CYP1A1 genetic polymorphisms, tobacco smoking and lung cancer risk in a French Caucasian population

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The *CYP1A1* gene encoding for an enzyme involved in the metabolic activation of important tobacco carcinogens could be implicated in smoking-induced lung cancer. Given the strong association between tobacco smoking and lung cancer, the effect of tobacco smoke exposure has to be taken into account when studying the potential association between lung cancer and *CYP1A1* genotypes. The effect of two *CYP1A1* genetic polymorphisms (*Msp*I and *Ile-Val*) on lung cancer risk were evaluated using peripheral blood DNA from 150 lung cancer patients and 171 controls. The *Msp*I site-present allele was found among 19.3% of both cases and controls and the variant allele *Val* among 6.7% of cases and 8.8% of controls. Lung cancer risks associated with the *Msp*I site-present allele (OR = 0.9; 95%CI: 0.5–1.8) or with the *Val* allele (OR = 0.8; 95%CI: 0.3–1.9) were not increased after adjustment for tobacco and asbestos exposures. These results persisted when analyses were stratified on smoking status, daily consumption of tobacco or duration of smoking. Similar findings were obtained when squamous cell or small cell carcinomas were studied separately. This study thus suggests a minor role for the known *CYP1A1* gene polymorphisms in predisposition to lung cancer among Caucasian populations.

Keywords: *lung cancer*, CYP1A1, *smoking, genetic polymorphism, cancer susceptibility.*

Introduction

Most of the procarcinogens contained in tobacco smoke must be activated in the organism to become ultimate carcinogens. Hence, the risk for tobacco-related cancers among individuals should depend on both their level of tobacco exposure and their genetically determined ability to metabolize the tobacco-derived carcinogens. The importance of enzymes of drug metabolism in tobacco-related cancer depends upon the role of genotoxic chemicals in the carcinogenic process. Since there are other mechanisms whereby tobacco smoke may induce lung cancer, the question of to what extent genetic variability in drug-metabolizing enzymes might influence susceptibility to this form of cancer remains to be established. The aryl hydrocarbon hydroxylase (AHH) is involved in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs), abundant in tobacco smoke (Yun *et al.* 1992). The *CYP1A1* gene encoding for AHH thus attracts particular interest for its potential role in pulmonary carcinogenesis. Apart from a genetic polymorphism in the *CYP1A1* gene specific to African-Americans (Crofts *et al.* 1993), an *Msp*I restriction fragment length polymorphism (RFLP) in the 3′ non-coding region and an *Ile-Val* polymorphism of exon 7, resulting in the replacement of the amino acid isoleucine by valine in the haem-binding region, have been found in all ethnic groups studied so far (Hayashi *et al.* 1991). These polymorphisms are in linkage disequilibrium, and since their functional significance is not verified, the variant allele *Val* located in the coding region appears as the more probable candidate as a modifier of the *CYP1A1* gene inducibility and the AHH enzymatic activity (Crofts *et al.* 1994).

Results of meta-analyses of epidemiological studies on lung cancer in relation to *Msp*I and *Ile-Val* polymorphisms have been recently reported (D'Errico *et al.* 1996). No association between lung cancer and each polymorphism was found among Caucasians, while in Japanese populations a significant increased risk was found with each variant allele. Although the potential association between *CYP1A1* polymorphisms and lung cancer should depend on the level of tobacco exposure, only a few of the previous studies have properly considered the concomitant effect of these genetic polymorphisms and tobacco exposure on lung cancer risk. Increased risks were reported to be associated, mainly in light smokers, with each *Msp*I and *Ile-Val* polymorphisms in Japan (Nakachi *et al.* 1993) and with *Ile-Val* polymorphism in the pluriethnic population of Brazil (Hamada *et al.* 1995). In studies involving Caucasian individuals with available data on smoking histories, the results on the concomitant effect of tobacco exposure and *CYP1A1* polymorphisms were either not reported (Hirvonen *et al.* 1992, Alexandrie *et al.* 1994), not interpretable (Drakoulis *et al.* 1994), or based on too small numbers (Shields *et al.* 1993). Therefore, previous results in Japanese or Brazilian populations still need to be confirmed in Caucasians.

We investigated the effects of both *Ile-Val* and *Msp*I polymorphisms on lung cancer risk according to the extent of tobacco exposure in a Caucasian population in France.

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MATERIALS AND METHODS

The study was drawn from a case-control study performed in France from 1988 to 1992. All case and control individuals were recruited in 10 private or public hospitals, of which nine are located in Paris. Cases were all eligible Caucasian patients with histologically confirmed squamous or small cell primary lung cancers. Control individuals were all eligible Caucasian patients without previous or actual malignant disease admitted in the same hospital (or, for anticancer centres, a hospital in the same geographical areas) and recruited accordin

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distributions observed in cases. The main medical diagnoses among controls were rheumatological, cardiovascular, respiratory, infectious and parasitic diseases. All cases and controls had to be regular smokers, defined as people having smoked five cigarettes or more (or cigars or pipes) per day for a least 5 years. Because the study design also included *CYP2D6* and *CYP2C19* phenotyping tests (using dextromenthorphan and mephytoin), the exclusion criteria equally related to subjects with severe renal disease, severe liver disease, or severe chronic heart failure or who had taken, during the last week, any drugs known or suspected to interfere with these tests (Bouchardy *et al.* 1996). Blood samples were available for 95% of subjects and DNA was then extracted by standard protocols from peripheral blood samples of 150 lung cancer patients and 171 controls. A questionnaire was filled out for each subject during a personal interview. Each interviewer had to include both cases and controls. Information on recent and past tobacco use (daily consumption, duration, age at the beginning of smoking, inhalation, type of tobacco and changes in smoking habits), as well as occupational exposure, was recorded.

The daily consumption of each type of tobacco was expressed in grams per day (1g for cigarette, 2g for cigar, and 3g for pipe) calculated by dividing the cumulative lifetime tobacco consumption by the overall duration of smoking. Ex-smokers were defined as people who had stopped smoking at least 1 year prior to the diagnosis. The duration of smoking and the mean daily consumption of tobacco were calculated from the age at the beginning of smoking till the age of smoking cessation (ex-smokers) or the age at diagnosis (current smokers).

Peripheral blood samples were collected into EDTA tubes and stored at –20 °C. Extraction and genotype determination were blindly performed on the total white blood cell DNA at the Finnish Institute of Occupational Health in Helsinki. The *CYP1A1* genotypes ascribed to the presence or absence of the *Msp*I site at the 264th base downstream from the additional polyadenylation signal and to the substitution of isoleucine for valine at residue 462 in the haem-binding region were studied as described (Hayashi *et al.* 1991, 1992, Oyama *et al.* 1995).

The odds ratio (OR) and 95% confidence interval (95%CI) for the presence of each variant allele were calculated using unconditional multivariate logistic regression (Breslow and Day 1980). All the ORs were adjusted for sex, age, as well as on all variables related to smoking and occupational exposure as confounding factors. Interactions between *CYP1A1* genotypes and each variable related to smoking were investigated to test the equality of the ORs for the presence of each variant allele across the different levels of tobacco exposure (heterogeneity test) and to evaluate a potential linear variation of *CYP1A1* genotypes effect with increasing levels of smoking (trend test) (Breslow and Day 1980). The average daily consumption of tobacco and the duration of smoking were expressed as continuous or as categorical variables as previously described (Bouchardy *et al.* 1996). In order to study the effect of the *CYP1A1* polymorphism among the lightest smokers, daily consumption was also expressed as a binary variable; since this study involved only regular smokers, the lowest class of tobacco consumption was defined by an average consumption of 15 g per day or less. All models were log-linear, fitted using the generalized linear interactive modelling statistical package (Baker and Nelder 1978).

Results

The main characteristics of lung cancer patients and controls are summarized in Table 1. The study population consisted almost entirely of males (93% of cases and 95% of controls). The mean age was slightly higher for lung cancer patients (58.4 years) than for controls (55.0 years). All individuals had a history of regular smoking, as defined above. About 75% of cases and of controls were exclusively cigarette smokers; the rest being almost entirely cigarette smokers associated with another tobacco product. Since we chose to recruit only regular smokers, the variability between cases and controls concerning tobacco exposure was very small. The average daily consumption of tobacco was similar among cases (26.3 g per day) and controls (25.1 g per day), but the cases had smoked longer than the controls (38.0 years versus 32.2 years; $p < 0.0001$). About 19% of the cases and 7% of the controls reported a history of occupational asbestos exposure ($p < 0.001$). The figures were 5% of cases and 1% of controls for arsenic exposure (not significant). No significant difference in either tobacco smoke, asbestos, or arsenic exposure was found between the two histological types of lung cancer, squamous cell and small cell carcinomas.

Table 2 shows the frequency distribution of *Msp*I and *Ile-Val* genotypes among lung cancer patients and controls. As expected, the two polymorphisms were in strong linkage disequilibrium both in cases and controls. Only three study subjects had homozygous variant genotypes *m2/m2* or *Val/Val*, all of whom were lung cancer patients. Therefore, all subjects with the variant allele for each polymorphism had to be combined for the statistical analyses. The variant allele *m2* was present among 19.3% of cases (allele frequency, 0.107) and among 19.3% of controls (allele frequency, 0.097). The genotypes in the control population were in Hardy–Weinberg equilibrium with observed frequencies of *m1/m1*, *m1/m2* and *m2/m2* genotypes (80.7%, 19.3% and 0% respectively) very close to those expected (81.6%, 17.4% and 1%). The variant allele *Val* was present in 6.7% of cases (allele frequency, 0.040) and in 8.8% of controls (allele frequency, 0.044). No significant difference was found between observed and expected frequencies of *Ile/Ile*, *Ile/Val* and *Val/Val* genotypes in controls (91.2%, 8.8% and 0% versus 91.4%, 8.4% and 0.2%).

Lung cancer risk in relation to *Msp*I and *Ile-Val* polymorphisms according to histology and variables related to

	All lung cancers (*n*=150)	Squamous cell carcinoma (*n*=98)	Small cell carcinoma (*n*=52)	Controls (*n*=171)
Mean age (years) ± SD	58.4 ± 9.9	58.6 ± 9.6	58.1 ± 10.4	55.0 ± 11.0
Mean daily consumption of tobacco (g per day) ± SD[a]	26.3 ± 13.4	27.3 ± 13.0	24.3 ± 13.9	25.1 ± 12.5
Mean duration of smoking (years) ± SD[b]	38.0 ± 9.5	37.7 ± 9.2	38.7 ± 9.9	32.2 ± 11.5

Table 1. Main characteristics of lung cancer patients and controls.

[a] Data were missing for four lung cancer patients and two controls.
[b] Data were missing for two lung cancer patients.

	Lung cancer patients			Controls		
Genotype	*Ile/Ile* (%)	*Ile/Val* (%)	*Val/Val* (%)	*Ile/Ile* (%)	*Ile/Val* (%)	*Val/Val* (%)
m1/m1[a]	121 (86%)[b]	0	0	138 (89%)	0	0
m1/m2	18 (13%)	8 (100%)	0	18 (11%)	15 (100%)	0
m2/m2	1 (1%)	0	2 (100%)	0	0	0
Total	140	8	2	156	15	0

Table 2. Frequency distribution of P450*CYP1A1* genotypes among lung cancer patients and controls.

[a] *m1/m1*: homozygous *Msp*I site-absent; *m1/m2*: heterozygote; *m2/m2*: homozygous *Msp*I site-present.

[b] The percentages of the *Msp*I among the *Ile-Val* genotypes are given in parentheses.

smoking is presented in Table 3. No significantly increased risk for lung cancer overall was found among individuals with the variant allele *m2* (OR = 0.9; 95%CI: 0.5–1.8) or with the *Val* allele (OR = 0.8; 95%CI: 0.3–1.9). Similar results were obtained when squamous and small cell carcinomas were studied separately. No significant interaction was observed between *CYP1A1* genotypes and variables related to smoking. However, analysis of interaction is probably hampered by small numbers. The lack of association between lung cancer and the presence of the *m2* allele or the *Val* allele persisted when analyses were stratified on smoking status, daily tobacco consumption or duration of smoking. Similar results were obtained among lightest smokers: for a daily consumption of 15 g or less, the ORs were 0.6 (95%CI: 0.1–2.1) among individuals with the variant allele *m2* and 0.9 (95%CI: 0.2–4.6) among those with the *Val* allele.

	*Msp*I genotypes		*Ile-Val* genotypes	
	m1/m1	*m1/m2* and *m2/m2*	*Ile/Ile*	*Ile/Val* and *Val/Val*
Histological types				
All lung cancers	1.0	0.9 (0.5–1.8)	1.0	0.8 (0.3–1.9)
	121/138	*29/33*	*140/156*	*10/15*
Squamous cell	1.0	1.2 (0.6–2.3)	1.0	1.1 (0.4–3.1)
	76/138	*22/33*	*89/156*	*9/15*
Small cell	1.0	0.7 (0.2–1.8)	1.0	0.2 (0.03–2.0)
	45/138	*7/33*	*51/156*	*1/15*
Smoking status				
Ex-smokers	1.0	0.9 (0.3–2.6)	1.0	1.7 (0.3–11.8)
	47/45	*13/11*	*55/54*	*5/2*
Current smokers	1.0	1.0 (0.4–2.1)	1.0	0.6 (0.2–1.8)
	74/93	*16/22*	*85/102*	*5/13*
Daily consumption of tobacco (g per day)				
< 20	1.0	0.8 (0.3–1.8)	1.0	0.4 (0.1–1.5)
	55/67	*14/18*	*64/74*	*5/11*
21–30	1.0	0.8 (0.2–3.0)	1.0	1.1 (0.1–22.9)
	26/29	*7/7*	*31/35*	*2/1*
> 30	1.0	1.0 (0.3–3.5)	1.0	1.5 (0.3–8.7)
	37/40	*7/8*	*41/45*	*3/3*
Duration of smoking (years)				
< 30	1.0	0.4 (0.1–1.9)	1.0	0.6 (0.1–4.7)
	31/65	*3/14*	*32/73*	*2/6*
> 30	1.0	1.2 (0.6–2.4)	1.0	0.8 (0.3–2.4)
	88/73	*26/19*	*106/83*	*8/9*

Table 3. Number of case/controls and OR[a] (95%CI) for lung cancer in relation to *CYP1A1* polymorphisms according to histology and tobacco exposure.

[a] Adjusted for all the variables in the table and for sex, age (< 50, 50–54, 55–59, 60–64, and ≥65), age at smoking initiation (> 18, 17–18, and < 16), inhalation (no/yes), asbestos exposure (no/yes) and arsenic exposure (no/yes).

Discussion

This case-control study failed to reveal any relationship between lung cancer risk and the known *CYP1A1* polymorphisms in a French Caucasian population. Our results agree with those previously reported for the *Ile-Val* polymorphisms in Scandinavian and North American Caucasian populations, but contrast with those found in the Japanese study and in the pluriethnic population of Brazil. They also agree with those previously reported for the *Msp*I polymorphisms in Norwegians and White Americans (Tefre *et al.* 1991, Shields *et al.* 1993).

The wide difference in the frequencies of the *Msp*I and *Ile-Val* polymorphisms between ethnic groups may in part explain the divergent findings. In our control group, as in other studies involving Caucasians (Tefre *et al.* 1991, Hirvonen *et al.* 1992, Shields *et al.* 1993, Alexandrie *et al.* 1994, Drakoulis *et al.* 1994), both polymorphisms were very rare with allele frequencies for *m2* (~10%) and *Val* (~5%), 2–3-fold lower than those reported for Japanese and Brazilians. Since the number of individuals homozygous for the rare alleles is very low among Caucasians, it is not possible to determine whether or not these individuals are more susceptible to lung cancer at lower levels of smoking, as was observed in the Japanese study (Nakachi *et al.* 1993). In this study, both heterozygous and homozygous carriers of the mutated alleles had to be included to the putatively 'at-risk' category. The estimates of lung cancer risk thus related predominantly to heterozygotes, leading to a possible dilution effect in the association between lung cancer and *CYP1A1* polymorphisms, since heterozygotes may be at lower lung cancer risk than homozygous carriers of the variant alleles. However, in a recent meta-analysis of Japanese studies, homozygous carriers of the variant allele *Val* appear to be at lower risk than heterozygous (D'Errico *et al.* 1996). Among Caucasians, the role of homozygosity has still to be evaluated and could be analysed only in a very large study. At present, it is not possible to determine whether the lack of an association between *CYP1A1* genotypes and lung cancer could be due to the rarity of these genotypes in Europeans or to a lack of an effect.

The discrepancies in the results could also be related to differences in levels of tobacco exposure between the studied populations. If the *CYP1A1* polymorphisms modulate the lung cancer risk mainly among lightest smokers, as suggested in previous studies, a higher average tobacco consumption in the Caucasian control populations would make it impossible to detect any significant association. In our study, the mean tobacco consumption among controls was high (~40 pack-years†; mean lifetime consumption: ~30×10^4 cigarettes), very

† Number of packs smoked per day multiplied by number of years of smoking; 20 cigarettes per pack.

close to that observed among controls of other negative studies, but higher than the one observed in the control population in Japan (mean lifetime consumption: 23×10^4 cigarettes) and in Brazil (~25 pack-years)†.

Methodological problems in analyses could also contribute to the discrepancies in previous findings between populations of different ethnicity. In the Japanese study, lung cancer risk associated with the *CYP1A1* polymorphism was estimated by subgroups of tobacco consumption. However, the mean tobacco consumption was higher among cases than among controls, and a residual effect of tobacco could explain the increased risk for lung cancer among lightest smokers. In the Brazilian study, the lung cancer risks associated with *CYP1A1* genotypes declined with increasing smoking levels. However, control individuals were pooled in the analyses, regardless of their smoking history. Given the very strong association between smoking and lung cancer in studies on the potential effect on lung cancer risk of *CYP1A1* genotypes, as well as on the interactive effect of *CYP1A1* and tobacco, the effect of smoking should be strictly controlled, as was done in our study. Without such an adjustment, an association observed between *CYP1A1* and lung cancer could in fact be artefactual.

Our data support the previous findings that these *CYP1A1* polymorphisms are not important population risk factors for lung cancer development in Europeans. Since lung cancer risk associated with homozygous variant genotypes could not be estimated in our study, the possibility exists that homozygosity for variant alleles is an individual risk factor. However, given the rarity of the variant alleles in European populations, the public health implication should be of less importance than in Asian populations.

Acknowledgements

We would like to thank Mrs S.T. Saarikoski for help in setting up the genotype assays, Dr C. Bonaïti-Pellié for her helpful comments on the manuscript, Mrs R. Striberni for her expert technical help, Mrs C. Paoletti and M. Labbé for technical assistance and Mrs T. Pamm for her editorial assistance.

We are also indebted to the consultants and chiefs of Clinical units who allowed us to study their patients for the purpose of the study: Drs G. Akoun, R. Arriagada, P. Baldeyrou, F. Besançon, A. Bisson, M. Bisson, F. Blanchet, F. Blanchon, A. Bouchiki, J. Brugère, C. Buffet, J. P. Camus, R. Caquet, Y. Chapuis, D. Chassagne, P. Constans, B. Dautzenberg, J. Debray, J. P. Derenne, P. Duroux, J. Fain, G. Freyss, A. Gerbaulet, Ph. Girard, J. Guerre, P. Guibout, H. Hamard, B. Housset, J. C. Imbert, F. Janot, A. Jardin, T. Le Chevalier, B. Lebeau, A. M. Leridant, Ph. Levasseur, V. G. Levy, A. Livartowski, G. Loyau, B. Loboinski, G. Mamelle, F. Mazas, P. Marandas, C. Menkes, H. Mondon, J. P. Passeron, J. Piquet, M. Robillard, J. Rochemaure, R. Roy-Camille, J. C. Saltiel, G. Schwaab, J. M. Segrestaa, D. Sereni, M. Spielmann, P. Testas, G. Tobelem, P. Vige.

This work was supported by the Swiss Cancer League, Switzerland (FOR063); League against Cancer of Fribourg, Switzerland (FOR381.88); Cancer Research, Switzerland (AKT617); and Fund for Clinical Research against Cancer, Gustave-Roussy Institute, Villejuif, France (88D28).

† Number of packs smoked per day multiplied by number of years of smoking; 20 cigarettes per pack.

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Received 16 September 1996, revised form accepted 11 November 1996