CYP1A1 and *CYP1B1* polymorphisms as modifying factors in patients with pneumoconiosis and occupationally related tumours: A pilot study

JOACHIM SCHNEIDER and ULRIKE BERNGES

Institut und Poliklinik für Arbeits und Sozialmedizin der Justus-Liebig Universität, D-35385 Giessen, Germany

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Abstract. *CYP1A1* and *CYP1B1* are involved in the metabolism of carcinogens. The effect of *CYP1A1* and *CYP1B1* polymorphisms as genetic modifiers of risk was investigated in individuals with asbestos, silica dust or ionizing radiation-induced occupational tumours compared to exposed non-cancer subjects suffering from pneumoconiosis, particularly in relation to tobacco smoking. *CYP1A1 T6235C, CYP1A1 A4889G* and *CYP1B1 codon 432* polymorphisms were determined by real-time PCR analysis in patients with asbestos-related lung cancer (n=39), patients with diffuse malignant mesotheliomas (n=19), lung cancer in silicosis patients (n=7), uranium miners with lung cancer (UMLC) (n=40), patients with asbestosis (n=181), and silicosis patients (n=204). The results were compared to those from a healthy unexposed control group (n=50) not exposed to carcinogenic (or fibrogenic) agents in the workplace. An additional healthy control group (n=134) comprised smokers and ex-smokers. Allele frequencies were within the range described for Caucasians. Multivariate analysis revealed that patients with occupational diseases with the susceptible *CYP1A1 T6235C* genotype had a calculated risk ranging from OR=0.5 (95% CI 0.18-1.36) for UMLC to OR=1.23 (95% CI 0.39-4.05) for uranium miners with silicosis. The risk for patients with the susceptible *CYP1A1 A4889G* allele was calculated as being between OR=0.39 (95% CI 0.10-1.54) for mesothelioma patients and OR=1.54 (95% CI 0.49-4.89) for UMLC. *CYP1B1 Val432Leu* polymorphisms were associated with a risk of OR=0.56 (95% CI 0.2-1.55) for UMLC and OR=1.52 (95% CI 0.68-3.39) for asbestos-exposed lung cancer patients. By analyzing the interaction between tobacco smoking, type of exposure to carcinogens and the genotypes, it was determined that smoking and the presence of the susceptible genotypes did not have a combined effect. In this pilot study, the analyzed polymorphism had no consistent modifying effect on pneumoconiosis or occupationally related tumours.

Correspondence to: Professor Joachim Schneider, Institut und Poliklinik für Arbeits- und Sozialmedizin der Justus-Liebig Universität, Aulweg 129/III, D-35385 Giessen, Germany
E-mail: joachim.schneider@arbmed.med.uni-giessen.de

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Introduction

Individual differences in susceptibility to occupationally induced carcinomas are in part ascribed to genetic differences in metabolic activity regarding the activation or detoxification of environmental carcinogens. Human lung cancer is caused by exposure to carcinogens such as polycyclic aromatic hydrocarbons (PAH), found mainly in cigarette smoke, but can also be caused by exposure to carcinogens in the workplace, such as asbestos, ionizing radiation or silica dust.

Most environmental carcinogens require metabolic activation by Phase I enzymes, cytochrome P-450s, to their reactive electrophilic intermediates. Several forms of P-450 have been identified. *CYP1A1* is involved in the bioactivation of carcinogenic PAH. In a Caucasian population, an association was found between *CYP1A1* homozygous *Msp*I RFLP and lung cancer (1,2).

In vitro studies have demonstrated that asbestos fibres exert a destructive effect through the generation of reactive oxygen species (ROS), which leads to alterations in mitochondrial function and the activation of several signal transduction pathways (3). Several mechanisms are likely to contribute to the synergistic carcinogenic effect of tobacco smoke and asbestos exposure (4). It has been demonstrated that cigarette smoke augments the penetration of asbestos fibres by an oxygen radical-mediated mechanism (5), while asbestos has been shown to cause the depletion of some antioxidants.

Respirable coal dust exposure inhibits the induction of pulmonary *CYP1A1*. Crystalline silica has been shown to be a negative modifier of pulmonary cytochrome P-4501A1 induction (6).

The expression levels of specific genes determine sensitivity to ionizing radiation. Cytochrome *CYP1B1* shows the greatest overexpression in radio-resistant cell lines (7).

CYP1B1 also induces the metabolic activation of carcinogens such as arylamines, nitroaromatics and PAH, participates in the metabolic activation of benzo(*a*)pyren, and catalyzes a number of PAH to ultimate carcinogenic epoxides. The *Val432Leu* polymorphism in particular has been associated

with the activity of the enzyme (8). Watanabe *et al* described an association between *CYP1B1* genetic polymorphisms and the incidence of breast and lung cancer (9).

In the present study, we investigated the role of *CYP1A1* and *CYP1B1* polymorphisms as genetic modifiers of risk in individuals with occupationally derived lung cancer and susceptible genotypes compared to exposed non-cancer subjects suffering from pneumoconiosis, particularly in relation to tobacco smoking.

The focus of the study was on these polymorphisms with known reported changes in microsomal enzyme activities. In epidemiological studies, genetic polymorphisms of the analyzed CYP genes were previously demonstrated to act as modifiers of risk in tobacco-related cancers.

Materials and methods

Study group and type of exposure (asbestos, ionizing radiation, silica dust, smoking). The study group consisted of 557 individuals who gave their written informed consent to participate in the study. All subjects were interviewed by means of a questionnaire to obtain data on lifestyle (including lifetime history of tobacco use) and occupational history.

To allow for discrimination between different kinds of exposure, several groups of tumour patients or patients suffering from fibrosis were created and compared to a non-exposed control group (n=184).

The tumour patients comprised individuals with asbestos-related lung cancer (n=39) (63.1±8.9 years of age) defined in a list of occupational diseases (i.e., asbestosis or pleural plaques or cumulative asbestos exposure of 25 fibres/ml x years) (10), patients with diffuse malignant mesotheliomas caused by asbestos dust (n=19) (64.4±7.7 years of age), lung cancer in silicosis patients (n=7) (65.9±4.2 years of age) and former uranium miners of SDAG Wismut (n=40) (67.8±5.6 years of age) with lung cancer related to ionizing radiation (radon and its decay products). Patients were diagnosed based on previously described criteria (11).

The patients with fibrosis comprised individuals with asbestosis (n=181) (66.2±7.4 years of age) and subjects suffering from silicosis (n=144) (70.3±6.7 years) as well as uranium miners with silicosis (n=60) (73.0±3.6 years of age) Patients were diagnosed based on previously described criteria (12).

The healthy unexposed control group (n=50) (58.4±7.0 years of age) comprised healthy subjects who were not exposed in the workplace to the carcinogenic (or fibrogenic) agents outlined above; in other words, they had no history of smoking and were never knowingly exposed to asbestos dust, silica dust or ionizing radiation. The additional healthy control group (n=134) (53.8±12.1 years) comprised subjects without occupational exposure to carcinogens who were smokers or ex-smokers.

Current smokers were defined as individuals who were smokers at the time of diagnosis. Ex-smokers were individuals who had previously smoked but no longer smoked at the time of diagnosis. Data were collected regarding the average number of cigarettes smoked per day, the age at which the subject had started smoking and the age at which the subject had stopped smoking if the person was an ex-smoker. Pack-years were calculated to determine cumulative cigarette smoking. One pack-year was defined as 20 cigarettes smoked per day over the period of a year. The smokers were categorized by their pack-year values.

There were no significant differences in the relevant characteristics of the study subjects between the tumour or non-tumour cases and the controls in terms of mean age. The lung cancer group comprised more smokers (84 smokers/ex-smokers, 2 never-smokers) than the non-tumour controls (451 smokers/ex-smokers, 146 never-smokers) ($p<0.001$). Lung cancer patients had higher values of pack-years smoked (42±23.6) than controls (22±16.3), which reflects tobacco smoking as the main factor of lung cancer. Among the mesothelioma patients, 11 were smokers/ex-smokers (15±9.4 pack-years) and 8 had never smoked.

Real-time PCR and polymorphism detection. Blood samples (~5 ml) were obtained from patients and controls. Genomic DNA was isolated from whole blood using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany). Real-time PCR analysis and melting-curve analysis of the cytochrome *P-4501A1 A4889G* and *T6235C* polymorphisms were performed as previously described by Harth *et al* (13), and of the cytochrome *P-4501B1 codon 432* polymorphism as previously described by Brüning *et al* (14). Both the PCR primers and the fluorescent-labelled detection probes were commercially synthesized by Tib Molbiol (Berlin, Germany).

Nomenclature. To describe the different polymorphic variants, the systematic nomenclature for *CYP1A1* polymorphisms according to Bartsch *et al* was used (15). For the *CYP1A1* polymorphisms at position *T6235C*, the genotypes were *CYP1A1*1/*1* for the wild-type (T/T), *CYP1A1*1/*2* for the heterozygous (T/C) and *CYP1A1*2/*2* for the mutant (C/C) genotypes. For the *CYP1A1 A4889G* polymorphism, the genotypes were *CYP1A1*1/*1* for the wild-type (A/A), *CYP1A1*1/*3* for the heterozygous (A/G) and *CYP1A1*3/*3* for the mutant (G/G) genotypes. For the *CYP1B1 Val432Leu* polymorphism, the genotypes were *CYP1B1*1/*1*, *CYP1B1*1/*2* and *CYP1B1*2/*2*.

Statistical analysis. The association between genotype distribution and patient status was assessed according to the odds ratios (ORs) and confidence intervals (CIs). The OR and CI were calculated by unconditional logistic regression and adjusted for age, gender and smoking in pack-years. The gene-smoking interaction, adjusted for age and gender, was also analyzed by logistic regression methods. All statistical analyses were performed using the statistical software SPSS 15.0. p-values were determined by the two-sided test. A p-value <0.05 was considered statistically significant.

Results

The prevalence of various genotypes in the control population and in patients with occupational diseases is shown in Table I. The homozygous mutant allele (mt/mt) of *CYP1A1* occurred at a low frequency; it was observed in only five individuals (one control subject, four lung cancer patients) for *CYP1A1*2/*2* and three individuals (one control, two lung cancer patients) for *CYP1A1*3/*3* (data not shown). As a result, cases with the mt/mt variant were analyzed together with the wt/mt variants. In the

Table I. Risk of occupational diseases associated with the *CYP1A1* or *CYP1B1* genotypes.

	CYP1A1 T6235C genotype			*CYP1A1 Ile462Val* genotype			*CYP1B1 Val432Leu* genotype		
	wt/wt[a]	wt/mt or mt/mt	OR[b] (95% CI)	wt/wt[a]	wt/mt or mt/mt	OR[b] (95% CI)	wt/wt[a]	wt/mt or mt/mt	OR[b] (95% CI)
Unexposed healthy controls	151 (82.1)	33 (17.9)	Reference	170 (92.4)	14 (7.60)	Reference	57 (31.0)	127 (69.0)	Reference
Asbestosis (No 4103 BKV)	147 (81.2)	34 (18.8)	0.56 (0.54-1.72)	166 (91.7)	15 (8.30)	0.81 (0.36-1.83)	58 (32.0)	123 (68.0)	1.06 (0.65-1.73)
Asbestos-induced lung cancers (No 4104 BKV)	31 (79.5)	8 (20.5)	0.70 (0.27-1.81)	33 (84.6)	6 (15.40)	0.51 (0.14-1.83)	14 (35.9)	25 (64.1)	1.52 (0.68-3.39)
Silicosis (No 4101 BKV)	122 (84.7)	22 (15.3)	1.15 (0.57-2.31)	130 (90.3)	14 (9.70)	0.63 (0.24-1.65)	50 (34.7)	94 (65.3)	1.25 (0.71-2.19)
Silicosis in uranium miners (No 4101 BKV)	52 (86.7)	8 (13.3)	1.26 (0.39-4.05)	56 (93.3)	4 (6.70)	0.89 (0.20-4.09)	26 (45.3)	34 (56.7)	1.20 (0.53-2.71)
Lung cancer in silicosis patients (No 4112 BKV)	7 (100)	0	Not defined	7 (100)	0	Not defined	2 (28.6)	5 (71.4)	0.89 (0.16-4.78)
Lung cancer due to ionizing radiation (No 2402 BKV)	32 (80.0)	8 (20.0)	0.50 (0.18-1.36)	37 (92.5)	3 (7.50)	1.54 (0.49-4.89)	10 (25.0)	30 (75.0)	0.56 (0.20-1.55)
Mesothelioma due to asbestos (No 4105 BKV)	16 (84.2)	3 (15.8)	1.12 (0.30-4.14)	15 (78.9)	4 (21.1)	0.39 (0.10-1.54)	4 (21.1)	15 (78.9)	0.63 (0.20-2.03)

[a]wt, wild-type genotype; mt, mutant genotype at the studied polymorphic site (% in parenthesis); [b]OR and CI were calculated by logistic regression and adjusted for age, gender and tobacco smoking in pack-years; BKV, Berufskrankheitenverordnung (German legal system for occupational diseases).

studied population, the *CYP1B1* mutant allele occurred with higher frequency (56.7-78.9%). There were no significant differences in *CYP1A1* or *CYP1B1* genotype distribution among the control subjects and tumour patients, and no differences in genotype frequency with respect to exposure among the various occupational diseases. The risk of lung cancer associated with the *CYP1A1* or *CYP1B1* polymorphisms is presented in Table I. Calculations were consistently performed for the wt/wt vs. wt/mt and mt/mt genotypes. The odds ratio (adjusted for age, gender and pack-years) of *CYP1A1 T6235C* was calculated as OR=0.50 (95% CI 0.18-1.36) for patients with lung cancer due to ionizing radiation and OR=1.26 (95% CI 0.39-4.05) for uranium miners suffering from silicosis. When *CYP1A1 A4889G* or *CYP1B1 Val432Leu* were analyzed according to occupational disease, no strong association with the polymorphisms was found (Table I). For the *CYP1A1 A4889G* polymorphisms, only lung cancer risk due to ionizing radiation was elevated (OR=1.54; 95% CI 0.49-4.89), whereas for the *CYP1B1 Val432Leu* polymorphisms risk did not decrease significantly in lung cancer due to ionizing radiation (OR=0.56; 95% CI 0.20-1.55), mesothelioma (OR=0.63; 95% CI 0.20-2.03) or lung cancers following silica dust exposure (OR=0.89; 95% CI 0.16-4.78). Gene-asbestos (no. 4103, 4104, 4105 BKV; BKV, Berufskrankheitenverordnung: German legal system for occupational diseases), gene-silica (no. 4101 BKV) or gene-radiation interactions (no. 2402 BKV) did not show consistent results. While patients with asbestosis or asbestos-exposed lung cancer patients had lower risk in terms of polymorphisms of *CYP1A1*, patients suffering from mesothelioma due to asbestos with the *CYP1A1 T6235C* genotype had an elevated risk.

Since smoking is such a prominent risk for lung cancer, the risk for occupational diseases was further examined with regard to smoking status. Only smokers with wild-type *CYP1A1 A4889G* (OR=4.94; 95% CI 1.53-15.95) showed a significantly elevated risk. Polymorphic mutant alleles of *CYP1A1 T6235C* or *CYP1A1 A4889G* could not be detected in lung cancer patients who had never smoked. Analysis in relation to type of exposure (asbestos, ionizing radiation or silica dust; adjusted for age and gender) revealed that none of the exposure types were strongly associated with the polymorphisms (Table II).

Among smokers carrying the *CYP1A1* (*T6235C* or *A4889G*) wt/wt polymorphism, a consistent elevated risk in terms of occupational disease was not detectable. Unexpectedly, the highest observed risk (OR=2.23; 95% CI 0.25-19.87) was calculated for smokers among the mesothelioma patients (*CYP1A1 T6235C*).

Gene-smoking interaction was also examined among patients with the *CYP1B1* polymorphism. When the combination of smoking and the variant *CYP1B1* genotypes was analyzed, no significant interaction between the susceptible genotypes and smoking status was observed; smoking and the presence of the *CYP1B1* polymorphism did not result in a combined effect on occupational disease.

When patients were sub-grouped into never-smokers and non-smokers, the data concerning *CYP1A1* or *CYP1B1* indicated that the interaction between occupational disease and the susceptible genotypes had no modifying effect on the risk of lung cancer. Additionally, the calculated risk concerning type of exposure did not show consistent results with regard to fibrogenic or malignant effects.

Table II. Interaction of the *CYP1A1* or *CYP1B1* genotypes and tobacco smoking on the overall risk of occupational diseases.

	CYP1A1 T6235C genotype			*CYP1A1 Ile462Val* genotype			*CYP1B1 Val432Leu* genotype		
	wt/wt[a]	wt/mt or mt/mt	OR[b] (95% CI)	wt/wt[a]	wt/mt or mt/mt	OR[b] (95% CI)	wt/wt[a]	wt/mt or mt/mt	OR[b] (95% CI)
Unexposed healthy controls									
Never smokers	40 (80.0)	10 (20.0)	Reference	42 (84.0)	8 (16.0)	Reference	16 (32.0)	34 (68.0)	Reference
Smokers/ex-smokers	111 (82.8)	23 (17.2)	1.17 (0.51-2.69)	128 (95.5)	6 (4.50)	4.94 (1.53-15.95)	41 (30.6)	93 (69.4)	0.94 (0.47-1.89)
Asbestosis (No. 4103 BKV)									
Never smokers	28 (82.4)	34 (17.6)	0.99 (0.29-3.37)	31 (91.2)	3 (8.80)	1.47 (0.35-6.17)	16 (47.1)	18 (52.9)	2.15 (0.79-5.80)
Smokers/ex-smokers	119 (81.0)	28 (19.0)	0.93 (0.39-2.22)	135 (91.8)	12 (8.20)	1.73 (0.64-4.67)	42 (28.6)	105 (71.4)	0.91 (0.44-1.91)
Asbestos-induced lung cancer (No. 4104 BKV)									
Never smokers	1 (100)	0	Not defined	0	1 (100)	Not defined	1 (100)	0	Not defined
Smokers/ex-smokers	30 (78.9)	8 (21.1)	0.84 (0.29-2.45)	33 (86.8)	5 (13.2)	1.21 (0.36-4.07)	13 (34.2)	25 (65.8)	1.14 (0.46-2.81)
Silicosis (No. 4101 BKV)									
Never smokers	23 (85.2)	4 (14.8)	1.60 (0.35-7.23)	23 (85.2)	4 (14.8)	0.87 (0.20-3.82)	12 (44.4)	15 (55.6)	2.49 (0.79-7.88)
Smokers/ex-smokers	99 (84.6)	18 (15.4)	1.48 (0.55-4.01)	107 (91.5)	10 (8.50)	1.55 (0.48-4.99)	38 (32.5)	79 (67.5)	1.17 (0.51-2.66)
Silicosis in uranium miners (No. 4101 BKV)									
Never smokers	5 (71.4)	2 (28.6)	0.40 (0.05-3.16)	6 (85.7)	1 (14.3)	0.76 (0.06-9.45)	2 (28.6)	5 (71.4)	1.29 (0.17-9.63)
Smokers/ex-smokers	47 (88.7)	6 (11.3)	2.01 (0.42-9.52)	50 (94.3)	3 (5.70)	2.21 (0.36-13.6)	24 (45.3)	29 (54.7)	1.97 (0.64-6.08)
Lung cancer in silicosis patients (No. 4112 BKV)									
Never smokers	1 (100)	0	Not defined	1 (100)	0	Not defined	0	1 (100)	Not defined
Smokers/ex-smokers	6 (100)	0	Not defined	6 (100)	0	Not defined	2 (33.3)	4 (66.7)	1.17 (0.19-7.25)
Lung cancer due to ionizing radiation (No. 2402 BKV)									
Never smokers	0	0	Not defined	0	0	Not defined	0	0	Not defined
Smokers/ex-smokers	32 (80.0)	8 (20.0)	1.06 (0.34-3.31)	37 (92.5)	3 (7.50)	2.10 (0.49-8.98)	10 (25.0)	30 (75.0)	0.82 (0.30-2.24)
Mesothelioma due to asbestos (No. 4105 BKV)									
Never smokers	6 (75.0)	2 (25.0)	0.70 (0.12-4.10)	7 (87.5)	1 (12.5)	1.23 (0.13-11.65)	2 (25.0)	6 (75.0)	0.76 (0.14-4.28)
Smokers/ex-smokers	10 (90.9)	1 (9.10)	2.23 (0.25-19.87)	8 (72.7)	3 (27.3)	0.45 (0.09-2.16)	2 (18.2)	9 (81.8)	0.52 (0.10-2.75)

[a]wt, wild-type genotype; mt, mutant genotype at the studied polymorphic site (% in parenthesis); [b]OR and CI were calculated by logistic regression and adjusted for age and gender; BKV, Berufskrankheitenverordnung (German legal system for occupational diseases).

Discussion

The number of cases of occupational cancer being compensated in Germany is rising. The most common occupational cancers are lung carcinomas (53.8%) and mesotheliomas of the pleura, peritoneum and pericardium (33.7%). Currently, approximately 2/3 (71.9%) of all occupational cancers eligible for compensation are the result of asbestos fibers. The second most common occupationally-derived lung cancer (13.7%) is caused by ionizing radiation, in particular radon and its decay products. Aromatic amines account for 4.7% and polycyclic hydrocarbons for 1.7% of cancer cases originating in the workplace (16). There is evidence from animal experiments that quartz dust is carcinogenic, and epidemiological studies have revealed an association between lung cancer and exposure to quartz dust. In recent years, respirable quartz dust has been classified as a carcinogen. A meta-analysis of epidemiological studies showed high risks (RR>2.0) of developing lung cancer in patients suffering from silicosis (17,18).

Many studies have focused on the identification of genes and their modifying effect on cancer risk due to environmental pollutants or exposure to occupational carcinogens. Genetic differences in the metabolism of carcinogens may co-determine individual predisposition to lung cancer. Since P-450s are the primary enzyme interface between environmental carcinogens and organisms, it is expected that genetic susceptibility attributed to this enzyme family will be associated with various types of carcinogenic exposure. To date, the extent of the association between human *CYP1A1* or *CYP1B1* genetic polymorphisms and occupational exposure to carcinogens has not been analyzed.

In the current pilot study, we investigated *CYP1A1* and *CYP1B1* polymorphisms as genetic modifiers of risk in individuals with occupationally-derived lung cancers or mesothelioma and in non-cancer controls. The aim was to estimate the gene-environment association, and to examine their relation to tobacco smoking as a synergistic effect. The observed genotype frequencies were within the range described for Caucasians (1).

It was previously reported that benzo(*a*)pyren-diol-epoxides-DNA adduct levels in the bronchial tissue of smokers with high pulmonary *CYP1A1* inducibility were elevated in comparison to non-inducible subjects with a similar smoking dose (15). Smokers with the *CYP1A1* exon 7 valine polymorphism had significantly (2-fold) higher levels of DNA damage than those without (5). A significant association was found between the combined heterozygous and homozygous *Msp*I variant of the *CYP1A1* gene (*CYP1A1*1/*2* or *CYP1A1*2/*2*) and lung cancer (OR=2.8; 95% CI 1.15-3.73) (19). In contrast, no significant differences in risk between cases and controls were reported for *CYP1A1* polymorphisms by Gsur *et al* (20), for *CYP1A1 Ile462Val* (*CYP1A1 iva*) by Carstensen *et al* (21), or for *CYP1A1 Msp*I (*CYP1A1*1/*2* or *CYP1A1*2/*2*) by Kelsey *et al* (22), Le Marchand *et al* (23) or Tefre *et al* (24).

The results of the present study did not reveal an association between *CYP1A1* polymorphisms at position *T6235C* or *A4889G* (*CYP1A1*1/*2* and *CYP1A1*2/*2* or *CYP1A1*1/*3* and *CYP1A1*3/*3*) or the *CYP1B1 codon 432* polymorphism and occupational lung cancer risk. Due to the relatively small sample number in some of the patients groups, significant results cannot be expected. In high-dimensional analysis, such as the investigation of all potential interactions, the number of hypotheses is greatly inflated. Additionally, a large population study (>10,000) is necessary to determine less frequent polymorphisms. Based on the number of patients with occupational tumours compensated annually in Germany, especially the relatively rare mesothelioma cases, these sample sizes will not be obtained. In this study, the results regarding gene-asbestos (no. 4103, 4104, 4105 BKV), gene-silica (no. 4101 BKV) or gene-radiation (no. 2402 BKV) interactions were inconsistent. While patients with asbestosis or asbestos-exposed lung cancer patients had lower risk in terms of *CYP1A1* polymorphisms, patients suffering from mesothelioma due to asbestos with the *CYP1A1 T6235C* genotype had an elevated risk (Table I).

Examination by histological subtype of cancer has also failed to identify significant associations (1,23,25). Among Asians, an association with squamous cell carcinoma only is apparent (1). Ko *et al* reported a significant increase in susceptibility associated with *CYP1B1 codon 432* polymorphisms in Caucasians smokers with head and neck squamous cell cancer (26). This was also associated with an increased frequency of smoking-induced p53 mutations.

In studies by Schneider *et al* (25) and Vineis *et al* (1), lung cancer risk increased significantly with higher cumulative cigarette smoking doses or longer duration of smoking. In Caucasians, a steeper increase in risk associated with higher smoking dose among patients with the variant genotype has been proposed. This phenomenon was less evident in Asians (1). It has been demonstrated that individuals with the susceptible *CYP1A1 Val/Val* (*CYP1A1*3/*3*) genotype have an increased risk even at lower cigarette dose levels (27). In the present study, no association between the *CYP1A1* or *CYP1B1* genotypes and smoking was observed, nor did we achieve consistent results regarding gene-asbestos, gene-silica or gene-radiation interactions.

Asbestos is not a substrate for the metabolic process, but one mechanism behind its destructive effect is through the generation of reactive oxygen (ROS) and nitrogen species (RNS), possibly modified by xenobiotic metabolizing enzymes (CYP) (4). Neri *et al* reported that metabolic genotypes were modulators of asbestos-related pleural malignant mesothelioma risk (28). In the present study, we did not detect an association between the CYP polymorphisms and the risk of developing malignant pleural mesothelioma, asbestos-induced lung cancer or (non-malignant) asbestosis. The lack of any association between the *CYP1A1* or *CYP1B1* genotypes and the risk of mesothelioma supports the notion that polycyclic aromatic hydrocarbons, the main metabolic substrates of CYP, may not have a direct effect on the development of this malignancy.

While crystalline silica is a negative modifier of pulmonary cytochrome *P-4501A1* induction, consistent results in silicosis patients were not acheived. For *CYP1B1* polymorphisms alone, the risk for silicosis patients was the same among smokers and non-smokers, and was slightly but not significantly elevated among uranium miners (Table II).

To detect possible synergistic effects by ionizing radiation, uranium miners were included in the study. Cytochrome *CYP1B1* showed the greatest overexpression in radioresistant cell lines (7). At low patient numbers, the results did not reveal a modified risk in former uranium miners in terms of *CYP1A1* or *CYP1B1* polymorphisms and smoking status.

The results also failed to provide any evidence of an association between *CYP1A1 T6235C, CYP1A1 A4889G* or *CYP1B1* and smoking status, susceptible genotypes and occupationally-derived lung or pleural disorders. In conclusion, neither the previously identified *CYP1A1*1/*2* and *CYP1A1*2/*2* nor the *CYP1A1*1/*3* and *CYP1A1*3/*3* polymorphisms were associated with tumour risk in occupationally induced tumours, nor did they significantly modify risk in relation to cigarette consumption. No association was found between *CYP1B1* polymorphisms, smoking and occupational diseases. This was independent of whether the exposure was to asbestos, silica dust or ionizing radiation.

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