Lung Cancer Risk in Nonsmokers and *GSTM1* and *GSTT1* Genetic Polymorphism[1]

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Abstract

Glutathione *S*-transferase (GST) polymorphism may contribute to the individual variability in detoxifying lung carcinogens. This effect might be particularly relevant at low-level exposure to environmental carcinogens, such as in nonsmokers exposed to environmental tobacco smoke (ETS). We conducted a case-control study among 122 nonsmoking lung cancer cases and 121 nonsmoking controls from eight countries. Information on environmental exposures was obtained through a personal interview. The presence of *GSTM1* and *GSTT1* genes was determined using multiplex PCR. *GSTM1*-positive samples were then analyzed for *1A and *1B polymorphism using an allele-specific amplification-PCR method. *GSTM1*2* (null) individuals had an odds ratio (OR) of lung cancer of 1.5 [95% confidence interval (CI), 0.9–2.7]; the risk associated with this genotype was higher for cases with squamous and small cell carcinomas (OR, 2.3; 95% CI, 0.9–6.1) than for cases with adenocarcinomas. It was also elevated in individuals with long-term exposure to indoor wood combustion (OR, 3.1; 95% CI, 0.9–9.9), in subjects who mainly lived in a rural setting (OR, 3.6; 95% CI, 1.0–13), and in cases exposed to occupational carcinogens (OR, 10.7; 96% CI, 0.4–260) but not in subjects exposed to ETS. *GSTT1*2* subjects did not show a risk of lung cancer. Our study suggests that the effect of *GSTM1* polymorphism in nonsmokers is similar to that found in smokers. It does not seem to interact with ETS exposure, although we cannot exclude that it does in association with exposure to other specific environmental carcinogens.

Introduction

A large body of evidence indicates that exposure to ETS[3] can cause several disorders, notably lung cancer (1, 2). The evidence of the role of passive exposure to tobacco smoke in lung carcinogenesis has clearly been confirmed by Hackshaw *et al.* (3) in a meta-analysis of 37 studies, on the basis of which the authors estimated an increased risk of 26% (95% CI, 7–47%) of lung cancer in lifelong nonsmokers whose spouses smoked. In addition, the IARC has recently coordinated a large case-control study in seven European countries (4). The study showed a 16% excess risk of lung cancer among those subjects exposed to ETS from the spouse, a 15% excess risk when the exposure to ETS was at the workplace, and a decrease in excess risk after cessation of exposure from both sources.

Lung cancer in nonsmokers has also been associated with dietary factors (OR range, 0.4–0.7 for consumption of vegetables; Refs. 5–7), indoor exposure to fumes from cooking oils (OR range, 1.4–3.8; Refs. 7–9), coal or wood combustion (OR range, 1.3–2.5; Refs. 7, 9), occupation (OR range, 1.5–3.0; Ref. 10), prior lung diseases (OR range, 1.5–5.9; Refs. 6, 7, and 9), and family history of lung cancer (OR, 2.3; Ref. 8).

Because the excess risk from exposure to the above-mentioned factors is relatively low, it is important to identify individuals that might be more susceptible to lung cancer development. Individual variability in activating and detoxifying carcinogens may explain varying susceptibilities of developing lung cancer. The equilibrium between phase I (activating) and phase II (detoxifying) enzymes may contribute to this variability. Phase II enzymes are involved in detoxification of mutagens, carcinogens, and other substances (11, 12). GST family members (mu and theta) are among the most studied phase II enzymes regarding cancer susceptibility (13). There are well-defined genetic polymorphisms in the expression of GSTM1 and GSTT1 enzymes. The *GSTM1*2* (null) genotype, inherited in a dominant fashion, determines the absence of the enzyme in approximately 45–55% of Caucasians (12, 14). Lack of GSTM1 enzyme has been associated with susceptibility to lung

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[3] The abbreviations used are: ETS, environmental tobacco smoke; GST, glutathione *S*-transferase; GSTM1*2, GST mu null; GSTT1*2, GST theta null; IARC, International Agency for Research on Cancer; ASA, allele-specific amplification; OR, odds ratio; CI, confidence interval.

cancer among smokers (12, 13). *GSTT1*2* homozygosity is associated with the absence of the GSTT1 enzyme. *GSTT1* null prevalence in Caucasians is ~20%, but its role as a susceptibility factor for lung cancer is not well established (12, 13).

The study of the effect of GST polymorphism in nonsmokers provides the opportunity to assess the role of genetic susceptibility factors in lung cancer among individuals exposed to a low level of carcinogens. We conducted an international collaborative study to investigate the role of metabolic polymorphism of GST enzymes in lung carcinogenesis among nonsmokers and their interaction with environmental risk factors.

Materials and Methods

The project consisted of a case-control study among nonsmokers. Cases were recruited among patients with histologically or cytologically confirmed diagnosis of primary lung cancer in hospitals from Sweden, Germany, France, Italy, Russia, Romania, Poland, and Brazil. Controls were selected either among patients from the same hospital as the cases or, in Sweden and Germany, from healthy individuals identified from population registries (4). Although diagnoses of hospital-based control subjects varied among centers, they were all tobacco-related free diseases, *i.e.*, injuries; diseases of the musculoskeletal system; diseases of the genitourinary system; diseases of the digestive system such as hernia, diverticula, and fistula; chronic sinusitis; benign neoplasms; and malignant melanoma of the skin. All study subjects were Caucasians.

Information on demographics and environmental exposures was obtained through a personal interview using a standard questionnaire. A screening questionnaire allowed us to distinguish between smokers (subjects who smoked >400 cigarettes in their lifetime), occasional smokers (smokers of up to 400 cigarettes in their lifetime), and nonsmokers (pure never smokers). Occasional smokers were considered together with nonsmokers on the basis that estimates for the risk of lung cancer were below one, and results did not change when they were included in the analyses. We collected information on exposure to ETS from several sources: parents, spouses, other cohabitants at the same house during adulthood life, workplace, and vehicles. We considered in particular exposure to ETS from the spouse and at the workplace because in previous studies these were the main predictors of urinary cotinine (15) and showed the strongest association with lung cancer risk in a previous IARC case-control study of nonsmokers (4). A weighted duration of ETS exposure was calculated as hours of exposure/day and times the years of exposure from the spouse, and as hours/day, times the days/week of exposure at the workplace. To reduce misclassification, which has been shown to act mainly at low levels of exposure (16), subjects were then categorized in three levels: never exposed and exposed below or above the 75th percentile of the distribution among controls. Furthermore, exposure to known and suspected occupational lung carcinogens was considered on the basis of a job exposure matrix (17). Residential history was categorized as living more than 75% lifetime in a rural setting (cities <50,000 inhabitants) or in an urban setting (cities >50,000 inhabitants) or mixed. Use of wood or coal for cooking or heating was considered as source of indoor air pollution: duration of exposure was categorized in two groups according to the median of years of exposure to both sources of combustion among controls. Hospital records were reviewed to collect information on histological type of tumors.

Samples of peripheral blood (30 ml) were collected before any treatment in most patients. Plasma, red, and WBCs were separated for all individuals except for some from Sweden, from whom only whole blood was available. Samples were frozen in each center and stored at −80°C.

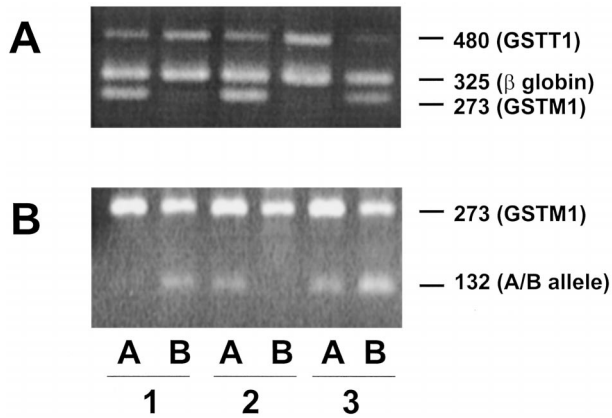

Fig. 1. Agarose gels with DNA from the amplification analysis of the *GSTM1* and *GSTT1* genes. *A*, the figure shows two DNA fragments of 273 and 480 bp that correspond to the presence of *GSTM1* and *GSTT1* genes, respectively. *B*, the ASA PCR analysis gives 132-bp fragments, corresponding to **A* and/or **B GSTM1* polymorphism, of three samples.

DNA was extracted from either whole blood or WBCs, using proteinase K (10 mg/ml) digestion at 55°C for 30 min. The presence of *GSTM1* and *GSTT1* genes was first tested using a multiplex PCR. To amplify both genes at the same time, primers M1E4 and M1E5, corresponding to exons 4 and 5 of the *GSTM1* gene (18, 19), and primers T11 and T12, corresponding to 3′ noncoding region of the *GSTT1* gene (19, 20), were used. In addition to specific *GST*s oligonucleotides, a pair of primers of β-globin (G3 and G4) was included as an internal amplification control (21). Ten μl of crude DNA extract (≅0.2 μg DNA) were added to a final volume of 50 μl containing 10× PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 1 mM $MgCl_2$], 5 mM deoxynucleoside triphosphate mixture, 10 μM of each primer, 1 unit of Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT), and sterile water. A PCR program of 30 cycles (94°C denaturation for 1 min, 52°C annealing for 1 min, and 72°C extension for 1 min) was conducted using a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer Cetus), preceded by a 2.5-min denaturation phase at 94°C and followed by an extension step of 72°C for 9 min. Ten μl of PCR product were analyzed by 3% agarose gel electrophoresis (NuSieve GTG), and gels were stained with ethidium bromide (20 μg/ml). An UV transilluminator was used to evaluate the PCR product content. Individuals carrying the *GSTM1* and *GSTT1* genes were identified by the presence of 273- and 480-bp DNA fragments, respectively (Fig. 1*A*). Eighty-six subjects from Sweden were reanalyzed in a different laboratory (22), and results were concordant in all but one. The case was not excluded from the study because identical results were obtained after a reanalysis of this sample.

GSTM1-positive samples were analyzed for the **A* and **B* genotype using a PCR ASA (23, 24). Briefly, two parallel assays were conducted for each sample: one with the reverse oligonucleotide specific for *GSTM1A* polymorphism (E7A) and the other with the primer specific for *GSTM1B* polymorphism (E7B); oligonucleotide I6 was used as the forward primer in both assays. In addition, the *GSTM1* gene was also amplified to verify its presence, and β-globin was coamplified as an internal standard. The PCR reactions were carried out in 50 μl contain-

ing 10 μl of crude DNA extract, 10× PCR buffer (50 mM KCl, 50 mM Tris-Cl, and 1.25 mM $MgCl_2$), 5 mM deoxynucleoside triphosphate mixture, 10 μM of each primer, 1 unit of Taq DNA polymerase (Perkin-Elmer Cetus), and sterile water. After a 2.5-min incubation at 94°C, a first round of 5 cycles was programmed (45 s at 94°C, 1 min at 58°C, and 1 min at 72°C), followed by a second round of 30 cycles (30 s at 94°C, 30 s at 58°C, and 45 s at 72°C, with a 3-s/cycle increase in extension time). PCR products were analyzed by 3% agarose gel electrophoresis, followed by ethidium bromide staining, and the presence of 132-bp fragments, corresponding to **A* and/or **B GSTM1* polymorphism, was determined using an UV transilluminator (Fig. 1*B*). Heterozygous null (*A0* and *B0*) and homozygous non-null (*AA* and *BB*) individuals couldn't be differentiated by this method. Analyses were conducted blindly to case-control status.

Statistical analyses were conducted using STATA software (STATA Corp., College Station, TX). Comparison of qualitative variables was performed using Pearson's χ^2 test or Fisher's exact test when 20% or more of the cells had expected counts of less than five. Student's *t* test and Mann-Whitney tests were used to assess the different distribution of a normal and nonnormal continuous variable, respectively (25). Age, gender, and center adjusted ORs of lung cancer and 95% CIs were calculated by unconditional logistic regression models (26). The association of *GST*s polymorphism to lung cancer was stratified by histological type, gender, age, and educational level. The role of *GST* polymorphism as a modifier of the effect of environmental exposures on lung cancer risk was analyzed both by stratification and by including the interaction term for each exposure-genotype combination in the logistic regression model. Results were considered significant at the two-sided *P* of 0.05 level.

Results

The study comprised 122 nonsmoking lung cancer cases and 121 nonsmoking controls. Participant centers contributed differently in the inclusion of study subjects: 36% of individuals came from Sweden, 16% from Germany, 12% from Russia, 11% from Romania, 8% from France, 8% from Poland, 5% from Italy, and 4% from Brazil. Among controls, 58 were healthy individuals, and 63 were hospital patients. According to histology, 65 (69%) cases were adenocarcinomas, 20 (21%) cases were squamous cell carcinomas, 5 (6%) cases were small cell carcinomas, 4 (4%) cases were large cell carcinomas, and 27 cases were classified as other or mixed histologies.

Table 1 shows the main demographic characteristics, as well as information on environmental exposures of cases and controls. Eighty-six % of cases and 72% of controls were women. Controls were younger than cases (mean age, 59 *versus* 64 years; $P = 0.004$). The proportion of subjects exposed to ETS from a spouse was higher among cases than among controls ($P = 0.04$). In addition, a higher proportion of cases were occasional smokers ($P = 0.1$), lived in a rural setting ($P = 0.004$), and were exposed to indoor pollution from wood combustion ($P = 0.001$) than controls.

Regarding GSTs genotype, 54% of cases were *GSTM1*2* (null) in comparison to 44% of controls (Table 2). The OR of lung cancer for the *GSTM1*2* genotype was 1.5 (95% CI, 0.9–2.7). The risk was higher for squamous and small cell carcinoma cases (OR, 2.3; 95% CI, 0.9–6.1) than for adenocarcinomas (OR, 1.2; 95% CI, 0.6–2.5). In comparison to *GSTM1*1A* individuals, *GSTM1*1B* and *GSTM1*AB* genotypes didn't represent a risk factor. The *GSTT1*2* genotype was less prevalent among cases than among controls (OR, 0.6; 95% CI, 0.3–1.2), and no difference was observed across histological types. When *GSTM1* and *GSTT1* genotypes were combined, *GSTM1*2/GSTT1*1* haplotype was associated with a nonsignificant increase of lung cancer risk, and again, the excess risk was higher among squamous and small carcinoma cases (OR, 2.6; 95% CI, 0.8–8.7).

The association between *GST*s null genotype and lung cancer was assessed across groups of subjects with different exposure to environmental risk factors (Table 3). In comparison to *GSTM1*1* subjects, *GSTM1*2* individuals who lived in a rural setting were over three times more likely to develop lung cancer (OR, 3.6; 95% CI, 1.0–13), and among subjects exposed >20 years to indoor wood combustion, *GSTM1*2* individuals

Table 1 Characteristics for the 122 nonsmoking cases of lung cancer and the 121 controls

Characteristics	Cases *n* (%)	Controls *n* (%)
Gender		
Men	17 (13.9)	34 (28.1)
Women	105 (86.1)	87 (71.9)
Age		
Minimum–49	19 (15.6)	26 (21.5)
50–59	26 (21.3)	34 (28.1)
60–69	26 (21.3)	36 (29.8)
70–maximum	51 (41.8)	25 (20.7)
Educational level		
<secondary	16 (25.0)	29 (31.2)
Some secondary	27 (42.2)	21 (22.6)
Secondary completed	12 (18.7)	14 (15.0)
>secondary	9 (14.1)	29 (31.2)
Missing	58	28
Exposure to environmental tobacco smoke from		
Spouse		
Never	64 (52.5)	79 (65.3)
Ever	58 (47.5)	42 (34.7)
1–119 h/day	41 (35.0)	27 (23.5)
>119 h/day	12 (10.3)	9 (7.8)
Missing[a]	5	6
Spouse or workplace		
Never	32 (26.2)	37 (30.6)
Ever	90 (73.8)	84 (69.4)
1–104 h/day	65 (55.5)	53 (49.1)
>104 h/day	20 (17.1)	18 (16.7)
Missing[a]	5	13
Occasional smokers		
Never	82 (68.9)	91 (78.3)
Ever	37 (31.1)	26 (21.7)
1–4.2 packs	20 (17.0)	12 (10.3)
>4.2 packs	16 (13.6)	11 (9.4)
Missing[a]	3	4
Exposure to known or suspected occupational carcinogens		
Never	105 (86.1)	105 (89.7)
Ever	17 (13.9)	16 (9.3)
Residence		
Rural (>75% lifetime)	42 (35.6)	24 (20.7)
Mixed rural and urban	44 (37.3)	38 (32.8)
Urban (>75% lifetime)	32 (27.1)	54 (46.6)
Missing	4	5
Indoor pollution from		
Wood (>20 yr)	47 (38.8)	27 (22.3)
Coal (>17 yr)	11 (9.1)	22 (18.2)

[a] Missing data refers to quantitative variables. h/day, hours/day times years.

Table 2 Distribution, ORs, and 95% CIs for lung cancer according to *GSTM1* and *GSTT1* genotypes

Genotype	All lung cancer		Squamous and small cell carcinomas		Adenocarcinomas	
	Cases/controls	OR[a] (95% CI)	Cases	OR[a] (95% CI)	Cases	OR[a] (95% CI)
GSTM1						
**1*	56/68	1 (ref.)[b]	9	1 (ref.)[b]	34	1 (ref.)[b]
**2*	66/53	1.5 (0.9–2.7)	16	2.3 (0.9–6.1)	31	1.2 (0.6–2.5)
GSTT1						
**1*	90/77	1 (ref.)[b]	18	1 (ref.)[b]	51	1 (ref.)[b]
**2*	32/44	0.6 (0.3–1.2)	7	0.6 (0.2–1.7)	14	0.5 (0.2–1.1)
M1 and T1						
**1*1*	41/44	1 (ref.)[b]	6	1 (ref.)[b]	26	1 (ref.)[b]
**1*2*	15/24	0.8 (0.3–1.9)	3	0.9 (0.2–4.8)	8	0.8 (0.3–2.5)
**2*1*	49/33	1.8 (0.9–3.5)	12	2.6 (0.8–8.7)	25	1.9 (0.7–3.7)
**2*2*	17/20	0.9 (0.4–2.1)	4	1.4 (0.3–6.9)	6	0.5 (0.2–1.7)

[a] Adjusted for gender, age, and center.
[b] ref., reference category.

Table 3 ORs of *GSTM1*2* and *GSTT1*2* in nonsmokers stratified by environmental exposures

	*GSTM1*2* OR[a] (95% CI)	*GSTT1*2* OR[a] (95% CI)
ETS spouse		
Never	1.5 (0.7–3.3)	1.0 (0.4–2.4)
Ever	1.4 (0.5–3.8)	0.3 (0.1–0.9)
<119 h/day[b]	1.0 (0.3–3.3)	0.8 (0.2–2.5)
>119 h/day	∞	∞
ETS spouse or work		
Never	1.2 (0.4–4.2)	0.8 (0.2–2.9)
Ever	1.7 (0.8–3.4)	0.7 (0.3–1.4)
<104 h/day	1.8 (0.8–4.4)	1.1 (0.5–2.8)
>104 h/day	0.3 (0–4.2)	0.1 (0–1.9)
Occasional smoking		
Never	1.4 (0.7–2.7)	0.5 (0.2–1.0)
Ever	2.1 (0.4–12)	1.0 (0.1–6.7)
<4.2 p/lt	4.7 (0.3–86)	2.0 (0.1–68)
>4.2 p/lt	0.5 (0–24)	3.4 (0–416)
Occupational		
No	1.5 (0.8–2.7)	0.7 (0.4–1.3)
Yes	10.7 (0.4–260)	∞
Residential		
Rural	3.6 (1.0–13)	0.1 (0–0.5)
Mixed	1.6 (0.5–5.3)	1.9 (0.5–6.7)
Urban	0.9 (0.3–2.9)	1.0 (0.3–3.4)
Indoor wood combust.		
No	1.2 (0.5–3.2)	0.5 (0.2–1.5)
<20 yr	1.3 (0.3–5.2)	0.5 (0.1–1.8)
>20 yr	3.1 (0.9–9.9)	0.5 (0.1–1.5)

[a] Adjusted for gender, age, and center.
[b] h/day, hours/day times years; p/lt, packs/lifetime; ∞, infinite OR, no controls exposed.

showed an OR of 3.1 (95% CI, 0.9–9.9). Results did not change after including both variables in the model. Although cases exposed to known or suspected occupational carcinogens had an OR of 11, this result is based on a small number of individuals (95% CI, 0.4–260). The risk of lung cancer associated with *GSTM1*2* genotype was higher among individuals exposed to ETS from the spouse or at the workplace than among unexposed individuals, but this difference was not statistically significant, and there was no relationship with the estimated duration of ETS exposure. *GSTT1*2* genotype was not significantly associated with lung cancer risk in any risk factor categories. *GST* null genotype and lung cancer association was also assessed across gender, age, and educational level strata, and no significant differences emerged (data not shown).

Table 4 shows the modifying effect of *GST*s genotypes on the association of several environmental exposures with lung cancer risk. *GSTM1*2* individuals presented a higher risk of lung cancer (OR, 6.2; 95% CI, 1.5–25) than *GSTM1*1* individuals (OR, 1.8; 95% CI, 0.5–7.1) after exposure to indoor wood combustion for >20 years. The risk for *GSTM1*2* cases decreased when the analysis was adjusted by residence history (OR, 3.0; 95% CI, 0.6–15). The same pattern was suggested for exposure to occupational carcinogens, although the small number of exposed subjects produced unstable risk estimates. Although weakly and nonsignificantly associated with lung cancer risk, similar results were observed for *GSTM1*2* cases exposed to >119 h/day times years of exposure to ETS from spouse, cases who smoked >4.2 packs/lifetime, and cases exposed to known or suspected occupational lung carcinogens as compared with the subjects in the low exposure category of each risk factor. Surprisingly, *GSTM1*2* individuals presented a stronger negative association with residence in urban settings than *GSTM1*1* individuals, and results did not change when they were adjusted for indoor pollution from wood or coal combustion. No effect modification of *GSTM1* polymorphism was suggested for exposure to ETS, not even when the duration of ETS exposure was considered (results not shown in detail). The effect of *GSTT1* genotypes on lung cancer risk according to environmental exposures lacked consistency and was difficult to interpret.

The estimates did not substantially change when the analysis was restricted to women. Multiplicative interactions tests and stratified analyses by age, center, or histological type were not significant, because of the small number of subjects in most strata.

To improve the statistical power of the study and to eliminate the potential control selection bias, a case-case analysis was conducted to assess the modifier effect of *GST* genotypes on the association of selected exposures with lung cancer. As expected, no important differences from the case-control approach were observed. Occupational exposures was the only variable in which an interaction with *GSTM1* genotype was observed (OR, 2.5; 95% CI, 0.7–8.8; $P = 0.156$). Although nonsignificant, possible because of the small number of subjects, occupational exposures deserve more attention in future studies.

Table 4 Effect modification of *GSTM1* and *GSTT1* genotypes on nonsmoking lung cancer risks attributable to environmental exposures

Exposures[a]	All subjects OR[b] (95% CI)	*GSTM1*1* OR[b] (95% CI)	*GSTM1*2* OR[b] (95% CI)	*GSTT1*1* OR[b] (95% CI)	*GSTT1*2* OR[b] (95% CI)
ETS from					
Spouse (ever)	1.5 (0.8–2.6)	1.7 (0.7–4.0)	1.3 (0.6–3.1)	2.3 (1.1–4.8)	0.5 (0.1–1.9)
Spouse or workplace (ever)	1.2 (0.6–2.3)	1.3 (0.4–3.7)	1.2 (0.5–2.7)	1.7 (0.8–3.9)	0.3 (0.1–1.5)
Occasional smokers (ever)	1.4 (0.7–2.9)	2.1 (0.7–6.2)	2.1 (0.7–6.4)	1.3 (0.5–3.0)	3.7 (0.5–27)
Occupational carcinogens	1.6 (0.6–4.0)	1.2 (0.3–5.2)	2.5 (0.7–9.0)	2.5 (0.8–7.8)	[c]
Residential history					
Mixed rural and urban	0.4 (0.2–0.9)	0.6 (0.2–1.8)	0.2 (0–0.6)	0.3 (0.1–0.7)	0.3 (0–3.0)
Urban (>75% lifetime)	0.2 (0.1–0.5)	0.3 (0.1–1.1)	0.1 (0.2–0.4)	0.1 (0–0.4)	0.5 (0.1–3.3)
Indoor pollution from					
Wood (>20 y)	2.5 (1.0–6.2)	1.8 (0.5–7.1)	6.2 (1.5–25)	3.0 (1.0–8.7)	1.7 (0.2–17)
Coal (>17 y)	0.4 (0.1–1.1)	0.3 (0.1–1.3)	0.4 (0.1–2.2)	0.5 (0.1–1.8)	0.2 (0–2.3)

[a] For reference categories, see Table 1.
[b] Adjusted for gender, age, and center.
[c] OR could not be estimated because of insufficient numbers in cells.

Discussion

It has been suggested (27, 28), and in some studies substantiated (19, 29, 30), that genetic predisposition to cancer may have a stronger effect on tumor development upon low-dose exposure to carcinogens. This hypothesis is based on the fact that high-dose exposure may saturate enzyme activity independently of the polymorphism, so that no effect of genotype would be apparent. This idea has been tested in this study in the context of lung cancer development in nonsmokers.

Our results show that, as in smokers (12), *GSTM1*2* genotype is associated with an increased risk for lung cancer in nonsmokers. The overall excess risk for this genotype is 50%, although it varies when subjects are stratified according to exposure. The factors that showed the greatest change in *GSTM1*-associated risk were indoor wood combustion, area of residence, and occupational exposure to carcinogens. However, when lung cancer risk because of indoor pollution from wood combustion was considered, taking into account the area of residence, the estimates became nonsignificant, although they still show an increase of risk: OR for all subjects, 1.7 (95% CI, 0.6–4.6), for *GSTM1*1* and *GSTM1*2* individuals 1.8 (95% CI, 0.4–7.6) and 3.0 (95% CI, 0.6–15), respectively. No confounding effect was observed when indoor pollution from coal combustion was modeled. ORs for residential history retained their former values in both models. Regarding this latter risk factor, we found a significantly decreased in risk of lung cancer among *GSTM1*2* patients who did live in urban areas in comparison to those who lived in rural areas. Furthermore and in agreement with the case-control results, a case-case design conducted within the series of cases of the present study showed that both occupational exposures (OR, 2.5; 95% CI, 0.7–8.8) and a residential history in a urban area (OR, 0.4; 95% CI, 0.1–1.3) presented interesting patterns of interaction with *GSTM1* genotype. The study Nielsen *et al.* (31) conducted in nonsmoker healthy residents of rural and urban areas found no effect of *GSTM1*2* genotype on DNA or protein adduct levels. Possible explanations of this discrepancy could be differences among centers in control selection criteria applied; in frequency of metabolic polymorphisms that could interact with *GSTM1* null genotype (29); and in prevalence of additional environment factors, such as vitamins, that could also modify the interaction of *GSTM1* and the study variables on lung cancer risk (32). Subjects exposed to passive smoking and occasional smokers did not show an excess risk. Nyberg *et al.* (22) have recently published the only study on *GSTM1* polymorphism and lung cancer in a nonsmoker series, and they found no association with the null genotype. In addition to random variation, the authors pointed out methodological issues to explain contradictory results among studies.

From our results, *GSTM1*2* genotype conferred a higher risk for squamous and small cell carcinomas than for adenocarcinoma. This finding agrees with our knowledge of the role of GSTs enzymes in detoxification of tobacco-related carcinogens because adenocarcinoma type is less associated with tobacco consumption, and consistent results regarding squamous and small cell histology have been shown from studies conducted in smokers (12).

In accordance with Bennett *et al.* (33), we found that *GSTT1*2* genotype did not present an increased risk for lung cancer. Our *a priori* hypothesis postulated that null *GSTT1* subjects (*GSTT1*2* genotype) would be at higher risk of cancer because they have a reduced ability to conjugate carcinogens to excretable hydrophilic metabolites (12). Previous epidemiological studies on the role of *GSTT1*2* genotype in lung carcinogenesis have not demonstrated a consistent increase of lung cancer risk in smokers (24, 34, 35). Rebbeck (12) points to the fact that some *GSTT1* metabolites could act as tissue-specific mutagens. Regarding other tobacco-related neoplasms, two studies (36, 37) have found a statistically significant increased risk for bladder cancer only among nonsmokers with *GSTT1* null genotype. In studies of other tumors, no consistent results on the role of *GSTT1* enzyme in cancer susceptibility have been reported (12, 38). This result is further substantiated by the study conducted by Rojas *et al.* (39) in which they did not observe differences in benzo[*a*]pyrene diol epoxide-DNA adduct level between *GSTT1* null and active genotypes in polycyclic aromatic hydrocarbon-exposed workers.

According to the hypothesis mentioned above, subjects with the *GSTM1*2-GSTT1*2* haplotype, 14% of lung cancer cases in our series, would show the highest risk for lung cancer. Nevertheless, our results do not support such a contention. In agreement with our findings, To-Figueras *et al.* (24) did not find an increased lung cancer risk among smokers with this haplotype. On the other hand, in our data the *GSTM1*2-GSTT1*1* haplotype had an overall 80% excess risk for lung cancer, although this difference did not reach statistical significance.

The following limitations should be considered in interpreting our results. The statistical power of the study is low, mainly for assessing the modifying role of *GST*s on the effect

of some environmental risk factors on lung cancer (40). Furthermore, false-positive results attributable to multiple comparisons cannot be discarded, although we present only data derived from a prespecified hypothesis. Controls were selected from two different sources, *i.e.*, hospital and population. GST null genotypes could be associated with illnesses among hospital controls or some characteristics, gender, age, and tobacco habits, in the general population (41). We observed different estimates for *GSTM1*2* genotype when we stratified the analysis according to the controls origin, population (sex and age adjusted OR, 2.36; 95% CI, 1.06–5.28; $P = 0.036$) *versus* hospital (adjusted OR, 1.13; 95% CI, 0.54–2.39; $P = 0.746$). Unfortunately, an insufficient number of subjects was available to stratify the analysis by this factor. Scarce bibliography is available on population heterogeneity of *GST* polymorphisms among European Caucasians (20). However, the possibility that, if present, this fact could bias our results was checked by assessing the differences among *GSTM1*2* and *GSTT1*2* prevalences among controls from population-based and hospital-based centers. No significant variability was observed; nevertheless, results were all adjusted for center. Finally, recall information on past exposure to ETS could be subject to exposure misclassification, resulting in bias (42). Because misclassification is more likely to be present at low doses of ETS (16), we addressed this potential problem by categorizing the ETS variables in three groups: never exposed, and subjects exposed to less and more than 75% percentile of the dose-index variable computed for each passive smoking source. In addition, the questionnaire used in this study allows the collection of detailed information on ETS exposure and has previously been applied and validated in a larger case-control study (4, 22).

Our study suggests that the effect of *GSTM1* polymorphism in nonsmokers is similar to that found in smokers. It does not seem to interact with ETS exposure, although we cannot exclude that it does in selected groups of individuals in association with exposure to other specific environmental carcinogens. Because of the complexity of lung cancer etiology, it is unlikely that a single polymorphism, either *GSTM1*2* or *GSTT1*2*, could explain most cancer susceptibility. The joint analysis of several metabolic gene polymorphisms implied in carcinogen activation and detoxification may provide new clues on lung carcinogenesis in nonsmokers.

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