

GSTM1, smoking and lung cancer: a case-control study

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Background	We conducted a case-control study to examine the risk of lung cancer in relation to GSTM1 polymorphism and cigarette smoking (primarily of black tobacco) in a French population.
Methods	The 611 subjects were 301 incident lung cancer cases and 310 hospital controls. We were able to constitute a DNA bank for 547 subjects (89.5%) and gather detailed information on smoking history for all of them. Results presented here concern 247 cases and 254 controls.
Results	Taking non- or light smokers as the reference category, we estimated odds ratios (OR) of 4.2 (95% CI : 2.6–6.7) and 5.2 (95% CI : 3.3–8.3) for the medium and heavy smokers respectively. On the other hand we estimated that the crude OR associating GSTM1 with lung cancer was 1.3 (95% CI : 0.9–1.8). Furthermore our data do not depart significantly from a multiplicative model of the combined effects of smoking and GSTM1 deficiency.
Conclusions	We conclude that smoking and the GSTM1 gene are each a risk factor for lung cancer, and that their combined effect does not differ significantly from that of a multiplicative model.
Keywords	Case-control study, GSTM1, tobacco, lung cancer, genetic-environment interaction
Accepted	11 February 1999

A growing body of experimental and epidemiological evidence suggests that cancer risk as well as lung cancer susceptibility results from the combined effect of genes and environment. Knowledge of genetic risk factors may increase the statistical power of future epidemiological studies, particularly those aimed at investigating common risk factors with only a moderate relation to lung cancer (e.g. passive tobacco, air pollution).

Many genes may be involved in cancer susceptibility at various stages of the carcinogenic process; these can cause differences in the metabolism of carcinogens and in DNA repair and may alter the expression of protooncogenes or tumour suppressor genes.¹ The possible host factors most thoroughly investigated so far are those both related to the metabolism of procarcinogens and for which polymorphisms have been identified.

Human exposure to polycyclic aromatic hydrocarbons (PAH), a widespread group of chemical compounds, comes from numerous sources in the general environment (air, water, soil, tobacco smoke, food consumption) and in occupational settings.²

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Cigarette smoking is the strongest known risk factor for lung cancer. The PAH comprise one of the most important families of carcinogens, and one of its major constituents, benzo(a)pyrene (B(a)P), has been classified as carcinogenic for humans.³ Several genes govern PAH metabolism. CYP1A1 is involved in its activation phase.⁴ The glutathione S-transferases (GST) are involved in the detoxification phase: they allow conjugation between electrophilic metabolites and glutathione molecules.⁴ In this superfamily of enzymes, the μ class GST catalyse the reaction with such electrophiles as polyaromatic hydrocarbon epoxides of B(a)P and therefore prevent the formation of its diol epoxide (BPDE), the powerful mutagen that is its terminal metabolite. One study has shown the formation of PAH-DNA adducts in lung tissue from BPDE.⁵

The class μ isozymes include those produced at the polymorphic GSTM1 locus. This gene has been shown to be deleted in approximately half the subjects studied.^{6,7} When it is, the enzyme is absent. A recent study examined specific PAH-DNA adducts (PAH-dGMP) in human lung tissues and found a clear association between adducts and the GSTM1 polymorphism.⁸

Several case-control studies have examined the effect of this polymorphism on the risk of lung cancer in various populations.^{9–24} A meta-analysis found that the odds ratio (OR) of the association between lung cancer and GSTM1 was 1.4 (95% CI : 1.2–1.6).²⁵ McWilliams's analysis also observed that the association seemed stronger for the Japanese subjects (OR = 1.6 [95% CI : 1.2–1.6]) than for whites (OR = 1.2 [95% CI : 1.0–1.4]).

Some studies have reported that the association between lung cancer and GSTM1 is strongest among heavy smokers.^{9,14,15,19} Others, however, have found a stronger relation among light smokers.^{18,20,22,24,26}

In this context, it appeared useful to us to analyse in detail the hypothesis that tobacco use may modify the lung cancer risk associated with this polymorphism.

Our results are based on a French lung cancer case-control study. The analysis presented here is directed toward two main goals: estimating the association between GSTM1 and lung cancer in a French population and studying the combined effect of smoking and GSTM1 on lung cancer risk.

Subjects and Methods

Subjects

All patients were recruited in three French hospitals, two in the Paris metropolitan area and one in Besançon (Eastern France). For slightly more than 3 years (September 1989–December 1992), all new patients in these hospitals who met the following criteria were prospectively recruited: a histologically-confirmed lung cancer diagnosis, male, <75 years old, no previous cancer diagnosis, no previous radio- or chemotherapy. Hospital controls were matched for sex, age (± 2 years), hospital, and residence area. All cases agreed to participate. A small number of controls refused. In this case, the next patient matched for the same criteria was asked to participate. Cases and controls were white, born in France of native French parents. Table 1 presents the diagnoses of the control subjects and the histological types of lung cancer among the cases. No control subjects had a non-cancerous lung disease or any kind of cancer. This study included 301 cases and 310 controls.

Interview

Cases and controls were interviewed using a standard questionnaire that included questions about demographic characteristics,

previous family history of cancer (parents and siblings), and lifetime occupational history. Detailed information was collected about cigar, pipe, and cigarette consumption and smoking habits. For each brand and type of tobacco patients had ever smoked, they were asked to specify their age when they started and, if applicable, stopped, usual daily consumption, use of filter tips (for cigarette smokers), and, lastly, if they inhaled usually, sometimes, or not at all. All cases and controls answered the questionnaire.

Blood sample

Once the lung cancer diagnosis was histologically confirmed, before the initiation of any chemo- or radiotherapy, blood samples (20 ml) were taken. Lymphocyte extraction was performed within 36 hours; the DNA was then isolated from the lymphocyte preparations. The DNA bank was established for only 552 of the 611 subjects (89.5%), because blood was not taken from the first 19 subjects (those who allowed us to refine the recruitment protocol), nor from another 23 patients for medical reasons; samples from 17 were lost or damaged. Finally, for 51 subjects for whom DNA extraction had been difficult, the available DNA was used up in studying other polymorphisms. The present study of the combined effect of smoking and GSTM1 deficiency on lung cancer risk is therefore based on the comparison of 247 cases and 254 controls.

Genotyping

Polymerase chain reaction (PCR) assays were performed with 50–100 ng of DNA in 10mM Tris-HCl, pH 8.3, 50 mM KCl, 1 mM MgCl₂, 0.2 mM of each deoxynucleotide triphosphate, 1 μ M of each primer, and 0.4 units of Taq-polymerase. Amplifications were performed with a 10-sec denaturation step at 95°C, one minute annealing at 53°C, and one minute extension at 74°C. The PCR products were detected and sized by agarose gel electrophoresis. We tested two combinations of primers (primers 1 and 2, primers 2 and 3) for each sample and confirmed each GSTM1 genotype with both combinations. In the genomic DNA of individuals with high GST activity, a fragment (of a trans-stilbene oxide conjugation with glutathione) could be amplified for each primer combination (273 bp for the first, 650 bp for the second). These fragments could not be amplified in people with impaired GST μ activity (13), that is, with a large deletion mutation with the GSTM1 gene (null allele). We used the following primer sequences:

Primer 1: CTGCCCTACTTGATTGATGGG
Primer 2: CTGGATTGTAGCAGATCATGC
Primer 3: CTCCTGATTATGACAGAAGCC

Cigarette smoking

Tobacco consumption was expressed either by two independent variables—mean lifetime quantity smoked, and total duration (duration in years of all periods during which the subject smoked)—or in terms of pack-years (sum of the product of quantity times duration for each of the smoking periods). Subjects were classified in three smoking categories according to the pack-years smoked, so that we might observe an approximate minimum of 50 cases and 50 controls in each class: light (<25 pack-years), medium (25–40 pack-years), and heavy (>40 pack-years) smokers.

Table 1 Diagnoses of controls and histological types of cases^a

	N (%)	Age m \pm sd
All lung cancer	247 (100)	59.7 \pm 9.5
Squamous cell carcinoma	113 (46)	60.3 \pm 9.0
Small cell carcinoma	49 (20)	62.3 \pm 9.1
Adenocarcinoma	57 (23)	56.6 \pm 10.3
Others ^b	28 (11)	59.0 \pm 9.3
All controls	254 (100)	59.3 \pm 10.0
Nervous system and sense organs (320–389) ^c	64 (25)	63.5 \pm 7.4
Circulatory system (390–459)	53 (21)	60.9 \pm 8.4
Musculoskeletal diseases (710–739)	16 (7)	53.9 \pm 12.5
Skin diseases (680–709)	30 (12)	56.5 \pm 12.2
Digestive system (520–579)	26 (10)	60.9 \pm 9.3
Injury and Poisoning (800–999)	23 (9)	56.3 \pm 9.8
Other	42 (17)	55.6 \pm 10.8

^a Pack-years are missing for 8 controls and 7 cases.

^b Including 8 subjects for whom histological type is missing, 10 large cell carcinoma, 6 non-small cell carcinoma, 1 bronchial carcinoma and 3 unspecified.

^c International Classification of Diseases code.

Statistical analysis

Statistical analysis was performed on a VAX (Digital Equipment). For the univariate analysis and the adjustment for single confounders, we used Statistical Analysis Software (SAS). The estimates we calculated were odds ratio (OR) with their 95% CI.

Potential confounding by the matching variables of age, hospital, and residence was accounted for by including them in an unconditional logistic regression, since the strata were large enough not to require matched analysis.²⁷ BMDP software performed logistic regression to adjust simultaneously for several confounders. The OR and 95% CI were calculated using the formulae: $OR = \exp(\beta)$ and 95% CI: lower limit = $\exp[\beta - 1.96 \times SE(\beta)]$, upper limit = $\exp[\beta + 1.96 \times SE(\beta)]$, with β the estimate of the regression coefficient and $SE(\beta)$ its standard error.

Table 2 Characteristics of tobacco consumption among cases and controls^a

	Cases		Controls	
Non smokers	0		51	
Ex-smokers	178		135	
Smokers	69		68	
Type of tobacco				
Dark	175 (71%)		132 (65%)	
Light	4 (1.6%)		16 (7.8%)	
Both	3 (15.8%)		31 (15.3%)	
Unknown	29 (11.7%)		24 (11.8%)	
	All	Smokers + ex-smokers	All	Smokers + ex-smokers
	m ± sd	m ± sd	m ± sd	m ± sd
Cigarettes/day				
Total	20.8 ± 9.8	20.8 ± 9.8	15.1 ± 13.8	19.1 ± 12.9
Dark	20.4 ± 9.8	20.8 ± 9.8	14.0 ± 14.7	20.0 ± 13.8
Light	3.5 ± 9.3	19.7 ± 13.0	2.7 ± 6.9	14.1 ± 9.5
Duration				
Total	37.1 ± 10.5	37.1 ± 10.5	23.4 ± 16.2	29.4 ± 12.5
Dark	35.1 ± 14.3	35.8 ± 13.5	19.3 ± 16.0	27.5 ± 13.0
Light	2.9 ± 8.2	14.8 ± 13.3	3.8 ± 10.1	19.8 ± 14.6
Pack-years				
Total	38.0 ± 19.5	38.0 ± 19.5	22.7 ± 23.1	29.4 ± 25.1
Dark	35.1 ± 14.3	36.6 ± 20.0	19.6 ± 23.6	28.0 ± 23.6
Light	2.9 ± 8.2	16.2 ± 17.5	3.0 ± 9.4	15.6 ± 16.2

^a One non-smoker excluded.

Results

As expected, case patients smoked more, by every measure, than the controls. Furthermore, there were no non-smokers among the cases, while 20% of the controls did not smoke. Mean pack-years of cigarette smoking was significantly higher among cases than controls (Table 2). The OR associated with pack-years of cigarette smoking, with the group with <25 pack-years as the reference category, was 4.1 (95% CI: 2.6–6.6) for the medium smokers (25–40 pack-years) and 5.1 (95% CI: 3.2–8.01) for the heavy smokers (>40 pack-years). Of 501 subjects, 247 had the null allele (49.3%). Table 3 shows the distribution of the GSTM1 genotype among cases and controls. The frequency of the null genotype was slightly higher among cases (52%) than controls (46%) (OR = 1.3 [95% CI: 0.9–1.8]) and, among cases, it was higher for small cell carcinoma and, especially, adenocarcinoma, but not for squamous cell carcinoma. The OR of GSTM1 was higher for subjects who were younger at diagnosis (<60 years OR = 1.6 [95% CI: 0.9–2.6]) than for the group ≥60 years (OR = 1.1 [95% CI: 0.6–1.7]). This was true overall and for each separate histological type.

Table 4 shows the relation between lung cancer and GSTM1 according to pack-years of cigarette smoking. For all lung cancer cases together, the GSTM1 null genotype was more frequent among cases than controls in all three categories. We observed OR of 1.2 (95% CI: 0.7–2.3), 2.1 (95% CI: 1.0–4.4), and 1.3 (95% CI: 0.6–2.6) for the light, medium, and heavy smokers, respectively. Using a logistic regression, we found no statistically significant interaction between smoking category and GSTM1 genotype. Results differed slightly according to histological type. For squamous cell carcinoma, we observed OR close to 1, and non-significant. For small cell carcinoma, the frequency of the null genotype was always higher among cases, but the only OR significantly greater than one concerned the medium smoking category (OR = 7.2 [95% CI: 1.3–39.4]). The highest frequencies of the null genotype were observed among the adenocarcinoma patients, and the OR increased with pack-year category. These OR were 1.8 (95% CI: 0.6–5.5), 2.2 (95% CI: 0.7–6.5), and 3.4 (95% CI: 1.0–12.2) for the light, medium and heavy smokers, respectively. The interaction terms between smoking and the GSTM1 polymorphism in each histological type considered separately were not significant. To verify that the lack of interaction was not related to the paucity of subjects in each category, particularly in view of the results for the adenocarcinoma patients, we looked for an interaction between the two risk

Table 3 Odds ratios (OR) of lung cancer related to GSTM1 genotype, according to histological type^a

	N	GSTM1(+)		GSTM1(–)		OR ^b (95% CI)
		n	%	n	%	
Controls	254	136	54	118	46	
Cases	247	118	48	129	52	1.3 (0.9–1.8)
SQCC ^c	113	63	56	50	44	0.9 (0.6–1.4)
SMCC ^d	49	20	41	29	59	1.7 (0.9–3.2)
ADK ^e	57	21	37	36	63	2.0 (1.1–3.6)

^a Histological type is missing for 28 subjects.

^b Odds ratio adjusted for age, hospital, mean daily cigarette consumption, lifetime duration of smoking.

^c Squamous cell carcinoma.

^d Small cell lung cancer.

^e Adenocarcinoma.

Table 4 Odds ratios (OR)^a of lung cancer related to GSTM1 genotype, according to histological type and pack-years^b

	Pack-years <25			Pack-years 25–40			Pack-years >40		
	N	% null	OR (95% CI)	N	% null	OR (95% CI)	N	% null	OR (95% CI)
Controls	145	49.0	1.0 (Ref)	49	40.8	1.0 (Ref)	52	42.3	1.0 (Ref)
All cases	58	53.5	1.2 (0.7–2.3)	79	58.2	2.1 (1.0–4.4)	103	48.5	1.3 (0.6–2.6)
SQCC ^c	25	44.0	0.8 (0.3–1.9)	32	53.1	1.8 (0.7–4.8)	52	40.4	0.9 (0.4–2.0)
SMCC ^d	12	58.3	1.5 (0.4–5.0)	12	83.3	7.2 (1.3–39.4)	23	47.8	1.3 (0.4–3.6)
ADK ^e	16	62.5	1.8 (0.6–5.5)	22	59.1	2.2 (0.7–6.5)	18	72.2	3.4 (1.0–12.2)

^a Adjusted for age, hospital, mean daily cigarette consumption, lifetime duration of smoking.

^b Pack-years is missing for 8 controls and 7 cases.

^c Squamous cell carcinoma.

^d Small cell lung cancer

^e Adenocarcinoma.

factors by using a logistic regression that considered the pack-years of cigarette smoking quantitatively rather than in three classes. In this case, the OR of interaction represent the ratio of the slopes of the relation between cigarette smoking (measured on a quantitative pack-years scale) and lung cancer among the GSTM1 active subjects and among those with null allele. They were very close to one for all lung cancer cases ($P = 0.84$) and for each histological type, considered separately. We obtained the same results for the interaction between GSTM1 and the mean number of cigarettes smoked per day (adjusted for the total duration) and between GSTM1 and the total duration of cigarette smoking (adjusted for the mean number of cigarettes smoked per day).

Table 5 shows the estimated OR of lung cancer, all types, according to both risk factors—smoking and GSTM1 polymorphism. The reference category was that of the light smokers who were GSTM1-active. We estimated combined OR of 1.0, 3.2 (95% CI: 1.6–6.3), and 5.0 (95% CI: 2.6–9.5) for GSTM1-active subjects who were light, medium and heavy smokers, respectively. They rose to 1.2 (95% CI: 0.7–2.2), 6.5 (95% CI: 3.3–13.0), and 6.4 (95% CI: 3.3–12.5) for subjects with the null allele. These results do not differ significantly from an independent multiplicative effect of smoking and the GSTM1 polymorphism. We observed similar results when we considered each histological type separately.

In a final logistic regression model that did not include interaction terms, we assessed the association of GSTM1 and lung cancer and obtained an OR of 1.4 (95% CI: 0.9–2.1). It was not possible to study this relation according to the type of tobacco smoked since too few subjects had smoked only blond tobacco (4 cases and 16 controls). Nonetheless, the final logistic regression

Table 5 Odds ratio^a of lung cancer according to GSTM1 genotype and pack-years of cigarettes smoked

	Pack-years of cigarettes smoked		
	Light smokers (<25)	Medium smokers (25–40)	Heavy smokers (>40)
GTSTMI (+)	(27/74) ^b	(33/29)	(53/30)
	1.0	3.2 (1.6–6.3)	5.0 (2.6–9.5)
GSTM1 (–)	(31/71)	(46/20)	(50/22)
	1.2 (0.7–2.2)	6.5 (3.3–13.0)	6.4 (3.3–12.5)

^a Odds ratio adjusted for age and hospital.

^b (Cases/controls).

model restricted to non-smokers or smokers of black tobacco only (170 cases and 180 controls) found an OR of 1.4 (95% CI: 0.8–2.3).

Discussion

This study was intended to answer two questions: (1) What is the OR of lung cancer related to the GSTM1 genotype in a French population? (2) What model best describes the combined effect of lifetime tobacco exposure and the GSTM1 genotype in the risk of lung cancer?

In this study of 247 lung cancer patients and 254 hospital-based control patients, the crude OR of lung cancer for the GSTM1 null allele was 1.3 (95% CI: 0.9–1.8). Examining the association according to histological type, we observed no relation between this allele and squamous cell carcinoma. For both small cell carcinoma and adenocarcinoma, on the other hand, the OR was slightly stronger, approximately 2.0. We estimated the OR of lung cancer, taking into account both the GSTM1 genotype and lifetime tobacco consumption. The OR for heavy smokers with the null allele was 5 (95% CI: 2.6–9.5) compared with 6.4 (95% CI: 3.3–12.5) for heavy smokers who were GSTM1-active. Finally our data do not depart significantly from a multiplicative model of the combined effects of these risk factors.

Our study found the null allele to be associated with a 1.4 increase in the risk of lung cancer after adjustment for tobacco smoking. A recent meta-analysis of the studies on this subject during this decade has calculated the OR of lung cancer to be 1.2 (95% CI: 1.0–1.4) among white populations.²⁵ Our results therefore seem to agree with the literature. This association, although it appears moderate, means that people with the null allele (50% of the white population) are at a 40% higher risk of developing lung cancer than GSTM1 carriers. The relation seems slightly stronger among people <60 years (OR = 1.6 [95% CI: 0.9–2.6]).

One of the particularities of smoking in the French population is the use of black (air-cured) tobacco. In our study, 77% of the smokers had smoked only black tobacco, and 5% only blond (flue-cured) tobacco. This distribution did not allow us to study the relation between GSTM1 and lung cancer separately for smokers of blond tobacco only. However, after excluding from the analysis subjects who had smoked blond tobacco (only or with black), we found an OR similar to that for the entire

study population. This result, together with the observation of an association between lung cancer and the GSTM1 polymorphism that was of the same order of magnitude as that reported generally in the literature, suggests that the type of tobacco smoked plays no special role.

Our results indicate that GSTM1 is associated with small cell lung cancer and adenocarcinoma, but not with squamous cell carcinoma. Further interpretation of this fact is difficult, especially since the results of such subgroup analyses in previous case-control studies have been inconsistent. The meta-analysis for histological types of lung cancer found similar OR for all three types, i.e. 1.5 for squamous cell carcinoma, 1.9 for small cell carcinoma, and 1.5 for adenocarcinoma, with nearly identical CI.²⁵ Our results are similar to those of Alexandrie *et al.*:¹⁶ no association between the null allele and squamous cell carcinoma, but an OR of 1.9 for small cell carcinoma. In view of the strength of the association between lung cancer and GSTM1 (i.e. 1.5–2), it seems unlikely that the available epidemiological studies, considered separately, could show a real difference in the OR of histological types of lung cancer for GSTM1 null subjects.

We also considered the question of the joint effect of smoking and the polymorphism and observed that the OR of lung cancer related to GSTM1 remained the same at the different levels of cigarette exposure (Table 4). Nonetheless, dividing the tobacco variable into three categories (light, medium, and heavy smokers) is not totally satisfactory, both because of the arbitrariness of the cutoff point and because of a residual heterogeneity in the number of cigarettes smoked within each

of the classes thus defined. Moreover, the lack of non-smokers among the cases meant our reference category had to include light smokers, which diminishes the differences in risk between classes. To address the joint effect of both risk factors, it thus appeared to us of particular interest to test for the existence of a statistical interaction, using a logistic model that allowed the exposure variable (i.e. pack-years of cigarette smoking) to be considered quantitatively. In such a model, what is tested is the departure from the multiplicative effect; the interaction term is the ratio of the slopes of the risk of cancer as a function of the exposure variable (i.e. pack-years) among patients carrying the null allele and among those who are GSTM1-active. Our results showed clearly that these slopes were essentially equal. Therefore, none of these ratios was significantly different from one, either for lung cancer overall or for any histological type. This finding indicates that our data do not depart significantly from a multiplicative model.

Ten studies including this one have examined the relation between the GSTM1 polymorphism and lung cancer risk according to tobacco consumption expressed in pack-years (Table 6). In five of the ten studies, the OR was higher among the heavier smokers than among light smokers. The other five studies found the opposite result, that is, a higher OR for the light smokers. These results must be interpreted cautiously, however, since most of these studies considered only two classes of smokers (light versus heavy), and the distribution of the two classes was not always well balanced.^{9,14} When we classified our subjects as light or heavy smokers, using the median pack-years as the cutoff point, the OR for adenocarcinoma was higher among

Table 6 Analysis of odds ratio (OR) observed in the literature for lung cancer related to GSTM1 genotype, among light smokers and heavy smokers; estimation of OR of interaction

Study	Cutoff point (pack-years)	OR (95% CI)		
		Light smokers	Heavy smokers	Interaction ^a
Seidegard, 1986	30	1.9 (0.5–8.1)	3.2 (1.4–7.5)	1.6 (0.3–8.6)
Hirvonen, 1993	40	0.8 (0.3–2.5)	4.0 (0.7–21.3)	4.8 (0.6–35.4)
Nakachi, 1993	32 10(4) ^b	2.5 (1.0–5.7)	1.3 (0.6–2.9)	0.5 (0.2–1.6)
Nazar-Stewart, 1993	54	1.6 (0.4–7.1)	6.7 (1.6–33.3)	4.1 (0.5–35.8)
Kihara, 1995	40, ≥60	1.0 (0.4–2.6)	3.3 (1.2–9.3)	3.1 (0.8–12.5)
London, 1995	40	1.8 (1.1–2.8)	0.9 (0.6–1.4)	0.5 (0.3–1.0)
To-Figueras, 1996	50	1.8 (1.0–3.1)	1.4 (0.7–2.5)	0.8 (0.3–1.7)
Garcia-Closas, 1997	40	1.3 (0.9–2.1)	0.7	0.5
Sun, 1997	NE, F	2.6 (1.4–4.8)	2.2 (1.5–3.2)	0.8 (0.4–1.7)
Present study	25, >40	1.2 (0.7–2.3)	1.3 (0.6–2.6)	1.1 (0.4–2.8)

^a OR of interaction = $\frac{\text{OR heavy smokers}}{\text{OR light smokers}}$

^b Original data = 32×10^4 (corresponding to the total amount of cigarettes consumed during their lifetime until the time of interview).

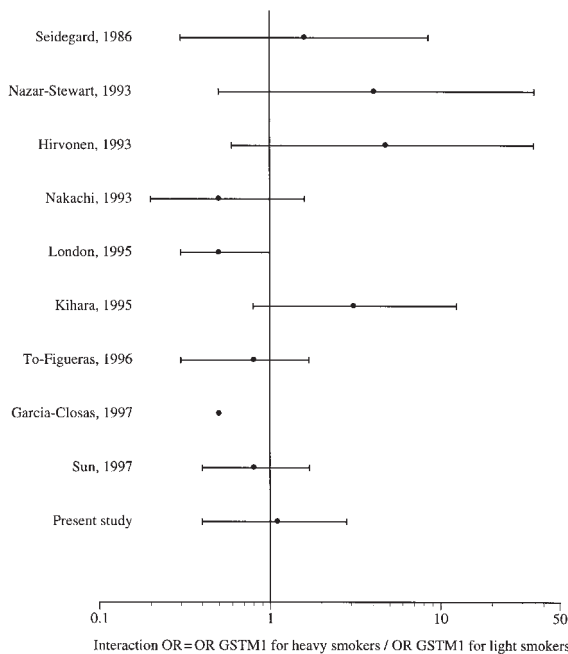


Figure 1 Interaction between GSTM1 genotype and smoking or lung cancer risk (odds ratio [OR] and 95% CI re-estimated from the published data)

the heavy smokers (OR = 3.5 [95% CI : 1.5–8.5]) than among light smokers (OR = 1.1 [95% CI : 0.4–2.7]). This OR, however, depended strongly on the cutoff point used to separate the two groups. We tested the existence of a statistically significant interaction in all the published results (Figure 1). It appeared in only one of the ten studies.²⁰ Overall, these results do not support the existence of an interaction between the level of smoking and the GSTM1 polymorphism. It thus seems reasonable to conclude that smoking and the GSTM1 gene are each a risk factor for lung cancer, and that their combined effect does not differ significantly from that of a multiplicative model.

In summary, our study shows a significant association between GSTM1 and lung cancer. The analysis of the combined effect of tobacco exposure and polymorphism produced results compatible with a multiplicative effect. Unfortunately, although the study included 247 cases and 254 controls, it could not address the question of multiplicative versus additive effect with sufficient statistical power. A sufficiently powerful consideration of this question would require pooling several case-control studies. It would also be very interesting to examine the effect on lung cancer risk of this gene together with others involved in PAH metabolism, such as CYP1A1 in the activation phase or epoxide hydrolase in the detoxification phase. The results would likely show that several of these genes, considered simultaneously, are more strongly associated with the risk of lung cancer than any of them considered alone.

Acknowledgements

This work was financed in part by the Caisse Nationale d'Assurance Maladie des Travailleurs Salariés (Collaboration

INSERM-CNAMTS) and by La Ligue contre le Cancer. We are grateful to Jo Ann Cahn for help in revising the manuscript.

References

- Perera FP, Santella R. *Molecular Epidemiology Principles and Practices*, New York: Academic Press, Inc., 1993, pp.277–92.
- Hemminki K, Pershagen G. Cancer risk of air pollution: epidemiological evidence. *Environ Health Perspect* 1994;**102**(Suppl.4): 187–92.
- IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Lyon, France: IARC, 1987.
- Bartsch H, Hietanen E. The role of individual susceptibility in cancer burden related to environmental exposure. *Environ Health Perspect* 1996;**104**:569–77.
- Bartsch H. DNA adducts in human carcinogenesis: etiological relevance and structure-activity relationship. *Mutat Res Rev Genet Toxicol* 1996;**340**:67–79.
- Seidegard J, Pero RW. The hereditary transmission of high glutathione transferase activity towards trans-stilbene oxide in human mononuclear leukocytes. *Hum Genet* 1985;**69**:66–68.
- Board P, Coggan M, Johnston P, Ross V, Suzuki T. Genetic heterogeneity of the human glutathione transferases: a complex of gene families. *Pharmacol Ther* 1990;**48**:357–69.
- Kato S, Browman ED, Harrington AM, Blomeke B, Shields PG. Human lung carcinogen-DNA adduct levels mediated by genetic polymorphisms in vivo. *J Natl Cancer Inst* 1995;**87**:902–07.
- Seidegard J, Pero RW, Miller DG, Beattie E. A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. *Carcinogenesis* 1986;**7**:751–53.
- Seidegard J, Pero RW, Markowitz MM, Roush G, Miller DG, Beattie EJ. Isoenzyme(s) of glutathione transferase (class Mu) as a marker for the susceptibility to lung cancer: a follow up study. *Carcinogenesis* 1990;**11**:33–36.
- Zhong S, Forbes-Howie A, Ketterer B *et al.* Glutathione S-transferase mu locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. *Carcinogenesis* 1991;**12**:1533–37.
- Heckbert SR, Weiss NS, Hornung SK, Eaton DL, Motulsky AG. Glutathione s-transferase and epoxide hydrolase activity in human leukocytes in relation to risk of lung cancer and other smoking-related cancers. *J Natl Cancer Inst* 1992;**84**:414–22.
- Brockmoller J, Kerb R, Drakoulis N, Nitz M, Roots I. Genotype and phenotype of glutathione S-Transferase class-mu isoenzyme-mu and isoenzyme-psi in lung cancer patients and controls. *Cancer Res* 1993;**53**:1004–11.
- Hirvonen A, Husgafvelpursiainen K, Anttila S, Vainio H. The GSTM1 null genotype as a potential risk modifier for squamous cell carcinoma of the lung. *Carcinogenesis* 1993;**14**:1479–81.
- Nazar-Stewart V, Motulsky AG, Eaton DL *et al.* The glutathione S-Transferase-mu polymorphism as a marker for susceptibility to lung carcinoma. *Cancer Res* 1993;**53**:2313–18.
- Alexandrie AK, Sundberg MI, Seidegard J, Tornling G, Rannug A. Genetic susceptibility to lung cancer with special emphasis on CYP1A1 and GSTM1: a study on host factors in relation to age at onset, gender and histological cancer types. *Carcinogenesis* 1994;**15**: 1785–90.
- Hayashi SI, Watanabe J, Kawajiri K. High susceptibility to lung cancer analyzed in terms of combined genotypes of P450IA1 and Mu-class glutathione S-transferase genes. *Jpn J Cancer Res* 1992;**83**: 866–70.
- Nakachi K, Imai I, Hayashi SI, Kawajiri K. Polymorphisms of the CYP1A1 and glutathione S-Transferase genes associated with

- susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Res* 1993;**53**:2994–99.
- ¹⁹ Kihara M, Noda K, Kihara M. Distribution of GSTM1 null genotype in relation to gender, age and smoking status in Japanese lung cancer patients. *Pharmacogenet* 1995;**5**:S74–S79.
- ²⁰ London SJ, Daly AK, Cooper J, Navidi WC, Carpenter CL, Idle JR. Polymorphism of glutathione s-transferase m1 and lung cancer risk among African/Americans and caucasians in Los Angeles County, California. *J Natl Cancer Inst* 1995;**87**:1246–53.
- ²¹ Moreira A, Martins G, Monteiro MJ *et al*. Glutathione S-transferase Mu polymorphism and susceptibility to lung cancer in the Portuguese population. *Teratogenesis Carcinog Mutagen* 1996; **16**:269–74.
- ²² Garcia-Closas M, Kelsey KT, Wiencke J, Xu X, Wain JC, Christiani DC. A case-control study of cytochrome P450 1A1, glutathione S-transferase M1, cigarette smoking and lung cancer susceptibility (Massachusetts, United States). *Cancer Causes Control* 1997;**8**:544–53.
- ²³ Kelsey KT, Spitz MR, Zuro ZF, Wiencke JK. Polymorphisms in the glutathione S-transferase class mu and theta genes interact and increase susceptibility to lung cancer in minority populations. *Cancer Causes Control* 1997;**8**:554–59.
- ²⁴ To-Figueras J, Gené M, Gomez-Catalan J *et al*. Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) polymorphisms and lung cancer risk among Northwestern Mediterraneans. *Carcinogenesis* 1997;**18**:1529–33.
- ²⁵ McWilliams JE, Sanderson BJ, Harris EL, Richert-Boe KE, Henner WD. Glutathione S-Transferase M1 (GSTM1) deficiency and lung cancer risk. *Cancer Epidemiol Biomarkers Prevent* 1995;**4**:589–94.
- ²⁶ GF Sun, Shimojo N, Pi JB, Lee S, Kumagai Y. Gene deficiency of glutathione S-transferase mu isoform associated with susceptibility to lung cancer in a Chinese population. *Cancer Lett* 1997;**113**:169–72.
- ²⁷ Rothmann KJ. *Modern Epidemiology*. Boston: Little, Brown and Co., 1986.