Influence of *CYP1A1*, *GSTM1*, *GSTT1*, and *NQO1* Genotypes and Cumulative Smoking Dose on Lung Cancer Risk in a Swedish Population

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Abstract

The major identified risk factor for lung cancer is tobacco smoking. We identified previously the possible modifying influence of *CYP1A1* and *GSTM1* polymorphisms on lung cancer risk in a Swedish population. The present study, extended by several study subjects and with analyses for polymorphisms in *GSTT1* and *NQO1*, includes 524 lung cancer cases and 530 control subjects. No evidence for an influence of genetic polymorphisms in *CYP1A1*, *GSTM1*, *GSTT1*, and *NQO1* on lung cancer risk overall was found. In smokers, there was, however, a suggestion that the variant *CYP1A1* and *NQO1* genotypes may confer an increased risk for squamous cell carcinoma. In ever smokers, the homozygously deleted *GSTM1* (*GSTM1*O/*O*) genotype was significantly associated with increased risk of small cell carcinoma (adjusted odds ratio 2.72, 95% confidence interval 1.32-5.90). The risks noted for the variant *CYP1A1* genotypes and the *GSTM1*O/*O* genotype seemed to be restricted to light smokers. The *GSTT1*O/*O* genotype also appeared to be a possible risk factor in light smokers, whereas, in heavy smokers, this genotype was associated with decreased risk for lung cancer overall (odds ratio 0.36, 95% confidence interval 0.13-0.99). Due to the multiple comparisons made, we cannot exclude the possibility that some of these associations may represent chance findings. (Cancer Epidemiol Biomarkers Prev 2004;13(6):908–14)

Introduction

Lung cancer is the leading cause of cancer death worldwide. Although tobacco smoking is causally implicated in the overwhelming majority of lung cancers, <20% of smokers develop this disease (1). Individual susceptibility to lung cancer may partly be explained by differences in metabolic efficiency in the formation and elimination of carcinogens in tobacco smoke. Functional polymorphisms in genes coding for enzymes involved in this process have been extensively studied in relation to lung cancer. An example of such a gene is the *CYP1A1* gene, the enzyme product of which mediates the activation of tobacco carcinogens, such as polycyclic aromatic hydrocarbons, into reactive intermediates capable of damaging DNA. Two closely linked polymorphisms in *CYP1A1*, the 3801T > C substitution creating a *Msp*I restriction site in the 3′ noncoding region and the 2455A > G substitution resulting in an amino acid change ($Ile^{462}Val$), were reported in 1990 to be associated with lung cancer in a Japanese population (2, 3). The *GSTM1* gene encodes an enzyme involved in detoxification of various reactive intermediates, including those of polycyclic aromatic hydrocarbons. In a study published in 1986, the homozygously deleted *GSTM1* gene was indicated as a risk factor for lung cancer (4). Another enzyme belonging to this family, GSTT1, is involved in the metabolism of other tobacco smoke constituents, such as 1,3-butadiene, ethylene oxide, and halogenated alkanes. Consequently, the *GSTT1* deletion polymorphism has also been extensively investigated in relation to lung cancer (5-11). The *NQO1* gene encodes an enzyme capable of reducing a wide variety of substrates, including quinones derived from polycyclic aromatic hydrocarbons. The functional 609C > T polymorphism causing an amino acid change ($Pro^{187}Ser$), resulting in loss of NQO1 activity (12), has also been studied for its role in lung cancer (11, 13-19).

Although many studies have assessed *CYP1A1*, *GSTM1*, *GSTT1*, and *NQO1* polymorphisms in relation to lung cancer, the results are conflicting. Besides different study designs, prevalence of genetic polymorphisms and linkage disequilibrium in different ethnic populations are possible explanations for the varying results obtained. Effect modifications by environmental or other genetic risk factors that differ between study populations are alternative causes. To avoid such influences, large studies on homogenous populations are warranted.

Our previous study on the effects of genetic polymorphisms in *CYP1A1* and *GSTM1* on lung cancer risk included 329 healthy subjects and 296 lung cancer cases of Swedish origin (20). The number of study subjects has been increased and the results presented here, on 530 control subjects and 524 lung cancer cases, also include analyses of the *GSTT1* and *NQO1* polymorphisms.

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We evaluated the influence of these genetic polymorphisms on lung cancer risk overall and performed analyses after stratification by histologic subtypes and smoking variables.

Materials and Methods

Study Population. Blood samples were obtained from 524 lung cancer patients and 530 control subjects between 1991 and 2000. Part of the study population was included in a previous study of the influence of genetic polymorphisms on lung cancer risk (20). All subjects were of Swedish origin and lived in the southern half of Sweden. The cases were recruited from eight different hospitals. At two of the hospitals, the majority of the patients completed a questionnaire about smoking habits. Information on smoking history and histologic subtypes was available to a lesser extent for the patients from the other six hospitals. The control subjects included laboratory workers, welders, and chimney sweeps from the former study. The group of laboratory staff was extended in the present study. Smoking history from the original and newly recruited laboratory workers was obtained by sending out a brief questionnaire. Smoking information from the sweeps and welders had previously been gathered via interview. In addition, blood samples and smoking history by interview were collected from elderly subjects visiting one of the hospitals for health checkups. The study was approved by the Ethics Committee of Karolinska Institutet (Stockholm, Sweden).

Genotyping. DNA was isolated from whole blood using phenol-chloroform extraction or by a modified salting out procedure (21, 22). The genotypes of the *CYP1A1* 3801T > C (*Msp*I) and Ile^{462}Val polymorphisms were determined using the *Msp*I RFLP-PCR method and an allele-specific PCR method, respectively, essentially as described by Hayashi et al. (2) and Carstensen et al. (23). The neighboring Thr^{461}Asp polymorphism does not interfere with the allele-specific method, as the primers used do not cover this polymorphic region. The predominant allele in Caucasians, lacking both of the investigated mutations, has been designated *CYP1A1*1*, while the allele with only the *Msp*I RFLP has been designated *CYP1A1*2A*. The *CYP1A1*2B* allele carries both the *Msp*I RFLP and the Ile^{462}Val polymorphism, whereas, in Caucasians, the extremely rare allele *CYP1A1*2C* only has the Ile^{462}Val polymorphism. Genotyping for the *GSTM1* and *GSTT1* null polymorphisms were performed using slightly modified published methods (24, 25), and detailed information of the PCR assays for *GSTM1* and *GSTT1* are given elsewhere (23, 26). The standard nomenclature for the functional *GST* alleles, *GSTM1*1* and *GSTT1*1*, and the *GST* null alleles, *GSTM1*O* and *GSTT1*O*, was used. The *NQO1* Pro^{187}Ser polymorphism was determined using the *Hin*fI PCR-RFLP method of Traver et al. (12). The common allele among Caucasians and the variant allele coding for the amino acid exchange Pro^{187}Ser have been designated *NQO1*1* and *NQO1*2*, respectively.

Statistical Analyses. Differences in distribution of age and cumulative smoking dose (pack-years) between cases and controls were tested by Student's *t* test or for nonnormally distributed variables by Wilcoxon rank sum test. Pearson χ^2 analysis was used to test the differences between cases and controls in the distributions of gender, smoking status, and genotypes. Population Hardy-Weinberg equilibrium was tested in controls using the χ^2 test. Odds ratios (ORs) with 95% confidence intervals (95% CI) were estimated by unconditional logistic regression adjusting for age (continuous variable) and gender. In some analyses, the cases and controls were stratified by cumulative smoking dose. Never smokers were defined as individuals who had never smoked or who had smoked 0.2 pack-years or less. Individuals at or below the median pack-years of cases and controls (median pack-years 21) were considered to be light smokers and individuals above median pack-years were considered heavy smokers. Statistical analyses were performed using the JMP Statistics software package (SAS Institute Inc., Cary, NC).

Results

The distributions of age, gender, and smoking variables of the subjects are shown in Table 1. Seventy-nine percent of the cases were diagnosed with one of the three main histologic subtypes (Table 1). The proportions of females and never smokers were higher among those with adenocarcinoma (47% and 17%, respectively) than among those with squamous (22% and 5%) and small cell carcinoma (35% and 1.5%). Data on age and gender were available for all subjects, whereas the information on smoking habits was incomplete in both cases and controls (Table 1).

Distribution of Genotypes in Controls. The distributions of the *CYP1A1*, *GSTM1*, *GSTT1*, and *NQO1* genotypes in controls (Table 2) below the median age did not differ significantly from those found in controls above median age. Furthermore, the genotype distributions did not differ significantly between women and men and between different control subpopulations. Overall, the estimated population frequency of the dominant *CYP1A1*1* allele was 0.91, whereas the frequencies for the variant alleles *CYP1A1*2A*, *CYP1A1*2B*, and *CYP1A1*2C* were 0.06, 0.03, and >0.001, respectively. The observed genotype frequencies were 82.4% *CYP1A1*1/*1*, 10.0% *CYP1A1*1/*2A*, 0.6% *CYP1A1*2A/*2A*, 6.6% *CYP1A1*1/*2B*, 0.2% *CYP1A1*2A/*2B*, and 0.2% *CYP1A1*1/*2C*. These genotype frequencies matched the prediction by the Hardy-Weinberg theorem based on the allele frequencies ($\chi^2 = 2.46$, $P = 0.86$). Deviations from Hardy-Weinberg equilibrium were not tested for the distributions of *GSTM1* and *GSTT1* genotypes because the PCR assays did not enable discrimination of heterozygous from homozygous carriers. The overall frequencies of the *NQO1*1* and *NQO1*2* alleles were 0.84 and 0.16, respectively. The genotype distribution of the *NQO1* polymorphism was consistent with the Hardy-Weinberg equilibrium ($\chi^2 = 2.37$, $P = 0.14$).

Influence of Genetic Polymorphisms on Lung Cancer Risk Overall. The genotype distributions of *CYP1A1*, *GSTM1*, *GSTT1*, and *NQO1* showed no significant differences between controls and all lung cancer cases or cases categorized by histologic subtypes (Table 2).

Table 1. Characteristics of control subjects and lung cancer cases

		Controls	Cases	*P*
Subjects, *n* (%)		530 (100)	524 (100)	
Squamous cell carcinoma			166 (31.6)	
Small cell carcinoma			102 (19.5)	
Adenocarcinoma			144 (27.5)	
Large cell carcinoma			16 (3.1)	
Mesothelioma			9 (1.7)	
Other/mixed			37 (7.1)	
No information			50 (9.5)	
Age (y)	Median (range)	44 (19-79)	66 (35-88)	$P < 0.001$*
Gender, *n* (%)	Female	117 (22.1)	180 (34.4)	$P < 0.001$†
	Male	413 (77.9)	344 (65.6)	
Smoking status, *n* (%)	Never	232 (43.8)	31 (5.9)	$P < 0.001$†
	Ever	273 (51.5)	312 (59.5)	
	No information	25 (4.7)	181 (34.6)	
Pack-years, *n*‡		272	196	
	Median (range)	11 (0.3-93)	34 (6-143)	$P < 0.001$§

*Student's *t* test.
†Pearson χ^2 analysis.
‡Smokers with information on pack-years.
§Wilcoxon rank sum test.

Influence of Genetic Polymorphisms on Lung Cancer Risk in Ever Smokers. In Table 3, 312 cases and 273 controls were included in the analysis. Due to fewer subjects, variant *CYP1A1* genotypes were combined. Likewise, variant *NQO1* genotypes were combined into one group.

In general, the effects of genotypes suggested in the total material (Table 2) were more pronounced in the ever smokers (Table 3). Stratification by histologic subtypes indicated an elevated OR for squamous cell carcinoma associated with the combined variant *CYP1A1* genotypes. The OR (95% CI) for small cell carcinoma associated with *GSTM1*O/*O* was significant with a value of 2.72 (1.32-5.90; $P = 0.008$). However, if adjustment for pack-years (continuous variable) was performed, the significance was lost (OR 1.97, 95% CI 0.72-5.80), but because of missing detailed smoking data, this analysis was based on only 31 of 65 ever smokers with small cell carcinoma. A protective role of the *GSTT1*O/*O* genotype that seemed to be most pronounced for small cell carcinoma and adenocarcinoma was observed, but the point estimate was below unity also for squamous cell carcinoma (Table 3). The frequency of the combined variant *NQO1* genotypes in patients with squamous cell carcinoma was significantly higher than in controls ($P = 0.04$). The age-adjusted and gender-adjusted OR (95% CI) for squamous cell carcinoma associated with these genotypes was 1.78 (0.95-3.34) and was significant when adjustment for pack-years was also included (OR 2.21, 95% CI 1.03-4.84; analysis based on 81 of the 115 ever smokers with squamous cell carcinoma).

Table 2. Adjusted ORs for lung cancer overall and for the main histologic subtypes in relation to genotypes

Genotype	Controls		Lung Cancer Cases			Squamous Cell Carcinoma			Small Cell Carcinoma			Adenocarcinoma		
	n	%	*n*	%	OR† (95% CI)	*n*	%	OR† (95% CI)	*n*	%	OR† (95% CI)	*n*	%	OR† (95% CI)
CYP1A1														
**1/*1*	437	82.4	440	84.0	1.00	136	81.9	1.00	88	86.3	1.00	121	84.0	1.00
**1/*2A*, **2A/*2A*	56	10.6	58	11.1	1.09 (0.62-1.92)	18	10.9	1.34 (0.61-2.89)	10	9.8	1.04 (0.42-2.43)	17	11.8	0.79 (0.35-1.69)
**1/*2B*, **2A/*2B*	37‡	7.0	26	4.9	1.20 (0.58-2.44)	12	7.2	1.55 (0.59-3.94)	4	3.9	0.70 (0.17-2.36)	6	4.2	0.90 (0.28-2.43)
GSTM1														
**1/*1*, **1/*O*	240	45.3	237	45.2	1.00	85	51.2	1.00	40	39.2	1.00	62	43.1	1.00
**O/*O*	290	54.7	287	54.8	1.02 (0.73-1.44)	81	48.8	0.81 (0.50-1.31)	62	60.8	1.61 (0.94-2.82)	82	56.9	1.07 (0.67-1.71)
GSTT1														
**1/*1*, **1/*O*	456	86.0	456	87.0	1.00	142	85.5	1.00	92	90.2	1.00	128	88.9	1.00
**O/*O*	74	14.0	68	13.0	0.85 (0.51-1.40)	24	14.5	0.94 (0.47-1.86)	10	9.8	0.57 (0.23-1.31)	16	11.1	0.61 (0.29-1.24)
NQO1														
**1/*1*	368	69.4	345	65.8	1.00	107	64.5	1.00	67	65.7	1.00	98	68.0	1.00
**1/*2*	153	28.9	168	32.1	1.11 (0.76-1.60)	55	33.1	1.33 (0.79-2.23)	32	31.4	0.97 (0.54-1.73)	43	29.9	1.21 (0.72-2.01)
**2/*2*	9	1.7	11	2.1	1.41 (0.45-4.36)	4	2.4	3.02 (0.60-12.4)	3	2.9	1.80 (0.32-7.88)	3	2.1	1.33 (0.24-5.73)

†Adjusted for age and gender.
‡Includes one subject with genotype *CYP1A1*1/*2C*.

Table 3. Adjusted ORs for lung cancer overall and for the main histologic subtypes in relation to genotypes in ever smokers

Genotype	Controls		Lung Cancer Cases			Squamous Cell Carcinoma			Small Cell Carcinoma			Adenocarcinoma		
	n	%	*n*	%	OR[†] (95% CI)	*n*	%	OR[†] (95% CI)	*n*	%	OR[†] (95% CI)	*n*	%	OR[†] (95% CI)
CYP1A1														
**1/*1*	230	84.2	261	83.6	1.00	95	82.6	1.00	58	89.2	1.00	66	80.5	1.00
**1/*2, *2/*2*	43	15.8	51	16.4	1.34 (0.72-2.49)	20	17.4	1.57 (0.67-3.63)	7	10.8	0.79 (0.26-2.16)	16	19.5	1.21 (0.51-2.79)
GSTM1														
**1/*1, *1/*O*	131	48.0	147	47.1	1.00	57	49.6	1.00	26	40.0	1.00	39	47.6	1.00
**O/*O*	142	52.0	165	52.9	1.22 (0.79-1.91)	58	50.4	1.12 (0.61-2.05)	39	60.0	2.72 (1.32-5.90)[‡]	43	52.4	1.21 (0.65-2.26)
GSTT1														
**1/*1, *1/*O*	232	85.0	271	86.9	1.00	99	86.1	1.00	59	90.8	1.00	73	89.0	1.00
**O/*O*	41	15.0	41	13.1	0.74 (0.38-1.43)	16	13.9	0.67 (0.27-1.59)	6	9.2	0.45 (0.13-1.34)	9	11.0	0.49 (0.17-1.27)
NQO1														
**1/*1*	194	71.1	205	65.7	1.00	69	60.0	1.00	44	67.7	1.00	58	70.7	1.00
**1/*2, *2/*2*	79	28.9	107	34.3	1.16 (0.72-1.88)	46	40.0	1.78 (0.95-3.34)	21	32.3	0.97 (0.45-2.01)	24	29.3	1.02 (0.51-2.02)

[†]Adjusted for age and gender.
[‡]$P = 0.008$.

Interaction between Genetic Polymorphisms and Cumulative Smoking Dose on Lung Cancer Risk. The study subjects were divided into three groups: never smokers, light smokers, and heavy smokers as described in Materials and Methods. The total number of cases and controls included was 227 and 504, respectively (Table 4). In never smokers, no differences in the distributions of the *CYP1A1*, *GSTM1*, *GSTT1*, and *NQO1* genotypes between cases and controls were observed. However, in light smokers, the distributions of the *CYP1A1*, *GSTM1*, and *GSTT1* genotypes in cases appeared to be different from that in controls (Table 4). The frequency of the variant *CYP1A1* genotypes was higher in cases than in controls, but the difference was not statistically significant. In addition, the frequency of the *GSTM1*O/*O* genotype was higher in cases than in controls ($P = 0.05$) and the adjusted OR for the *GSTM1*O/*O* genotype showed borderline significance (2.68, 95% CI 1.00-7.94). We also found an increased frequency of the *GSTT1*O/*O* genotype in cases compared with controls, although this difference was not statistically significant.

In contrast to the observations in light smokers, the genotypes associated with increased risk (variant *CYP1A1*, *GSTM1*O/*O*, and *GSTT1*O/*O*) appeared to be underrepresented in cases classified as heavy smokers. In heavy smokers, the frequency of the variant *CYP1A1* genotypes was low in the cases, but as this also was apparent in the controls, the adjusted OR equaled unity (Table 4). The *GSTM1*O/*O* genotype frequency in cases was lower than in controls, but the difference was not statistically significantly. A lower frequency of the *GSTT1*O/*O* genotype in cases compared with conxtrols was also found, and after adjustment, the *GSTT1*O/*O* genotype was significantly associated with a decreased risk in heavy smokers (OR 0.36, 95% CI 0.13-0.99; $P = 0.04$).

Table 4. Distributions of genotypes and adjusted ORs among cases and controls stratified by cumulative cigarette dose

Genotype	Never Smokers					Light Smoker (≤21 Pack-Years)[†]					Heavy Smokers (>21 Pack-Years)				
	Controls		Cases		OR[‡] (95% CI)	Controls		Cases		OR[‡] (95% CI)	Controls		Cases		OR[‡] (95% CI)
	n	%	*n*	%		*n*	%	*n*	%		*n*	%	*n*	%	
CYP1A1															
**1/*1*	185	79.7	24	77.4	1.00	173	82.8	23	76.7	1.00	56	88.9	145	87.3	1.00
**1/*2, *2/*2*	47	20.3	7	22.6	1.28 (0.42-3.61)	36	17.2	7	23.3	2.37 (0.72-7.47)	7	11.1	21	12.6	1.00 (0.36-3.00)
GSTM1															
**1/*1, *1/*O*	99	42.7	13	41.9	1.00	102	48.8	9	30.0	1.00	28	44.4	93	56.0	1.00
**O/*O*	133	57.3	18	58.1	0.86 (0.34-2.17)	107	51.2	21	70.0	2.68 (1.00-7.94)[§]	35	55.6	73	44.0	0.82 (0.42-1.63)
GSTT1															
**1/*1, *1/*O*	200	86.2	26	83.9	1.00	178	85.2	23	76.7	1.00	53	84.1	149	89.8	1.00
**O/*O*	32	13.8	5	16.1	1.37 (0.37-4.43)	31	14.8	7	23.3	2.56 (0.71-8.79)	10	15.9	17	10.2	0.36 (0.13-0.99)[¶]
NQO1															
**1/*1*	160	69.0	21	67.7	1.00	146	69.9	20	66.7	1.00	47	74.6	109	65.7	1.00
**1/*2, *2/*2*	72	31.0	10	32.3	1.24 (0.47-3.18)	63	30.1	10	33.3	0.91 (0.31-2.47)	16	25.4	57	34.3	1.23 (0.58-2.66)

[†]21 is the overall median pack-years among smokers.
[‡]Adjusted for age and gender.
[§]$P = 0.06$.
[¶]$P = 0.04$.

The *NQO1* genotype distribution in cases was similar to that found in controls in never, light, and heavy smokers with adjusted ORs close to unity within each smoking group.

Distribution of Genotypes in Cases Stratified by Cumulative Smoking Dose. When analyzing the case group only, a difference in the genotype frequencies between light and heavy smokers was observed (Table 4). The frequency of the variant *CYP1A1* genotypes in heavy smokers (12.6%) was only half of that found in light smokers (23.3%), but the difference was not statistically significant ($P = 0.12$). The *GSTM1*O/*O* genotype was significantly less common in heavy smokers (44.0%) compared with light smokers (70.0%; $P = 0.009$). The prevalence of the *GSTT1*O/*O* genotype was also significantly lower in the heavy smokers (10.2%) compared with that found in the light smokers (23.3%; $P = 0.04$).

When the heavy smokers (pack-years >21) were divided into three equally sized groups, the frequencies of the variant *CYP1A1*, *GSTM1*, and *GSTT1* genotypes were higher in light smokers compared with those found in each subgroup of heavy smokers (Fig. 1), although each comparison was no longer significant due to the lower numbers in these subgroup comparisons. In contrast, the variant *NQO1* genotypes appeared to be similarly distributed in all five groups.

Gene-Gene Interaction. All possible combinations of two genotypes for *CYP1A1*, *NQO1*, *GSTM1*, and *GSTT1* were examined. No evidence for effects of gene-gene interactions on lung cancer risk overall was found. However, the suggested increased risk for squamous cell carcinoma in ever smokers with the variant *CYP1A1* genotypes was only present in subjects also carrying the *GSTM1*O/*O* genotype. The age-adjusted and gender-adjusted OR (95% CI) associated with this combined genotype was 1.89 (0.63-5.71). In comparison, the OR (95% CI) for the variant *CYP1A1* genotype in combination with the presence of at least one *GSTM1*1* allele was 1.13 (0.35-3.50) when using the genotype combination *CYP1A1*1/*1* and presence of at least one *GSTM1*1* allele as reference group. The adjusted OR for squamous cell carcinoma was substantially higher in the group with combined variant genotypes of *CYP1A1* and *NQO1* (3.54, 95% CI 0.88-14.3) compared with the ORs in the groups with only one of these variant genotypes (1.36, 95% CI 0.46-3.90 for variant *CYP1A1* only and 1.69, 95% CI 0.85-3.39 for variant *NQO1* only). The adjusted OR (95% CI) for squamous cell carcinoma associated with the combined genotype *CYP1A1*1/*1* and *GSTT1*O/*O* was 0.33 (0.10-0.95) with the combined genotype *CYP1A1*1/*1* and presence of at least one *GSTT1*1* allele as reference group. This apparent protective effect of the *GSTT1*O/*O* genotype was not found in combination with the variant *CYP1A1* genotype. The OR (95% CI) for squamous cell carcinoma for this combined genotype was 3.89 (0.80-17.6).

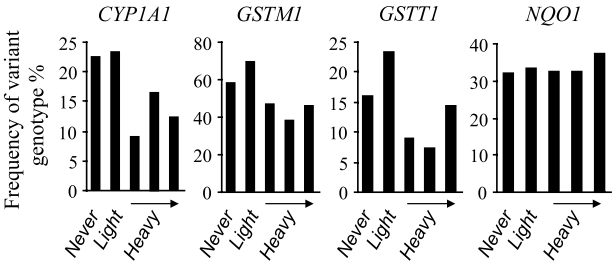

Figure 1. Frequencies of variant genotypes among lung cancer cases categorized by cumulative smoking dose (pack-years) into never smokers ($n = 31$), light smokers (pack-years ≤ 21; $n = 30$), and heavy smokers (pack-years > 21; $n = 166$), the latter being further subdivided in three equally sized groups with increasing number of pack-years in the direction of the *arrow* ($21 <$ pack-years ≤ 32, $n = 55$; $32 <$ pack-years ≤ 43, $n = 55$; and $43 <$ pack-years ≤ 143, $n = 56$). The genotypes defined as variant were *CYP1A1*1/*2*, *CYP1A1*2/*2*, *GSTM1*O/*O*, *GSTT1*O/*O*, *NQO1*1/*2*, and *NQO1*2/*2*.

Discussion

Numerous studies have investigated the association between *CYP1A1* and *GSTM1* polymorphisms and lung cancer with conflicting results (27). In agreement with our result, an early meta-analysis restricted to non-Asians suggested a small increased lung cancer risk among light smokers associated with *CYP1A1* variant genotypes (28). Also in accordance with our study, an elevated risk for squamous cell carcinoma associated with these genotypes was found (28). Pooled analyses of individual data of lung cancer cases and controls from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens found a significant association between variant *CYP1A1* and lung cancer risk in Caucasians, particularly in never and light smokers (29-31). That the effect of polymorphisms in biotransformation enzymes is stronger at comparably lower exposures has been observed in several epidemiologic studies on cancer risk (32), suggesting that, at high exposures, sufficiently high levels of reactive metabolites are formed in all individuals, regardless of the biotransformation genotypes.

Several meta-analyses of *GSTM1* polymorphism and lung cancer risk have suggested a modest increased risk for lung cancer associated with the *GSTM1*O/*O* genotype (28, 33-35). The association appeared to be more pronounced in Asians than in Caucasians and concerned all three main histologic subtypes. In our study, the increased risk associated with *GSTM1*O/*O* appeared to be limited to small cell carcinoma. The only meta-analysis addressing the effect of smoking suggested, in an analysis restricted to Asians, that the association between the *GSTM1*O/*O* genotype and lung cancer risk was stronger in heavy smokers than in light smokers (28). However, some studies, including the present one, have found the opposite association with an increased risk for lung cancer associated with the *GSTM1*O/*O* genotype in light smokers (36-38). In a pooled analysis from Genetic Susceptibility to Environmental Carcinogens, ever smokers with the *GSTM1*O/*O* genotype had an increased risk for small cell carcinoma, in agreement with our study, although, in that study, the strongest effect was seen at 20 to 39 pack-years. One explanation for the inconsistent results may be a difference in the definition of light and heavy smokers. For example, in

our study, light smokers were defined as those who had smoked equal or below the median pack-years of cases and controls (median pack-years 21). By choosing the median pack-years of the cases only (median pack-years 34), the association observed between the genotypes *GSTM1*O/*O*, *GSTT1*O/*O*, and variant *CYP1A1* and increased risk in light smokers disappeared. Further, as in most studies, a limitation of our exposure categorization is that smokers included in the light smokers category may not actually have been light smokers for a long time but could have been heavy smokers for a short time with the same cumulative dose.

In agreement with most previous studies, no significant association between *GSTT1* polymorphism and lung cancer overall was found. In contrast, in an analysis of smokers only, we found the *GSTT1*O/*O* genotype to be underrepresented in all three main histologic subtypes. A decreased risk associated with the *GSTT1*O/*O* genotype has also been observed in other studies (8, 10). This effect by the *GSTT1*O/*O* genotype was found to be restricted to heavy smokers in our study. Surprisingly, the *GSTT1*O/*O* genotype was suggested to be a risk factor for lung cancer in light smokers. In agreement with our result, an increased risk for head and neck cancer associated with the *GSTT1*O/*O* genotype was only evident in light smokers (39). On the other hand, in another Swedish study, an opposite result was observed [i.e., the *GSTT1*O/*O* genotype was associated with increased risk for lung cancer in heavy smokers (9)]. That study had population-based matched controls, which should contribute to higher study validity, but was much smaller than the present study. In addition, it included only a limited number of heavy smokers, as mainly elderly women were studied. An explanation for the fact that the GSTT1 protein seems to act both as a risk factor and as a factor protecting against lung cancer may be that it is involved in both detoxification and activation of carcinogens (5). It is also known to metabolize protective dietary antioxidants (6, 7).

In studies performed in Asians, the *NQO1*1/*1* genotype was found to be associated with lung cancer, particularly adenocarcinoma (11, 13-15), whereas the variant *NQO1* allele (*NQO1*2*) has been suggested to be a risk factor for lung cancer in Caucasians (16-19). The only previous study on Caucasians with sufficient number of cases that performed an analysis of histologic subtypes was in agreement with our study that the variant *NQO1* genotypes were overrepresented in squamous cell carcinoma (16).

The gene-gene interactions between *CYP1A1* and *GSTM1* and between *CYP1A1* and *NQO1*, observed in the present study, support the plausibility of a combined role of these enzymes in lung cancer susceptibility. Thus, the presence of the variant CYP1A1 protein may result in an increased formation of carcinogenic metabolites, and the detoxification of these reactive metabolites are restrained by the absence of functional GSTM1 and NQO1 enzymes.

The present study benefited from consisting of an ethnically homogenous population. This is of importance as ethnic-specific effects on lung cancer susceptibility related to polymorphism in genes of xenobiotic-metabolizing enzymes have been shown to exist. Further, the relatively large sample size of our study made it possible to divide the material by histologic subtypes and smoking status, which revealed some associations not seen in the total material. The selection of cases in the study may not have been optimal, but it is, on the other hand, almost never possible to ascertain all cases from a study base. We consider it unlikely that the procedures for recruiting cases are differential with regard to the genetic polymorphisms studied. The control selection occurred separate from the case selection, but as the controls came from the same ethnic population and the same geographic areas as the cases, they should be genetically representative of the study base. The controls may, however, be unrepresentative of the study base for nongenetic factors (e.g., age, gender, and smoking), so that estimation of main effects of these variables or a full set of interaction parameters in the case-control analysis was not possible. Adjusting or stratifying for age and gender and (where appropriate) for smoking ensures that any comparison of genotypes between cases and controls occurs within levels of these variables.

The case series included a few (n = 9) cases of mesothelioma, which has been associated more with asbestos exposure than with smoking. All mesothelioma cases lacked smoking information and were consequently not included in statistical analyses where this information was considered. Among the cases that lacked information of histologic subtype, it cannot be excluded that one or two actually had mesothelioma. The possible inclusion of a few mesotheliomas in the study would not affect results appreciably.

In conclusion, the variant *CYP1A1* and *NQO1* genotypes were suggested to confer increased risk for squamous cell carcinoma, whereas the *GSTM1*O/*O* genotype was associated with small cell carcinoma risk. Stratification by cumulative smoking dose revealed that the risks noted for the variant *CYP1A1* genotypes and *GSTM1*O/*O* genotype seemed to be restricted to light smokers. The *GSTT1*O/*O* genotype also appeared to be a possible risk factor in light smokers, whereas, in heavy smokers, this genotype decreased the risk for lung cancer overall. One important observation, which is corroborated by findings of DNA adducts (40), is that the variant *CYP1A1* genotypes only seem to be risk factors when present in individuals with the *GSTM1*O/*O* genotype. Due to the many comparisons made in the study and the small number of light smokers, caution in interpretation of the results is required.

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References

1. Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 2002;3:461-9.
2. Hayashi S, Watanabe J, Nakachi K, Kawajiri K. Genetic linkage of lung cancer-associated *Msp*I polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. J Biochem 1991;110:407-11.
3. Kawajiri K, Nakachi K, Imai K, Yoshii A, Shinoda N, Watanabe J. Identification of genetically high risk individuals to lung cancer by

DNA polymorphisms of the cytochrome P450IA1 gene. FEBS Lett 1990;263:131-3.
4. Seidegard J, Pero RW, Miller DG, Beattie EJ. A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. Carcinogenesis 1986;7:751-3.
5. Landi S. Mammalian class θ GST and differential susceptibility to carcinogens: a review. Mutat Res Rev Mutat Res 2000;463:247-83.
6. Spitz MR, Duphorne CM, Detry MA, et al. Dietary intake of isothiocyanates: evidence of a joint effect with glutathione *S*-transferase polymorphisms in lung cancer risk. Cancer Epidemiol Biomarkers & Prev 2000;9:1017-20.
7. London SJ, Yuan JM, Chung FL, et al. Isothiocyanates, glutathione *S*-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet 2000;356: 724-9.
8. Risch A, Wikman H, Thiel S, et al. Glutathione-*S*-transferase M1, M3, T1 and P1 polymorphisms and susceptibility to non-small-cell lung cancer subtypes and hamartomas. Pharmacogenetics 2001;11:757-64.
9. Hou SM, Falt S, Nyberg F, Pershagen G, Lambert B. Interaction between GSTT1 genotype and smoking on lung cancer risk. Chem Biol Interact 2001;133:106-8.
10. Stucker I, Hirvonen A, de Waziers I, et al. Genetic polymorphisms of glutathione *S*-transferases as modulators of lung cancer susceptibility. Carcinogenesis 2002;23:1475-81.
11. Sunaga N, Kohno T, Yanagitani N, et al. Contribution of the NQO1 and GSTT1 polymorphisms to lung adenocarcinoma susceptibility. Cancer Epidemiol Biomarkers & Prev 2002;11:730-8.
12. Traver RD, Siegel D, Beall HD, et al. Characterization of a polymorphism in NAD(P)H:quinone oxidoreductase (DT-diaphorase). Br J Cancer 1997;75:69-75.
13. Hamajima N, Matsuo K, Iwata H, et al. NAD(P)H:quinone oxidoreductase 1 (NQO1) C609T polymorphism and the risk of eight cancers for Japanese. Int J Clin Oncol 2002;7:103-8.
14. Lin PP, Wang HJ, Lee H, et al. NAD(P)H:quinone oxidoreductase polymorphism and lung cancer in Taiwan. J Toxicol Environ Health 1999;58:187-97.
15. Chen HW, Lum A, Seifried A, Wikens LR, Le Marchand L. Association of the NAD(P)H:quinone oxidoreductase C-609 -> T polymorphism with a decreased lung cancer risk. Cancer Res 1999; 59:3045-8.
16. Xu LL, Wain JC, Miller DP, et al. The NAD(P)H:quinone oxidoreductase 1 gene polymorphism and lung cancer: differential susceptibility based on smoking behavior. Cancer Epidemiol Biomarkers & Prev 2001;10:303-9.
17. Lewis SJ, Cherry NM, Niven RM, Barber PV, Povey AC. Polymorphisms in the NAD(P)H:quinone oxidoreductase gene and small cell lung cancer risk in a UK population. Lung Cancer 2001;34:177-83.
18. Benhamou S, Voho A, Bouchardy C, Mitrunen K, Dayer P, Hirvonen A. Role of NAD(P)H:quinone oxidoreductase polymorphism at codon 187 in susceptibility to lung, laryngeal and oral/pharyngeal cancers. Biomarkers 2001;6:440-7.
19. Rosvold EA, Mcglynn KA, Lustbader ED, Buetow KH. Identification of an NAD(P)H:quinone oxidoreductase polymorphism and its association with lung cancer and smoking. Pharmacogenetics 1995; 5:199-206.
20. 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.
21. Gustafson S, Proper J, Bowie E, Sommer S. Parameters affecting the yield of DNA from human blood. Anal Biochem 1987;165:294-9.
22. Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
23. Carstensen U, Alexandrie A-K, Högstedt B, Rannug A, Bratt I, Hagmar L. B- and T-lymphocyte micronuclei in chimney sweeps with respect to genetic polymorphism for CYP1A1 and GST1 (class μ). Mutat Res 1993;289:187-95.
24. Brockmoller J, Kerb R, Drakoulis N, Nitz M, Roots I. Genotype and phenotype of glutathione *S*-transferase class-μ isoenzyme-μ and isoenzyme-ψ in lung cancer patients and controls. Cancer Res 1993; 53:1004-11.
25. Pemble S, Schroeder KR, Spencer SR, et al. Human glutathione *S*-transferase θ (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 1994;300:271-6.
26. Warholm M, Rane A, Alexandrie AK, Monaghan G, Rannug A. Genotypic and phenotypic determination of polymorphic glutathione transferase T1 in a Swedish population. Pharmacogenetics 1995; 5:252-4.
27. Smith GBJ, Harper PA, Wong JMY, et al. Human lung microsomal cytochrome P4501A1 (CYP1A1) activities: impact of smoking status and CYP1A1, aryl hydrocarbon receptor, and glutathione *S*-transferase M1 genetic polymorphisms. Cancer Epidemiol Biomarkers & Prev 2001;10:839-53.
28. d'Errico A, Malata N, Vineis P, Boffetta P. Chapter 23. Review of studies of selected metabolic polymorphisms and cancer. In: Vineis P, Malats N, Lang M, et al, editor. Metabolic polymorphisms and susceptibility to cancer. IARC Scientific Publications No. 148. Lyon (France): IARC; 1999. p. 323-93.
29. Vineis P, Veglia F, Benhamou S, et al. CYP1A1 T3801C polymorphism and lung cancer: a pooled analysis of 2,451 cases and 3,358 controls. Int J Cancer 2003;104:650-7.
30. Hung RJ, Boffetta P, Brockmoller J, et al. CYP1A1 and GSTM1 genetic polymorphisms and lung cancer risk in Caucasian non-smokers: a pooled analysis. Carcinogenesis 2003;24:875-82.
31. Le Marchand LC, Guo CF, Benhamou S, et al. Pooled analysis of the CYP1A1 exon 7 polymorphism and lung cancer (United States). Cancer Causes & Control 2003;14:339-46.
32. Vineis P. The relationship between polymorphisms of xenobiotic metabolizing enzymes and susceptibility to cancer. Toxicology 2002; 181-182:457-62.
33. McWilliams JE, Sanderson BJ, Harris EL, Richert-Boe KE, Henner WD. Glutathione *S*-transferase M1 (GSTM1) deficiency and lung cancer risk. Cancer Epidemiol Biomarkers & Prev 1995;4:589-94.
34. Benhamou S, Lee WJ, Alexandrie AK, et al. Meta- and pooled analyses of the effects of glutathione *S*-transferase M1 polymorphisms and smoking on lung cancer risk. Carcinogenesis 2002;23:1343-50.
35. Houlston RS. Glutathione *S*-transferase M1 status and lung cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers & Prev 1999;8: 675-82.
36. Nakachi K, Imai K, Hayashi S, 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;53:2994-9.
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.
38. 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.
39. Hamel N, Karimi S, Hebert-Blouin MN, et al. Increased risk of head and neck cancer in association with GSTT1 nullizygosity for individuals with low exposure to tobacco. Int J Cancer 2000;87:452-4.
40. Alexandrov K, Cascorbi I, Rojas M, Bouvier G, Kriek E, Bartsch H. CYP1A1 and GSTM1 genotypes affect benzo[*a*]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. Carcinogenesis 2002;23:1969-77.

Influence of *CYP1A1*, *GSTM1*, *GSTT1*, and *NQO1* Genotypes and Cumulative Smoking Dose on Lung Cancer Risk in a Swedish Population

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