Pergamon

European Journal of Cancer Vol. 32A, No. 10, pp. 1701–1706, 1996
Copyright © 1996 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0959–8049/96 $15.00+0.00

PII: S0959-8049(96)00172-4

Original Paper

Correlation Between P450 CYP1A1 Inducibility, MspI Genotype and Lung Cancer Incidence

M. Jacquet,[1] V. Lambert,[1] E. Baudoux,[3] M. Muller,[2] P. Kremers[1] and J. Gielen[1]

[1]Service de Chimie Médicale, Institut de Pathologie B23, CHU Sart-Tilman, B-4000 Liège; [2]Service de Physiologie du Travail, Institut Malvoz, B-4020 Liège; and [3]Department of Blood Transfusion, CHU Sart-Tilman, B-4000 Liège, Belgium

The aim of this study was to verify a possible correlation between *CYP1A1* induction, MspI genotype and lung cancer incidence. A case–control study was performed on 48 lung cancer patients and 81 healthy subjects to test the existence of a correlation, within a European population. The hyperinducible group exhibited a significantly higher risk of lung cancer (odds ratio = 3.41; $P = 0.036$), especially for adenocarcinoma (odds ratio = 5.29; $P = 0.033$). In contrast with the situation observed in Asian populations, the frequency of the M2 allele did not differ significantly in the total lung cancer population (7.82%) and the group of healthy subjects (10.71%). The median inducibility value was slightly higher among cancer patients with one or two M2 alleles than among patients homozygous for the wild-type allele ($P = 0.09$). However, the percentage of individuals possessing at least one mutated allele was not significantly higher among hyperinducible patients (37.5%) than among non-hyperinducible patients (16.0%). No significant correlation could be found between M2 allele and lung cancer or between M2 allele and *CYP1A1* inducibility; the only positive correlation found was between *CYP1A1* hyperinducibility and lung cancer incidence. Our observations do not support the view that the presence of the M2 allele at the MspI site of the *CYP1A1* gene constitutes a significant lung cancer risk in Caucasians.
Copyright © 1996 Elsevier Science Ltd

Key words: lung cancer, smoking, *CYP1A1*, polymorphism, inducibility, lymphocytes, EROD
Eur J Cancer, Vol. 32A, No. 10, pp. 1701–1706, 1996

INTRODUCTION

As a result of the widespread contamination of our environment, we are continuously exposed to a wide variety of pollutants, notably polycyclic aromatic hydrocarbons (PAHs) such as benzo(*a*)pyrene, produced by combustion of organic compounds and present especially in cigarette smoke. This exposure, which can become major in some workplaces, plays a considerable role in the development of various forms of cancer, particularly lung cancers [1–3]. Most of these products are hydrophobic and the host organism needs the P450 enzyme battery to eliminate them. For instance, benzo(*a*)pyrene metabolism via the CYP1A1 enzyme generates a highly electrophilic diol-epoxide metabolite capable of creating DNA adducts [4, 5]. In addition to environmental risk factors, inherited or acquired host factors also play an important role in lung cancer development. Expression of the *CYP1A1* gene is upregulated by certain foreign chemicals, including PAHs, but the extent to which PAHs induce *CYP1A1* varies considerably in human populations. This may explain the variability of the genetic predisposition to this kind of epithelial cancer [1, 6]. As first discovered in human lymphocytes [2, 7], some individuals display very high inducibility of *CYP1A1* by PAHs. The high inducibility values recorded in 10% of the human population define what is called the 'hyperinducibility phenotype'. Kellerman and colleagues have shown that this phenotype is more frequent in lung cancer patients than in healthy individuals [8]. We have shown that mitogen-activated lymphocytes are a good model for evaluating *CYP1A1* inducibility by PAHs in subjects' lungs (data not shown). The method involves determining the ratio of ethoxyresorufin-*O*-deethylase (EROD) activity, supported by the CYP1A1 enzyme, to cyt. c red. (cytochrome c reductase) activity, supported by a microsomal enzyme unaffected by PAH induction. Experiments aimed at optimising this method led us to measure both activities on cultured

Correspondence to P. Kremers.
Received 4 Sep. 1995; revised 22 Mar. 1996; accepted 25 Apr. 1996.

mitogen-activated lymphocytes after a 96-h induction by benzanthracene. Unfortunately, this procedure is long, tedious, and requires large blood samples. Many investigators have therefore sought a correlation between the hyperinducibility phenotype and a particular genotype, easier to determine. Several polymorphisms of the Ah (Aryl hydrocarbon) locus have been studied [7, 9–12]. In particular, a point mutation called 'M2', located 300 bp downstream of the *CYP1A1* gene polyadenylation site, has raised hopes of explaining hyperinducibility by PAHs. In the Japanese population, this mutation, which generates an MspI restriction fragment length polymorphism (RFLP), is more frequent among lung cancer sufferers, principally those with squamous cell carcinoma, than among healthy individuals [11, 13]. This mutated genotype might thus be responsible for increased susceptibility to tobacco-related lung cancer [10]. Much work has focused on possible links between lung cancer development and either the M2 allele [11, 14–18] or hyperinducibility [1, 2, 8]. Although in the Japanese population the link between the M2 allele and lung cancer seems well established [11, 13], the existence of such a link in the Caucasian population is much debated [12, 19]. Hyperinducibility of *CYP1A1* is considered a risk factor for this type of cancer [2], but no clear correlation has been established between high inducibility of *CYP1A1* and the presence of the M2 allele.

In the present work, we have compared two groups of Caucasians: a group of 81 blood donors and a group of 48 lung cancer patients. The MspI genotype and the inducibility of the *CYP1A1* gene were determined on lymphocytes isolated and cultured after blood sampling. This case–control study was performed to confirm or invalidate the hypothesis that, within the European population, there is a correlation between the presence of the *CYP1A1* M2 allele and either lung cancer or the hyperinducibility phenotype.

MATERIALS AND METHODS

Materials

All chemicals and biochemicals were of analytical quality and were purchased from Sigma (München, Germany), GIBCO (Gent, Belgium), Boehringer (Mannheim, Germany) or Merck (Darmstadt, Germany). Ficoll-Paque was purchased from Pharmacia (Uppsala, Sweden). Disposable materials used for cell culture were purchased from Becton-Dickinson (Helsinki, Finland).

Biological samples

Blood samples were obtained from: (a) a blood donor population (81 healthy persons); (b) a lung cancer patient population (48 patients hospitalised for bronchopulmonary cancer). Lymphocytes from blood samples freshly collected on heparin were rapidly isolated on Ficoll-Paque gradients as recommended by the manufacturer. They were frozen in liquid nitrogen [20] until cultured.

For lymphocyte culture and induction, the procedure used was a modified version of that described by Kouri and colleagues [21]. The lymphocytes were rapidly thawed at 37°C and suspended at a cell density of 0.5×10^6 cells/ml in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated human AB serum, phytohaemaglutinin PHA-M, and 12 μM benzanthracene. Aliquots of 7 ml were seeded into culture dishes (diameter: 35 mm) and incubated for 96 h at 37°C in an atmosphere of humidified air (95%) and CO_2 (5%). All assays were performed in triplicate.

Inducibility of CYP1A1 in lymphocytes was evaluated by measuring a CYP1A1-specific enzyme activity (EROD) and comparing it with another enzyme activity unaffected by PAH induction (cyt. c red.). The inducibility factor was defined as the ratio of EROD activity to cyt. c red. activity determined on the same cells. For each lymphocyte sample, the enzyme activities were determined on three different culture dishes. EROD activity was expressed in pmoles of resorufin formed per minute and per dish. Cytochrome c reductase activity was expressed in nmoles of cytochrome c reduced per minute and per dish. The activity ratios were calculated from the means of the three independent measurements.

EROD activity assay. EROD activity was measured directly on the ongoing cultures. Ethoxyresorufin and dicoumarol (a stabiliser of the reaction product) were added to each cell suspension (respective final concentrations: 8 and 10 μM) and the samples were incubated for 45 min at 37°C. A 1 ml aliquot of cell suspension directly withdrawn after addition of the substrate provided a blank (*T*0). At the end of the incubation, the cell suspensions (6 ml) were rapidly centrifuged to separate the cells from the medium. The supernatants and cell pellets were frozen separately and stored at −80°C. Overnight hydrolysis of the conjugated metabolites was carried out at 37°C on 1 ml aliquots of supernatant using a mix of β-glucuronidase/arylsulphatase in a 0.1 M acetate buffer, pH 5.5. Proteins were precipitated with methanol, and the resorufin formed was finally measured by fluorimetry according to Burke and Mayer [22].

Cytochrome c reductase. This was measured on whole cell pellets according to Peterson and coworkers [23] (ϵ = 21 mM^{-1} cm^{-1}). The quantitation threshold was 0.25 nmoles of cytochrome c reduced per minute and per culture dish.

DNA extraction was carried out on 5 ml blood samples collected on heparin. A conventional phenol-chloroform method [24] was used as follows: after centrifugation and elimination of the plasma, the erythrocyte pellet was lysed by dilution in a buffer containing 2.5 mM Tris–HCl (pH 7.6), 80 mM sucrose, 1.25 mM $MgCl_2$, 0.25% Triton X-100 and 0.005% sodium azide. The leucocyte pellet, obtained after a 15-min centrifugation at 300***g***, was digested overnight with proteinase K (0.1 mg/ml) at room temperature in a buffer containing 50 mM Tris–HCl (pH 8.0), 20 mM EDTA and 2% SDS. Phenol-chloroform extractions were carried out until a clear lysate was obtained. The pH of the aqueous phase was adjusted to 5.6 with 0.1 M ammonium acetate and one volume of isopropanol was added. The precipitated DNA was removed with a glass loop and washed once with 70% ethanol before solubilisation in a buffer containing 10 mM Tris–HCl (pH 8.0) and 0.1 mM EDTA. DNA concentration and purity were estimated by UV spectrophotometry.

The MspI genotype was elucidated by amplifying a 338 bp DNA fragment encompassing the site of interest by the polymerase chain reaction (PCR). The primers used were 5′-AGT GAA GAG GTG TAG CCG CT-3′ and 5′-CGC TAA GAC AGC ACA GTG ATT-3′. The PCR mix contained 1 × PCR Gene Amp buffer (Perkin Elmer), 2.5 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM primers, 0.15 μg genomic DNA and 1.25 units of Ampli Taq polymerase (Cetus); it was subjected to 35 thermocycles of 2 min at 94°C, 1 min at 57°C and 2 min at 72°C. The elongation cycle was ended by heating at 72°C for 5 min. The PCR products were digested with MspI restriction

enzyme. When the mutated allele (M2) is present, this yields two DNA fragments (205 and 133 bp, see Figure 1).

Statistics

Hardy-Weinberg's equation was used to calculate allele frequencies. A subject was considered to display the hyperinducibility phenotype when the inducibility factor measured on lymphocytes fell within the 90th quantile of inducibility values recorded in the unilateral healthy population. Different statistical analyses were used to assess correlations between the CYP1A1 genotype, *CYP1A1* inducibility and the lung cancer risk: (1) Unpaired *t*-tests or Mann–Whitney Rank Sum analyses were used to compare inducibility value distributions in subgroups segregated on the basis of genotype and of the absence/presence of lung cancer; (2) Chi-square of Fisher's exact analyses were performed on contingency tables. The odds ratios (relative risk estimates) and 95% confidence intervals were simultaneously computed.

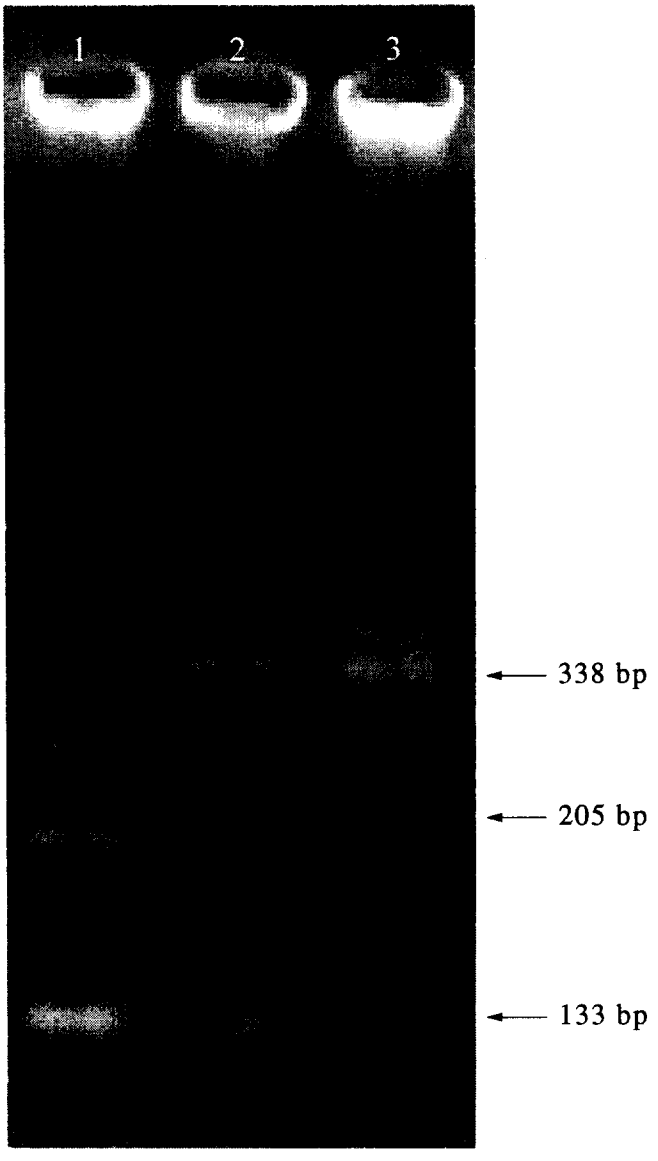

Figure 1. Determination of the CYP1A1 MspI genotype. Patterns obtained after MspI digestion of the PCR product (see Methods): (1) mutant homozygous (M2/M2): two fragments of 205 and 133 bp, respectively. (2) Heterozygous (M1/M2): three fragments of 338, 205 and 133 bp, respectively. (3) Wild-type homozygous (M1/M1): only one fragment of 338 bp.

RESULTS

The patients: age, sex, smoking habits, and distribution of lung cancer histological types

In the whole lung cancer population, the mean age was 62 years and the average tobacco consumption expressed in pack-years was 43. Among these patients, 53% were current smokers, 43% were ex-smokers and 4% were non-smokers. The average age of non-smokers was slightly higher (71 years) than that of patients having stopped smoking more than a year prior to lung cancer diagnosis (67 years), which in turn was distinctly higher than that of current smokers (59 years). The accelerating effect of smoking on lung cancer development was most obvious in the mixed-type lung cancer group, where the mean age of patients varied from 48 among smokers to 71 among ex-smokers (no cases being observed among the non-smokers).

The distribution of lung cancer histological types differed according to the sex: 70% of the women patients had adenocarcinoma, while a more homogeneous distribution of the various histological types was seen among the male patients.

Frequency of the CYP1A1 *M2 allele in healthy and lung cancer populations*

The MspI genotype was determined on blood samples from healthy individuals and lung cancer patients. In the healthy population (81 individuals), we found 64 wild-type homozygotes (M1/M1), 15 heterozygotes (M1/M2) and two mutant homozygotes (M2/M2) (Table 1). The frequency of the rare mutant allele (M2) was thus 10.71%. Among the cancer patients, 37 were wild-type homozygotes, six were heterozygotes for the M2 allele and one was an M2/M2 mutated homozygote. In this population, the frequency of the M2 allele was 7.8%, not significantly different from the control value.

When the results for the primary lung cancer patients were broken down according to the histological subclass of the tumour, the rare M2 allele appeared slightly more frequent among the adenocarcinoma patients (10.91%) and much more frequent in the mixed-type lung cancer subgroup (40%). It was absent among patients with small cell carcinoma or with secondary lung cancer. Its frequency was 4.1% in the squamous cell carcinoma subgroup. It must be said, however, that none of these values differs significantly from the reference value, given the small size of the various subgroups and the resulting limited power of our statistical analyses.

Variability of the inducibility factor in healthy individuals and lung cancer patients

The inducibility factor measured on mitogen-activated lymphocytes after BA induction varied considerably among healthy individuals and cancer patients (Figure 2). The variability range was greater in the lung cancer population (0.04–1.16) than in the healthy population (0.02–0.85), but the general median values for the healthy and lung cancer populations, 0.303 and 0.329, respectively, were not significantly different (Mann–Whitney Rank Sum Test; $P > 0.05$).

All subjects exhibiting an inducibility factor of at least 0.503 were considered to display the hyperinducibility phenotype (90th quantile of inducibility factor values recorded in healthy subjects). According to this criterion, the proportion of hyperinducible individuals was 8.64% in the healthy group and

Table 1. M1 and M2 allele distribution in lung cancer and healthy populations

MspI genotype	Healthy population	Cases of primary lung cancer Sq.C.C.	Sm.C.C.	Adeno.C.	Mixed	Undefined	All subtypes	2d L.C.	All populations
M1/M1	64 (79.0)	11 (84.6)	8 (100)	12 (80.0)	2 (50.0)	4 (100)	37 (84.1)	4 (100)	105 (81.4)
M1/M2	15 (18.5)	1 (7.7)	0 (0.0)	3 (20.0)	2 (50.0)	0 (0.0)	6 (13.6)	0 (0.0)	21 (16.3)
M2/M2	2 (2.5)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)	3 (2.3)
Total	81 (100)	13 (15)	8 (100)	15 (100)	4 (100)	4 (100)	44 (100)	4 (100)	129 (100)
M2 frequency	10.71%	4.10%	0.00%	10.91%	40.00%	0.00%	7.82%	0.00%	9.36%
OR	1.00 (ref.)	0.68	–	0.94	3.76	–	0.71	–	–
95% CI	–	[0.14–3.35]	–	[0.36–2.46]	[0.49–28.58]	–	[0.27–1.87]	–	–

Sq.C.C., squamous cell carcinoma; Sm.C.C., small cell carcinoma; Adeno.C., adenocarcinoma; 2d L.C., secondary lung cancer; O.R., odds ratio calculated after combining subjects carrying one or two MspI mutations.
x (y) with x = number of cases and y = percentage of total number of cases for the subgroup.
95% C.I., confidence interval at 95%.

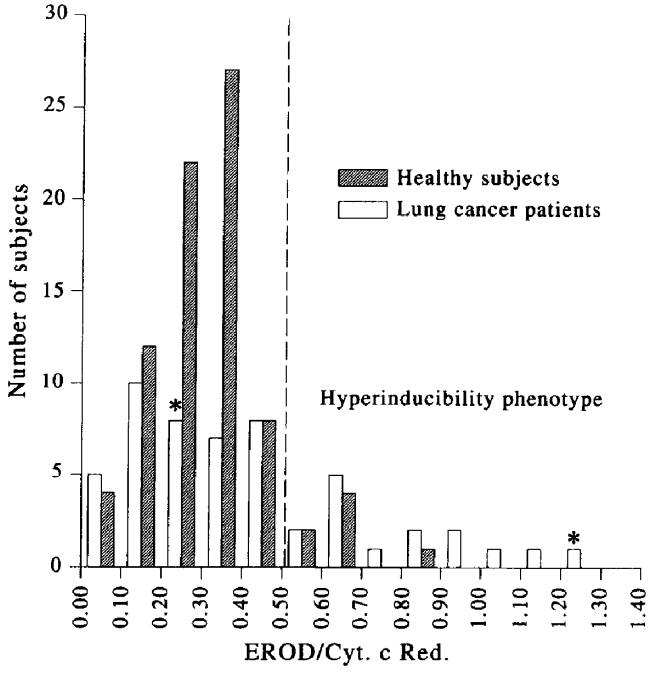

Figure 2. *CYP1A1* inducibility distribution in healthy and lung cancer populations. The inducibility factor is defined as the ratio of EROD to cytochrome c reductase activity as described under Materials and Methods. The broken line represents the minimal inducibility factor defining the hyperinducible phenotype. *, one secondary lung cancer patient.

24.39% in our lung cancer group (Table 2). Chi-square analysis showed this difference to be significant (P = 0.036; odds ratio = 3.41). When the results were broken down according to the histological type of the tumour, the respective percentages for the squamous cell, small cell and adenocarcinoma subgroups were 25, 12.5 and 33.33%, but the only group of cancer patients to display a significantly higher hyperinducibility rate than the reference population was the adenocarcinoma group (P = 0.033). Hyperinducibility thus appears as a major risk factor for adenocarcinoma (odds ratio (OR): 5.29; 95% confidence interval (CI): [1.27–22.00]).

Relationship between the MspI genotype and the inducibility phenotype

Figure 3 illustrates, for healthy subjects and lung cancer patients, the variability of the inducibility factor according to the MspI genotype. When heterozygotes and homozygotes for the M2 allele were compared separately with non-mutant homozygotes (M1/M1), statistical analyses of the data revealed no significant effect of the MspI genotype on the inducibility value distribution, in either healthy subjects or lung cancer patients. However, when the data for the M1/M2 and M2/M2 genotypes were combined, there appeared a slight upward shift of the distribution median; this effect was non-significant (P = 0.09).

When the data for each population were broken down according to the inducibility phenotype of the subjects, the mutant rates were, respectively, 28.6 and 19.2% in the healthy group (OR:1.69; CI [0.30–9.61]) and 37.5 and 16.0% in the lung cancer population (OR: 3.15; CI [0.53–18.76]), but according to the statistical analysis, the risk of presenting the hyperinducible phenotype was not significantly increased by the presence of the M2 allele (P >0.10).

Thus, the presence of one or two *CYP1A1* M2 mutations in the genotype does not seem to influence PAH inducibility in lung cancer patients.

DISCUSSION

One fact clearly emerges from our analysis of age and smoking habits in our lung cancer patients: the average age of the smokers is lower than that of the non-smokers. This holds true for all histological subtypes of cancer studied here. These results suggest that tobacco smoking accelerates lung cancer development. Our data further show a sex-dependent distribution of the histological subtypes: adenocarcinoma is by far the most frequently encountered subtype in women (70%), while the distribution is more homogeneous in men.

Our results corroborate the conclusion of Kellerman and Kouri [2, 8] that *CYP1A1* hyperinducibility is linked with a higher risk of developing lung cancer. Not only did our lung cancer population exhibit a wider range of inducibility values than the healthy reference group, but 'hyperinducible' individuals also display a 3.41-fold higher lung cancer risk (P = 0.036). This increase factor is in keeping with that recorded by Kellerman. In agreement with the results of Anttila and associates [1], our data further suggest that humans with the hyperinducibility phenotype have an increased risk of developing one particular form of lung cancer, adenocarcinoma (relative risk factor = 5.29; P = 0.033). This conclusion is strengthened by the fact that tobacco consumption was relatively low in this subgroup. These data, however, must be

Table 2. Correlation between the CYP1A1 *hyperinducibility phenotype and lung cancer susceptibility*

Parameters	Lung cancer population				Reference population
	Sq.C.C.	Sm C.C.	Adeno. C.	Total	
Hyperinducibility rate	25.00%	12.5%	33.33%	24.39%	8.64%
Odds ratio (cancer:hyperinducibility)	3.52	1.51	5.29	3.41	1.00
95% confidence limits	0.77–16.03	0.16–14.05	1.27–22.00	1.19–9.75	–
P value*	ns	ns	0.033	0.036	–

Sq.C.C., squamous cell carcinoma; Sm C.C., small cell carcinoma; Adeno. C., adenocarcinoma; ns, not significant ($P > 0.05$).
*Obtained after comparison of two hyperinducibility rates (case:reference) by the Fisher's exact test or Chi-square test.

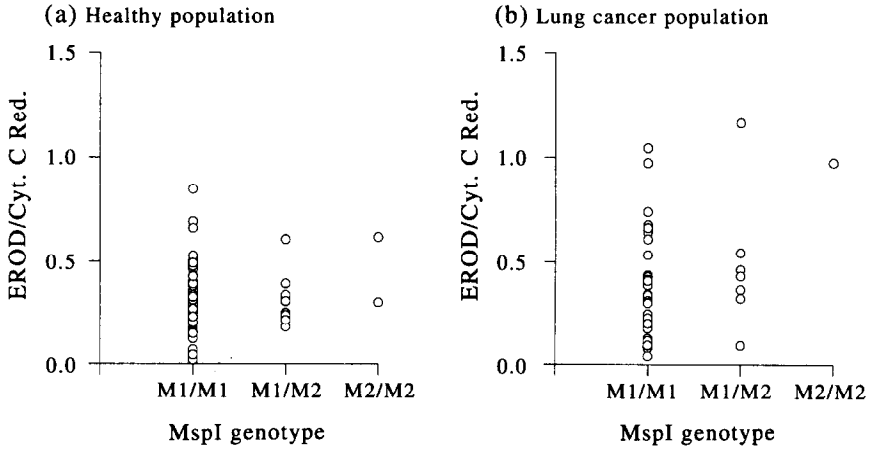

Figure 3. Variability of *CYP1A1* inducibility according to the MspI genotype in healthy (a) and lung cancer (b) populations. EROD, ethoxyresorufin-*O*-deethylase; Cyt. C. Red., cytochrome c reductase.

interpreted with caution. *CYP1A1* inducibility is not the only factor to be taken into account when examining benzo(*a*)pyrene toxicity. Anttila and colleagues [25] report a protective effect of the gene that expresses GSTM1 against bronchial lung cancer in hyperinducible patients. Moreover, we found no significant correlation between *CYP1A1* hyperinducibility and lung cancer subtypes other than adenocarcinoma.

The frequency of the M2 allele was not significantly different in the two tested populations (7.82% in the cancer patients, 10.71% in the healthy population). Breakdown according to the histological subtype revealed a higher, but not significantly higher, frequency among patients with mixed-type lung cancer (40.00%; OR = 3.76). In other studies on Caucasians [14, 15], the frequency of the M2 allele was similarly not significantly different in the total lung cancer population, but it did appear more frequent among squamous cell carcinoma patients. These divergences between results obtained after breakdown by histological subtype probably reflect the small number of cases and the low frequency of the M2 allele among Caucasians. This contrasts with the results for Asian populations [11], where the M2 allele is significantly more frequent in lung cancer patients than in healthy subjects. The corresponding lung cancer risk for M2/M2 homozygotes is 2.6-fold higher than for M2/M1 heterozygotes and 3.1-fold higher than for wild-type homozygotes. The risk of squamous cell lung cancer is 5-fold higher for M2/M2 than for M1/M1 homozygotes. This ethnic difference might be due to the higher frequency of the M2 allele in Asian populations (36%).

We found no influence of the *CYP1A1* MspI genotype on PAH inducibility value distributions in our Caucasian population when the results were broken down according to the three possible genotypes. Furthermore, the percentage of individuals possessing at least one mutated allele was not significantly higher among hyperinducible than among non-hyperinducible subjects. In the lung cancer patients, we did note a slight upward shift of the median inducibility value among patients with one or two M2 alleles compared with patients homozygous for the wild-type allele ($P = 0.09$).

Landi and associates [20] have also observed, in Caucasians, an association between the presence of the M2 allele and high CYP1A1 enzyme activity, but they did not find a higher level of *CYP1A1* mRNA. This supports the controversial hypothesis that this minor allele is genetically linked with an A-G transition in exon 7 which leads to an Ile-Val substitution [13]. The consequence of this substitution in the haem-binding catalytic region of the CYP1A1 enzyme could be to increase the affinity of the variant CYP1A1 enzyme for its substrate or to decrease its turnover rate [20]. Most of these observations discredit the view that the presence of the M2 allele at the MspI site of the *CYP1A1* gene significantly increases the risk of lung cancer in Caucasians. Even if the rare M2 allele does slightly affect the inducibility phenotype, its rôle is probably minor, *CYP1A1* expression being regulated by complex transregulatory machinery. Further studies concerning the expression of ARNT and the Ah receptor will certainly contribute to our knowledge of the regulatory mechanism.

1. Anttila S, Hietanen E, Vainio H, *et al.* Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P450IA in lung cancer patients. *Int J Cancer* 1991, **47**, 681–685.
2. Kouri RE, McKinney CE, Slomiany DJ, Snodgrass DR, Wray NP, McLemore TL. Positive correlation between high aryl hydrocarbon hydroxylase activity and primary lung cancer as analyzed in cryopreserved lymphocytes. *Cancer Res* 1982, **42**, 5030–5037.
3. McLemore TL, Adelberg S, Liu MC, *et al.* Expression of *CYP1A1* gene in patients with lung cancer: evidence for cigarette smoke-induced gene expression in normal lung tissue and for altered gene regulation in primary pulmonary carcinomas. *J Natl Canc Inst* 1990, **82**, 1333–1339.
4. Dahl AR, Lewis JL. Respiratory tract uptake of inhalants and metabolism of xenobiotics. [Review]. *Ann Rev Pharm Toxicol* 1993, **33**, 383–407.
5. Phillips DH. Fifty years of benzo(a)pyrene. *Nature* 1983, **303**, 468–472.
6. Bartsch H, Castegnaro M, Rojas M, Camus AM, Alexandrov K, Lang M. Expression of pulmonary cytochrome P4501A1 and carcinogen DNA adduct formation in high risk subjects for tobacco-related lung cancer. *Toxicol Lett* 1992, 64–65 Spec, No: 477–483.
7. Nebert DW, Petersen DD, Puga A. Human AH locus polymorphism and cancer: inducibility of *CYP1A1* and other genes by combustion products and dioxin. *Pharmacogenetics* 1991, **1**, 68–78.
8. Kellermann G, Shaw CR, Luyten-Kellerman M. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N Engl J Med* 1973, **289**, 934–937.
9. Crofts F, Cosma GN, Currie D, Taioli E, Toniolo P, Garte SJ. A novel *CYP1A1* gene polymorphism in African-Americans [published erratum appears in *Carcinogenesis* 1993, **14**(12), 2652]. *Carcinogenesis* 1993, **14**, 1729–1731.
10. Kawajiri K, Nakachi K, Imai K, Hayashi S, Watanabe J. Individual differences in lung cancer susceptibility in relation to polymorphisms of P-450IA1 gene and cigarette dose. *Int Sympos of the Princess Takamatsu Cancer Research Fund* 1990, **21**, 55–61.
11. 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 Letters* 1990, **263**, 131–133.
12. Wedlund PJ, Kimura S, Gonzalez FJ, Nebert DW. 1462V mutation in the human CYP1A1 gene: lack of correlation with either the Msp I 1.9 kb (M2) allele or CYP1A1 inducibility in a three-generation family of east Mediterranean descent. *Pharmacogenetics* 1994, **4**, 21–26.
13. Hayashi S, Watanabe J, Nakachi K, Kawajiri K. Genetic linkage of lung cancer-associated MspI polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. *J Biochem* 1991, **110**, 407–411.
14. 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**, 1785–1790.
15. Drakoulis N, Cascorbi I, Brockmoller J, Gross CR, Roots I. Polymorphisms in the human CYP1A1 gene as susceptibility factors for lung cancer: exon-7 mutation (4889 A to G), and a T to C mutation in the 3'-flanking region. *Clin Invest* 1994, **72**, 240–248.
16. Hayashi S, Watanabe J, Nakachi K, Eguchi H, Gotoh O, Kawajiri K. Interindividual difference in expression of human Ah receptor and related P450 genes. *Carcinogenesis* 1994, **15**, 801–806.
17. Kelsey KT, Wiencke JK, Spitz MR. A race-specific genetic polymorphism in the *CYP1A1* gene is not associated with lung cancer in African Americans. *Carcinogenesis* 1994, **15**, 1121–1124.
18. Sugimura H, Suzuki I, Hamada GS, *et al.* Cytochrome P-450 Ia1 genotype in lung cancer patients and controls in Rio de Janeiro, Brazil. *Cancer Epidemiol Biomarkers Prevention* 1994, **3**, 145–148.
19. Hirvonen A, Husgafvel-Pursiainen K, Karjalainen A, Anttila S, Vainio H. Point-mutational MspI and Ile-Val polymorphisms closely linked in the *CYP1A1* gene: lack of association with susceptibility to lung cancer in a Finnish study population. *Cancer Epidemiol Biomarkers Prevention* 1992, **1**, 485–489.
20. Landi PJ, Bertazzi PA, Shields PG, *et al.* Association between *CYP1A1* genotype, mRNA expression and enzymatic activity in humans. *Pharmacogenetics* 1994, **4**, 242–246.
21. Kouri RE, Oberdorf J, Slomiany DJ, McKinney CE. A method for detecting aryl hydrocarbon hydroxylase activities in cryopreserved human lymphocytes. *Cancer Lett* 1981, **14**, 29–40.
22. Burke MD, Mayer RT. Ethoxyresorufin: direct fluorimetric assay of a microsomal o-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Met Disp* 1974, **2**, 583–588.
23. Peterson JA, Ebel RE, O'Keefe DH. Dual-wavelength stopped-flow spectrophotometric measurement of NADPH-cytochrome P-450 reductase. *Method Enzymol* 1978, **52**, 221–226.
24. Maniatis T, Fritsch EF, Sambrook J. *Molecular Cloning–A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1982.
25. Anttila S, Hirvonen A, Husgafvel-Pursiainen K, *et al.* Combined effect of CYP1A1 inducibility and GSTM1 polymorphism on histological type of lung cancer. *Carcinogenesis* 1994, **15**, 1133–1135.

Acknowledgements—This study was supported by a grant from the Belgian Prime Minister's Federal Office for Scientific, Technical and Cultural Affairs and BioMed P1 931622, program of the European Community.