	0.89 (0.54–1.48)
2	12/17	0.75 (0.31–1.82)	1/0	f	0.81 (0.35–1.90)
GSTT1 and *GSTP1*[g]					
0	26/46	1.00 (ref.)	7/5	1.00 (ref.)	1.00 (ref.)
1	63/80	1.49 (0.81–2.74)	15/19	0.62 (0.13–3.05)	1.33 (0.76–2.33)
2	10/18	1.00 (0.39–2.56)	3/4	0.50 (0.06–4.00)	0.91 (0.39–2.14)
GSTM1 and *GSTP1*[g]					
0	16/30	1.00 (ref.)	5/5	1.00 (ref.)	1.00 (ref.)
1	55/69	1.62 (0.78–3.36)	14/17	0.69 (0.13–3.57)	1.41 (0.73–2.71)
2	32/47	1.43 (0.65–3.14)	6/6	1.74 (0.24–12.65)	1.43 (0.70–2.93)

[a]Missing household ETS exposure estimates for six Caucasian cases and one Caucasian control.
[b]Missing household ETS exposure estimates for three African American cases and one African American control.
[c]Adjusted for age at diagnosis (cases)/interview (controls), sex and years of household ETS exposure (continuous).
[d]Adjusted for age at diagnosis (cases)/interview (controls), race, sex and years of household ETS exposure (continuous).
[e]Testing for joint effects by the following scheme: 0 = no risk genotypes, 1 = only one gene exhibits a risk genotype, 2 = both genes exhibit a risk genotype.
[f]Not enough events to calculate.
[g]Ile/Val and Val/Val are classified as risk alleles for *GSTP1*.

years was significant in the full analysis model ($P = 0.01$). To investigate this possible interaction and still maintain study power, individuals were stratified into no household ETS exposure, <20 household ETS years (the median level among exposed controls and ≥20 years of household ETS). Among individuals with no household ETS exposure, there appeared to be a protective effect for the *GSTM1* and *GSTT1* null genotypes individually, although these associations were not statistically significant (*GSTM1* OR = 0.52, 95% CI: 0.22–1.23; *GSTT1* OR = 0.32, 95% CI: 0.10–1.03). Carrying the null genotype in both of these genes was associated with reduced risk of lung cancer in those not exposed to ETS (OR = 0.16, 95% CI: 0.03–0.93), however, this group included only two cases and six controls. No single genotype or combination of genotypes was significantly associated with lung cancer among individuals with greater than zero and less than the median exposure (Table V). Among individuals with the median ETS exposure or greater, however, having the null variant of *GSTM1* more than doubled the risk of lung cancer (OR = 2.32, 95% CI: 1.05–5.13). Similarly, carrying both the *GSTM1* null variant and a valine residue at the *GSTP1* locus was associated with a 4-fold increased risk of lung cancer among those with greater ETS exposure (OR = 4.56, 95% CI: 1.21–17.21). Power calculations based on available sample size indicated 70% power to detect a true OR of 2.08 among individuals with one or two risk genotypes for the *GSTM1*/*GSTP1* combination or a 60% power to detect a true OR of 1.67. Stratification by histologic type did not reveal any significant differences in the results (data not shown).

Discussion

Numerous previous studies of *GSTM1* genotype in smokers have reported moderate increases in lung cancer risk associated with the null genotype [meta-analysis (7)]. Less has been reported for *GSTT1* and *GSTP1* polymorphisms, particularly in never smokers, and for combinations of all these polymorphisms. In our study, the *GSTM1* null genotype and the *GSTM1* null/*GSTP1* Val combination were associated with increased lung cancer risk in never smoking individuals with 20 or more years of household ETS exposure. Studies focusing solely on the risk of lung cancer in never smokers have been limited in size and in number. A recent report by Hung *et al*. used pooled data collected on non-smoking lung cancer cases and controls from 14 primarily European studies and reported no significant association between the *GSTM1* null genotype and lung cancer (17). Through stratification of cases by ETS levels, Bennett *et al*. showed a strong interaction between the *GSTM1* null genotype and higher ETS exposure, including a significant trend. Kiyohara *et al*. reported no significant increase in lung cancer risk associated with the *GSTM1* null genotype in non-smoking individuals with low ETS exposure, but an over 2-fold increase among individuals with 40 or more packyears of ETS exposure in a hospital-based case-control study conducted in Japan (18). Similar suggestive, but not statistically significant, findings for increased risk associated with ever exposure to ETS among non-smokers carrying the *GSTM1* null genotype were reported in a primarily European case-control study (19). Our population-based study is one of

Table V. OR and associated CI for lifetime non-smokers by ETS exposure

	No household ETS		1–19 household ETS years		20+ household ETS years	
	Cases/Controls	OR[a] (95% CI)	Cases/Controls	OR[a] (95% CI)	Cases/Controls	OR[a] (95% CI)
GSTM1 gene present	34/27	1.0 (ref.)	23/34	1.0 (ref.)	22/31	1.0 (ref.)
GSTM1 null	16/23	0.52 (0.22–1.23)	18/27	1.07 (0.44–2.61)	43/35	2.32 (1.05–5.13)
GSTT1 gene present	42/39	1.0 (ref.)	30/48	1.0 (ref.)	49/51	1.0 (ref.)
GSTT1 null	6/12	0.32 (0.10–1.03)	7/9	0.77 (0.22–2.71)	14/16	1.16 (0.48–2.79)
GSTP1 Ile/Ile	15/20	1.0 (ref.)	11/23	1.0 (ref.)	18/25	1.0 (ref.)
GSTP1 Ile/Val	21/27	1.01 (0.40–2.54)	22/28	1.58 (0.57–4.35)	29/35	1.29 (0.56–3.00)
GSTP1 Val/Val	6/6	1.57 (0.40–6.19)	6/10	1.39 (0.38–5.09)	8/6	1.72 (0.48–6.12)
GSTP1 Ile/Val or Val/Val	27/33	1.11 (0.46–2.66)	28/38	1.52 (0.59–3.94)	37/41	1.36 (0.61–3.07)
Joint effects[b]						
GSTM1 and *GSTT1*						
0	28/22	1.0 (ref.)	20/25	1.0 (ref.)	17/24	1.00 (ref.)
1	17/20	0.60 (0.24–1.49)	11/30	0.53 (0.20–1.42)	36/33	1.88 (0.80–4.42)
2	2/6	0.16 (0.03–0.93)	6/2	2.38 (0.35–16.43)	7/9	1.89 (0.50–7.13)
GSTT1 and *GSTP1*[c]						
0	13/14	1.0 (ref.)	9/19	1.0 (ref)	11/18	1.00 (ref.)
1	24/29	0.95 (0.35–2.53)	21/32	1.31 (0.45–3.78)	35/40	1.91 (0.73–5.00)
2	4/8	0.38 (0.08–1.73)	5/6	1.01 (0.19–5.39)	6/8	1.73 (0.43–7.00)
GSTM1 and *GSTP1*[c]						
0	10/9	1.0 (ref.)	7/11	1.0 (ref.)	4/15	1.00 (ref.)
1	24/27	0.86 (0.29–2.56)	18/35	0.85 (0.26–2.78)	28/25	4.64 (1.25–17.22)
2	7/14	0.45 (0.12–1.67)	13/15	1.59 (0.40–6.33)	21/25	4.56 (1.21–17.21)

[a]Adjusted for age at diagnosis (cases)/interview (controls), race, sex and years of exposure to household ETS (continuous).
[b]Testing for joint effects by the following scheme: 0 = no risk genotypes, 1 = only one gene exhibits a risk genotype, 2 = both genes have a risk genotype.
[c]Ile/Val and Val/Val are classified as risk alleles for GSTP1.

the largest of never smokers and our findings support an association between the *GSTM1* null genotype and increased lung cancer risk among individuals with high ETS exposures. This is consistent with the hypothesis that reduced ability to detoxify tobacco carcinogens in ETS through the enzymatic activity of GSTM1 contributes to lung cancer susceptibility.

Increased risk is even more evident in never smokers with 20 or more years of household ETS exposure who carry both the *GSTM1* null and at least one Val allele at *GSTP1*. Similar findings of increased lung cancer risk associated with carrying the *GSTM1* null/*GSTP1* Val allele genotype were reported by Wang *et al*., however this study included only 64 non-smoking cases (20). Miller *et al*. report increased (although not statistically significant) risk of lung cancer associated with ETS exposure (OR = 1.62, 95% CI: 0.51–5.14) among non-smokers (n = 66 cases) carrying the *GSTP1* Val/Val genotype, while those carrying the Ile/Ile genotype had lower risks associated with ETS exposure (21). While Miller's results are not statistically significant, they support the findings reported here. Studies of *GSTP1* in smokers, either alone or in combination with other genotypes, have reported conflicting results. In only two of nine studies of *GSTP1* alone (reviewed in ref. 22), the exon 5 *GSTP1* Val low activity allele was associated with an ~2-fold increased risk of developing lung cancer. In a recent hospital-based study, Wang *et al*. reported no association between the exon 5 polymorphism and lung cancer risk, but did find evidence of risk associated with the exon 6 Ala114Val polymorphism (OR = 1.40, 95% CI: 1.03–1.91) (10). Nazar-Stewart *et al*. evaluated *GSTP1* in combination with *GSTM1* and found no increased risk associated with the exon 5 polymorphism (6). Two other studies of this combined genotype support our findings (23,24). The apparently conflicting findings in the literature suggest complex interactions between genetic polymorphisms, metabolism of tobacco carcinogens and smoking patterns. More than one GST isoform is responsible for the detoxification of tobacco smoke carcinogens. *GSTP1* codes for the most abundant isoform in the lung and it would be expected that further reduction in detoxification ability from carrying risk genotypes at two loci would increase risk under certain exposure conditions. The increase in risk associated with decreased detoxification activity may only be evident when exposures are relatively low, as we see in never smokers exposed to ETS, and when multiple enzymes in a pathway are lacking.

Findings of associations between *GSTT1* and lung cancer have been less consistent than findings for *GSTM1*, some suggesting a possible interaction with smoking. Among studies of mostly smokers, the *GSTT1* null genotype has been associated with increased lung cancer risk (25,26) and no difference in risk (6). Although not statistically significant, our study found that never smokers who reported no household ETS exposure and who were *GSTT1* null were at one-third the risk of lung cancer than individuals not *GSTT1* null. A possible protective effect of being *GSTT1* null in non-smokers has also been reported by Hou *et al*. (27). The potential protective effect of the *GSTT1* null genotype in never smokers without ETS exposure suggests that there are non-tobacco related exposures that need to be explored. One possibility is that never smokers deficient in GSTT1 (*GSTT1* null) who consume foods rich in isothiocyanates (ITC) are protected against lung cancer. ITCs are found in cruciferous vegetables, are substrates for GSTs, and are associated with reduced cancer risk. Reduced intake of ITCs has been associated with increased risk of lung cancer in current smokers who are either *GSTM1* or *GSTT1* null (26). London *et al*. demonstrated in a cohort of

Chinese men that individuals with detectable levels of ITC who were either *GSTM1* or *GSTT1* null were at reduced risk of lung cancer (28). We did not measure ITC intake so we cannot look at this directly in our study.

While differences in histology types and possible carcinogenesis pathways between non-smokers and smokers are hypothesized, differences in risk related to varying levels of ETS in non-smokers have not been well categorized. Stratifying this population of never smokers by histology did not reveal significant differences in risk by ETS levels or GST genotype.

Despite the large sample of Caucasian and African American population-based never smokers included in this study, analyses by race and ETS exposure were limited by the small number of cases. In particular, our power to detect a true difference in lung cancer risk for *GST* combinations was limited. Our finding of a 4-fold increase in lung cancer risk associated with the combination of *GSTM1* and *GSTP1* risk genotypes must therefore be considered with caution. In addition, estimates of ETS exposure are subject to misclassification. Both estimates of lifetime years and hours of ETS exposure were collected, however, years of exposure was chosen for the analysis because we believe that it was a more reliable measure than the estimates of hours exposed. Another potential limitation of this study is the difference in mean ages of the cases and controls. Younger controls were more likely to provide a blood sample than older controls and were oversampled. However, none of the genotype distributions varied by age (comparing those <55 years, the mean age in the controls, to those age 55 and older) in either cases or controls, so it is unlikely that this age difference confounded the results (data not shown). Additionally, age was included in all the multivariate models. While not all subjects interviewed provided biologic specimens for genotyping, the observed allele frequencies for each genotype in our controls are comparable with those reported in the literature (6,17).

It is only recently that studies have evaluated combinations of polymorphisms for a possible association with lung cancer risk and few studies have evaluated these combinations among never smokers. Our study of lifetime never smokers has identified significant differences in lung cancer risk associated with both *GSTM1* null and *GSTM1* null/*GSTP1* Val combined genotypes in the presence of high levels of ETS exposure. Because these polymorphisms play important overlapping roles in detoxifying tobacco carcinogens and because risk associated with these polymorphisms might only be evident at low exposure levels (such as ETS exposure rather than active smoke exposure) (29), the continued study of risk associated with multiple polymorphisms simultaneously among low exposure groups is essential. While studies of high frequency, low penetrant candidate susceptibility genes, including the *GST*s, have not been as revealing as originally hoped (30,31), continued study in large populations is needed to fully understand the complexities underlying susceptibility. This is likely to be best accomplished through collaborative consortiums.

Acknowledgements

The authors thank Donald R.Schwartz, PhD, for his technical assistance. This work was supported by NCI Grant R01-CA60691, Contract N01-CN65064 and Contract N01-PC-35145.

References

1. Ries,L.A.G., Eisner,M.P., Kosary,C.L., Hankey,B.F., Miller,B.A., Clegg,L.X. and Edwards,B.K.E. (2000) *SEER Cancer Statistics Review, 1973–1997*. National Cancer Institute, Bethesda, MD.
2. Hammond,E.C. and Seidman,H. (1980) Smoking and cancer in the United States. *Prev. Med.*, **9**, 169–174.
3. Strange,R.C. and Fryer,A.A. (1999) *The Glutathione S-Transferases: Influence of Polymorphism on Cancer Susceptibility*. IARC Scientific Publications, IARC, Lyon, pp. 231–249.
4. Ali-Osman,F., Akande,O., Antoun,G., Mao,J.X. and Buolamwini,J. (1997) Molecular cloning, characterization and expression in *Escherichia coli* of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. *J. Biol. Chem.*, **272**, 10004–10012.
5. Sunaga,N., Kohno,T., Yanagitani,N., Sugimura,H., Kunitoh,H., Tamura,T., Takei,Y., Tsuchiya,S., Saito,R. and Yokota,J. (2002) Contribution of the NQO1 and GSTT1 polymorphisms to lung adenocarcinoma susceptibility. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 730–738.
6. Nazar-Stewart,V., Vaughan,T.L., Stapleton,P., Van Loo,J., Nicol-Blades,B. and Eaton,D.L. (2003) A population-based study of glutathione S-transferase M1, T1 and P1 genotypes and risk for lung cancer. *Lung Cancer*, **40**, 247–258.
7. Benhamou,S., Lee,W.J., Alexandrie,A.K. *et al.* (2002) Meta- and pooled analyses of the effects of glutathione S-transferase M1 polymorphisms and smoking on lung cancer risk. *Carcinogenesis*, **23**, 1343–1350.
8. Taioli,E., Gaspari,L., Benhamou,S. *et al.* (2003) Polymorphisms in CYP1A1, GSTM1, GSTT1 and lung cancer below the age of 45 years. *Int. J. Epidemiol.*, **32**, 60–63.
9. Miller,D.P., Neuberg,D., de Vivo,I., Wain,J.C., Lynch,T.J., Su,L. and Christiani,D.C. (2003) Smoking and the risk of lung cancer: susceptibility with GSTP1 polymorphisms. *Epidemiology*, **14**, 545–551.
10. Wang,Y., Spitz,M.R., Schabath,M.B., Ali-Osman,F., Mata,H. and Wu,X. (2003) Association between glutathione S-transferase p1 polymorphisms and lung cancer risk in Caucasians: a case-control study. *Lung Cancer*, **40**, 25–32.
11. Kelsey,K.T., Spitz,M.R., Zuo,Z.F. and Wiencke,J.K. (1997) Polymorphisms in the glutathione S-transferase class mu and theta genes interact and increase susceptibility to lung cancer in minority populations (Texas, United States). *Cancer Causes Control*, **8**, 554–559.
12. Miller,D.P., Liu,G., De Vivo,I., Lynch,T.J., Wain,J.C., Su,L. and Christiani,D.C. (2002) Combinations of the variant genotypes of GSTP1, GSTM1 and p53 are associated with an increased lung cancer risk. *Cancer Res.*, **62**, 2819–2823.
13. Schwartz,A.G., Yang,P. and Swanson,G.M. (1996) Familial risk of lung cancer among nonsmokers and their relatives. *Am. J. Epidemiol.*, **144**, 554–562.
14. Lo,Y.M., Lau,T.K., Chan,L.Y., Leung,T.N. and Chang,A.M. (2000) Quantitative analysis of the bidirectional fetomaternal transfer of nucleated cells and plasma DNA. *Clin. Chem.*, **46**, 1301–1309.
15. Chen,C.-L. (1996) Simultaneous characterization of glutathione S-transferase M1 and T1 polymoprhisms by Polymerase chain reaction in American whites and blacks. *Pharmacogenetics*, **6**, 187–191.
16. Schlesselman,J.J. (1982) *Sample Size. Case Control Studies: Design, Conduct, Analysis*. Oxford University Press, New York, pp. 148–160.
17. Hung,R.J., Boffetta,P., Brockmoller,J. *et al.* (2003) CYP1A1 and GSTM1 genetic polymorphisms and lung cancer risk in Caucasian non-smokers: a pooled analysis. *Carcinogenesis*, **24**, 875–882.
18. Kiyohara,C., Wakai,K., Mikami,H., Sido,K., Ando,M. and Ohno,Y. (2003) Risk modification by CYP1A1 and GSTM1 polymorphisms in the association of environmental tobacco smoke and lung cancer: a case-control study in Japanese nonsmoking women. *Int. J. Cancer*, **107**, 139–144.
19. Malats,N., Camus-Radon,A.M., Nyberg,F. *et al.* (2000) Lung cancer risk in nonsmokers and GSTM1 and GSTT1 genetic polymorphism. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 827–833.
20. Wang,J., Deng,Y., Cheng,J., Ding,J. and Tokudome,S. (2003) GST genetic polymorphisms and lung adenocarcinoma susceptibility in a Chinese population. *Cancer Lett.*, **201**, 185–193.
21. Miller,D.P., De Vivo,I., Neuberg,D., Wain,J.C., Lynch,T.J., Su,L. and Christiani,D.C. (2003) Association between self-reported environmental tobacco smoke exposure and lung cancer: modification by GSTP1 polymorphism. *Int. J. Cancer*, **104**, 758–763.
22. Stucker,I., Hirvonen,A., de Waziers,I., Cabelguenne,A., Mitrunen,K., Cenee,S., Koum-Besson,E., Hemon,D., Beaune,P. and Loriot,M.A.

(2002) Genetic polymorphisms of glutathione S-transferases as modulators of lung cancer susceptibility. *Carcinogenesis*, **23**, 1475–1481.
23. Perera,F.P., Mooney,L.A., Stampfer,M. *et al*. (2002) Associations between carcinogen-DNA damage, glutathione S-transferase genotypes and risk of lung cancer in the prospective Physicians' Health Cohort Study. *Carcinogenesis*, **23**, 1641–1646.
24. Kihara,M. and Noda,K. (1999) Lung cancer risk of the GSTM1 null genotype is enhanced in the presence of the GSTP1 mutated genotype in male Japanese smokers. *Cancer Lett*., **137**, 53–60.
25. Sorensen,M., Autrup,H., Tjonneland,A., Overvad,K. and Raaschou-Nielsen,O. (2004) Glutathione S-transferase T1 null-genotype is associated with an increased risk of lung cancer. *Int. J. Cancer*, **110**, 219–224.
26. Spitz,M.R., Duphorne,C.M., Detry,M.A., Pillow,P.C., Amos,C.I., Lei,L., de Andrade,M., Gu,X., Hong,W.K. and Wu,X. (2000) Dietary intake of isothiocyanates: evidence of a joint effect with glutathione S-transferase polymorphisms in lung cancer risk. *Cancer Epidemiol. Biomarkers Prev*., **9**, 1017–1020.
27. Hou,S.M., Falt,S. and Nyberg,F. (2001) Glutathione S-transferase T1-null genotype interacts synergistically with heavy smoking on lung cancer risk. *Environ. Mol. Mutagen*., **38**, 83–86.
28. London,S.J., Yuan,J.M., Chung,F.L., Gao,Y.T., Coetzee,G.A., Ross,R.K. and Yu,M.C. (2000) Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms and lung-cancer risk: a prospective study of men in Shanghai, China. *Lancet*, **356**, 724–729.
29. Vineis,P. (1997) Molecular epidemiology: low-dose carcinogens and genetic susceptibility. *Int. J. Cancer*, **71**, 1–3.
30. Garte,S., Gaspari,L., Alexandrie,A.K. *et al*. (2001) Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol. Biomarkers Prev*., **10**, 1239–1248.
31. Caporaso,N.E. (2002) Why have we failed to find the low penetrance genetic constituents of common cancers? *Cancer Epidemiol. Biomarkers Prev*., **11**, 1544–1549.

Received June 28, 2004; revised September 30, 2004; accepted October 26, 2004