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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

N. Drakoulis, I. Cascorbi, J. Brockmöller, C.R. Gross, I. Roots

Institut für Klinische Pharmakologie, Medizinische Fakultät (Charité), Humboldt-Universität zu Berlin, D-10098 Berlin, Germany

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Abstract. Genetic differences in the metabolism of carcinogens may codetermine individual predisposition to cancer. Cytochrome P-4501A1 (CYP1A1) metabolically activates precarcinogens in cigarette smoke, such as benzo(a)pyrene, which is also an inducer of CYP1A1. Two point mutations have been reported, m1 in the 3'-flanking region (6235T to C), and m2 within exon 7 (4889A to G), the latter leading to an isoleucine to valine exchange. In the Japanese population m1 and m2 are correlated with lung cancer, suggesting an increased susceptibility to cigarette smoking related lung cancer. We studied 142 lung cancer and 171 reference patients in an ethnically homogeneous German group for m1 and m2 mutations by restriction fragment length polymorphism and allele-specific polymerase chain reaction, respectively. No statistically significant difference was found in the distribution of m1 alleles between lung cancer and controls; the frequency was 8.5% and 7.3% of the alleles, respectively (odds ratio = 1.17). A trend to an overrepresentation of m1 alleles was observed among 52 squamous cell carcinoma patients (odds ratio = 1.65). In contrast, the frequency of m2 alleles in lung cancer patients was twofold higher (6.7%) than in the reference group (3.2%; odds ratio = 2.16; 95% confidence limits 0.96–5.11, $P = 0.033$); the odds ratio of m2 alleles in squamous cell carcinoma was 2.51 (95% confidence limits 0.85–7.05, $P = 0.05$). There was a close genetic linkage of m2 to m1 (10 of 11 reference patients), but a significantly higher number of cancer patients showed no linkage compared to the controls (odds ratio = 8.89, 95% confidence limits 0.83–433, $P = 0.04$). Thus no association was found between presence of m1 alleles and lung cancer, but, in contrast, m2 alleles proved as a hereditary risk factor, especially if not linked with m1 alleles.

Key words: Cytochrome P-450 – CYP1A1 – Polymorphism – Lung cancer – Polymerase chain reaction – Cancer epidemiology – Risk factor

The human cytochrome P-450 genes (*CYP*) regulate the most important enzymes in the oxidative metabolism, not only of drugs and other foreign compounds including many chemical carcinogens from the environment but also of endogenous substances such as steroids, fatty acids, and prostaglandins. The various isoenzymes of CYP differ in their substrate specificity, and most of them are subjected to distinct genetic regulation. Within a population there exist genetic variations of cytochrome P-450 enzymes leading to interindividually different metabolic capacity. Since cytochromes P-450 may either detoxify carcinogens or transform precarcinogens to ultimate carcinogens, the genetic variability of *CYP* may partly explain the interindividual difference in susceptibility to carcinogens. The main hypothesis explaining such a connection between hereditary deficiency of a certain enzyme and susceptibility to certain cancers is based on the assumption that carriers of a deficiency are not capable of toxifying certain carcinogens. On the other hand, the carriers of this special deficiency may not be able to detoxify other carcinogens and may in this way be more susceptible to cancer. Research has thus far centered particularly on the cytochrome P-450 2D6 (*CYP2D6*) deficiency found in about 10% of the Caucasian population [21]. In addition, the polymorphisms of ary-

Abbreviations: Ah = aryl hydrocarbon; CYP1A1 = cytochrome P4501A1; *CYP1A1* = CYP1A1 gene; PCR = polymerase chain reaction; PY = pack years; RFLP = restriction fragment length polymorphism

Correspondence to: N. Drakoulis

lamine *N*-acetyltransferase [3, 21] and glutathione *S*-transferase class μ [2] have been investigated. Molecular genetic methods are now available to study these polymorphisms in patients – in terms of molecular epidemiology – and it has been shown that carriers of the CYP2D6 deficiency (poor metabolizers) are significantly underrepresented among lung cancer patients [20]. This indicates that the poor metabolizer trait provides some protection from lung cancer.

To detect further host factors of lung cancer susceptibility we focused our interest on the cytochrome P-450 1A family that comprises of two functional genes. *CYP1A1*, highly expressed in pulmonary tissue [15], is responsible for the metabolic activation of several environmental chemicals such as benzo(a)pyrene and numerous other polycyclic aromatic hydrocarbons occurring e.g. in cigarette smoke, motor exhaust, and roasted meat [6, 27]. CYP1A2, for example, *N*-demethylates caffeine and is also involved in arylamine metabolism. Both CYP1A isoenzymes are induced (up-regulated) by certain xenobiotics such as benzo(a)pyrene, 3-methylcholanthrene, β-naphthoflavone, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [5, 6]; moreover CYP1A activities are also enhanced by certain drugs such as omeprazole [22]. The induction process is complex and relates to interaction of the cytosolic aryl hydrocarbon (Ah) receptor, liganded by the inducer molecule, with regulatory elements in the 5' region of the *CYP1A1* gene [4]. A polymorphism of inducibility has been reported previously. Approximately 10% of the Caucasian population exhibits high CYP1A1 inducibility [17, 18], leading to an increased activation of polycyclic hydrocarbon precarcinogens to reactive ultimate carcinogens; this trait may thus result in greater risk of cigarette smoke induced bronchogenic carcinoma [14].

The human *CYP1A1* gene is localized on the long arm of chromosome 15 (15q22-q24) and comprises seven exons and six introns with a total length of 5810 bp [10, 11]. Two point mutations have recently been demonstrated [7, 12]. The mutation discovered first, m1, is situated at position 6235 in the 3' flanking region (i.e., 1194 bp downstream from exon 7) and represents a thymine to cytosine (T to C) transition, providing a new restriction endonuclease cleavage site for *Msp*I [1, 16, 26]. The second mutation, m2, is a replacement of adenine by guanine (A to G) and is localized in the coding region at position 4889 within exon 7 [7]. This mutation results an amino acid substitution of isoleucine to valine (Ile to Val) at residue 462 in the heme binding region of *CYP1A1* (Fig. 1).

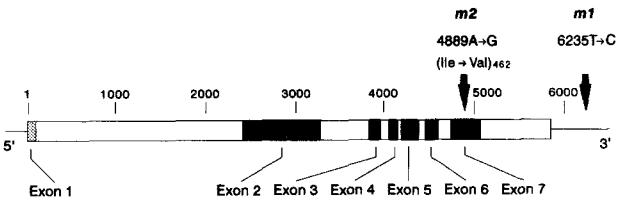

Fig. 1. Gene map of human cytochrome P4501A1 (*CYP1A1*). There are seven exons and six introns. The first exon is not translated. Mutations are described in the 3' flanking region (m1), creating an *Msp*I cleavage site and inside exon 7 (m2). Xenobiotic responsive elements are located upstream of exon 1. Data were communicated from European Molecular Biology Laboratory Gene Data Bank (accession numbers X04300, D12525, D01198, provided by DKFZ, Heidelberg, Germany)

Both mutations of *CYP1A1* have been frequently observed in the Japanese population at large [7, 16]. Interestingly, their prevalence is significantly higher among lung cancer patients in Japan [13], indicating that the presence of these traits represents a risk factor for lung cancer. Mutations m1 and m2 generally occur jointly so that their individual impacts cannot easily be discerned. In the European (Caucasian) population, the allelic frequency of m1 is low (around 11%), and an association with lung cancer has not been detected [8, 28].

Our aim was to establish a fast and efficient method for detecting both these mutations of the *CYP1A1* gene and to determine their frequency in an ethnically uniform German study group. We investigated whether the mutation m1 is linked to m2 and examined the frequency of both of these mutations in bronchial carcinoma patients to clarify whether these *CYP1A1* polymorphisms can be established as a susceptibility factor for bronchial carcinoma in the Caucasian population.

Materials and methods

Patients and controls

The study included 142 patients with histologically verified bronchogenic carcinoma and as a control group 171 healthy individuals or hospital patients with various other pulmonary diseases but no known malignancy. Both lung cancer and control patients were selected within the same time period between 1991 and 1992 from the Department of Pulmonology of the Zehlendorf Hospital and from the Klinikum Steglitz in Berlin, Germany. All patients gave their informed consent, and the study was approved by the Ethics Committee of the Klinikum Steglitz, Free University of Berlin. Demographic data of the patients are given in Table 1. The histological subdivision of bronchogenic car-

Table 1. Demographic data of the lung cancer and control patients included in the study

	Lung cancer ($n=142$)	Reference ($n=171$)
Male/female	115/27	99/72
Median age (range) (years)	65 (38–87)	59 (18–86)
Males	65 (38–87)	60 (18–84)
Females	65 (41–87)	57 (23–86)
Nonsmokers	10	34
Moderate smokers (1–10 PY)	9	16
Smokers (>10 PY)	123	121

Table 2. Histological classification of the lung cancer group

	n	Males	Females
Squamous cell carcinoma	52	49	3
Large cell carcinoma	23	17	6
Small cell carcinoma	26	22	4
Adenocarcinoma	34	20	14
Mixed and others	7	7	0
Total	142	115	27

cinomas follows the WHO classification and is listed in Table 2. Patients of mixed histology and two patients with bronchiolo alveolary carcinoma were included in the "mixed and others" group. Total cigarette consumption is expressed in pack years (PY; 1 PY = daily consumption of 20 cigarettes for 1 year). Nonsmokers were defined as persons who never smoked, moderate smokers had 0–10 PY, and smokers over 10 PY. AB0 blood groups and the rhesus factor (Rh, + or –) were recorded.

DNA isolation and PCR analyses

Venous blood samples (5–10 ml), drawn in EDTA as anticoagulant, were obtained from patients and controls, and DNA was isolated from leukocytes by standard phenol/chloroform extraction after digestion with proteinase K (1 mg/ml, Boehringer, Mannheim, Germany) overnight at 37°C and subsequent ethanol precipitation [23]. After extraction DNA was dissolved in 10 mM Tris buffer (pH 8.0) and stored at 4°C until further analysis.

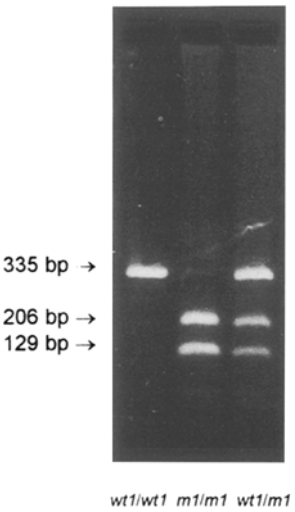

Fig. 2. Agarose gel electrophoresis of *CYP1A1* DNA fragments stained with ethidium bromide. *Msp*I restriction fragment length polymorphism of the 3' flanking region of *CYP1A1*, preamplified with primer pairs P79/P80: wt1, wild type; m1, mutation at 6235 nt

The identification of *CYP1A1* genotypes ascribed to the mutation at position 6235 in the 3' flanking region (reported first in [12], therefore termed m1, wt1, respectively) was carried out by restriction fragment length polymorphism analysis (RFLP) after amplification of a 335-bp fragment by polymerase chain reaction (PCR; Fig. 2). Target DNA (about 1 µg) was amplified in a 50-µl reaction mixture containing 10 µM of each primer P79 and P80 (Table 3), 1 U *Taq* DNA polymerase (Gibco, Gaithersburg, MD, USA), 0.2 mM KCl, 10 mM Tris-HCl (pH 8.8), 0.01% gelatin, and 2.4 mM $MgCl_2$. Finally, 50 µl mineral oil (Sigma, St. Louis, MO, USA) was added to prevent evaporation and

Table 3. List of oligonucleotide primers used for DNA amplification to detect polymorphism sites of *CYP1A1* (3' = downstream-primer, 5' = upstream-primer)

Primer	Position (nt)	Fragment length (bp)	Nucleotide sequence	Specificity
P79 (5')	6106-6126	335	5'-AAGAGGTGTAGCCGCTGCACT-3'	m1 fragment
P80 (3')	6440–6420	–	5'-TAGGAGTCTTGTCTCATGCCT-3'	
P71 (5')	4394–4417	1472	5'-ATTAGGGTTAGTGGGAGGGACACG-3'	m2 fragment
P57 (5')	4870–4889	996	5'-GAAGTGTATCGGTGAGACCA-3'	4889 A (wt2)
P58 (5')	4870–4889	996	5'-GAAGTGTATCGGTGAGACCG-3'	4889 G (m2)
P72 (3')	5865–5841	–	5'-GCTCAATGCAGGCTAGAATAGAAGG-3'	

32 cycles of amplification were carried out in a programmable thermocycler water bath (Autogene II, Grant, Cambridge, UK; DNA denaturation 92°C, 30 s; primer annealing 63°C, 1 min; polymerization 72°C, 1 min). The amplified fragments were incubated with 40 U restriction endonuclease *Msp*I (Boehringer Mannheim) overnight at 37°C. Evaluation was performed by gel electrophoresis (1.8% w/v agarose; Gibco). The wild type, wt1, was defined as the absence, where the m1 allele provided the *Msp*I-specific GGCC motif.

The m2 mutation within exon 7 [7] resulting the amino acid substitution of isoleucine to valine at amino acid position 462 in the heme binding region was determined by the use of allele-specific PCR. Since this mutation was the second discovered in the *CYP1A1* gene, it was termed m2, and the wild type for this allele is termed wt2. Genomic DNA was first amplified with the upstream primer P71 and the downstream primer P72 (Table 3) to produce a 1472-bp fragment including the possible adenine to guanine transition at position 4889 (Fig. 1). The PCR profile was identical to the scheme mentioned above. The amplified fragments were then reamplified for 12 cycles under the same conditions alternatively either with the 5' wt2 wild type specific primer P57 or the 5' m2 mutation-specific primer P58, and the common 3' primer P72 (Table 3).

Statistical evaluation

The data were recorded in a database system developed by our Department. The frequencies of alleles or genes in different groups were compared by calculating odds ratios of observed versus expected frequencies and their 95% confidence limits. When the frequency (f) of the wild-type configuration (wt/wt) is given, the frequency of the mutated allele configuration (m/m) can be calculated by the following equation according the Hardy-Weinberg equilibrium: $f_{m/m} = (1 - \sqrt{f_{wt/wt}})^2$.

Fisher's test was used to evaluate statistical significance, and the statistical level of significance was fixed at $P = 0.05$; type II error and sample size estimations were calculated according to Schlesselman [24].

Results

Detection and prevalence of m1

Mutation m1 was characterized by the presence or absence of the *Msp*I site located 1194 bp downstream from exon 7 of the *CYP1A1* gene by PCR RFLP. Absence of the *Msp*I site in both alleles represents the homozygous wild-type genotype (wt1/wt1) and is characterized by a single 335-bp fragment. Persons with the m1 mutation in homozygous state (m1/m1) showed 206- and 129-bp fragments. Heterozygous persons with a wild-type and a m1-mutated allele (wt1/m1) showed an uncleaved 335-bp fragment and in addition 205- and 134-bp fragments (Fig. 2).

Table 4 demonstrates the distribution of these *CYP1A1* allele conformations in 142 lung cancer and 171 control patients. No differences between expected and observed frequencies were determined. The allele frequencies for wt1 and m1 were 0.927 and 0.073, respectively, in the control subjects, and 0.915 and 0.085 in the lung cancer patients (Table 5). The allelic distribution of wt1 and m1 among lung cancer patients and controls showed no statistically significant difference (odds

Table 4. Frequencies of polymorphic *CYP1A1* allelic conformations in 142 lung cancer and 171 control patients: wt1 = wild type (6235 T), m1 = 6235 C; wt2 = wild type (4889 A), m2 = 4889 G

	wt1/wt1		wt1/m1		m1/m1		Total	
	n	%	*n*	%	*n*	%	*n*	%
Lung cancer patients								
wt2/wt2	111	78.2	14	9.9	0	0.0	125	88.0
wt2/m2	8*	5.6	6	4.2	1	0.7	15	10.6
m2/m2	0	0.0	2	1.4	0	0.0	2	1.4
Total	119	83.8	22	15.5	1	0.7	142	100.0
Reference patients								
wt2/wt2	145	84.8	15	8.8	0	0.0	160	93.6
wt2/m2	1	0.6	10	5.8	0	0.0	11	6.4
m2/m2	0	0.0	0	0.0	0	0.0	0	0.0
Total	146	85.4	25	14.6	0	0.0	171	100.0

* Compared to all remaining genotypes; odds ratio = 10.15 (95% confidence limits = 1.33–453; $P = 0.009$)

Table 5. Allele frequency of the m1 mutation at position 6235 (*Msp*I site) in the 3′ flanking region of *CYP1A1* in histologically classified lung cancer patients in relation to the reference group

		Alleles				Odds ratio m1:wt1	95% confidence limits	*P*
		m1		wt1				
	n	*n*	%	*n*	%			
Lung cancer	142	24	8.5	260	91.5	1.17	0.62–2.19	NS
Squamous cell carcinoma	52	12	11.5	92	88.5	1.65	0.73–3.57	NS
Large cell carcinoma	23	4	8.7	42	91.3	1.21	0.29–3.75	NS
Small cell carcinoma	26	3	5.8	49	94.2	0.78	0.14–2.69	NS
Adenocarcinoma	34	4	5.9	64	94.1	0.79	0.19–2.41	NS
Mixed and others	7	1	7.1	13	92.9	0.98	0.02–7.02	NS
Reference	171	25	7.3	317	92.7	1.00	–	

ratio = 1.17; 95% confidence limits = 0.62–2.19). The division into histologically defined subclasses of bronchogenic carcinoma showed a particular, albeit statistically nonsignificant increase in the m1 allele in squamous cell cancer patients (odds ratio = 1.65). No statistically significant difference in the distribution of m1 allele carriers in male or female bronchogenic carcinoma and control patients was observed. Also the division into subjects with various categories of smoking led to no significant results.

Detection and prevalence of m2

The A to G transition in exon 7 (m2) was detected by the presence or absence of a 996-bp fragment after allele specific PCR. Homozygous wt2/wt2 persons yielded a 996-bp fragment with the wild type specific primer pair P57/P72. Homozygous m2/m2 carriers yielded a 996-bp fragment with the mutation-specific primer pair P58/P72. The formation of a 996-bp fragment in both reactions, i.e., with the wild type and the mutation-specific primer pairs, attested to heterozygosity (wt2/m2) (Fig. 3).

The observed allele frequencies for wt2 and m2 were 0.968 and 0.032, respectively, for the controls, and 0.933 and 0.067 for the lung cancer cases (Table 6). The calculated odds ratio was 2.16 (95% confidence limits = 0.96–5.11, $P = 0.032$), indicating a significant overrepresentation of m2 among lung cancer patients. When not the m2 alleles but persons carrying at least one m2 allele are compared (17 lung cancer and 11 reference patients; Table 4), an odds ratio of 1.98 (95% confidence limits = 0.84–4.84, $P = 0.066$) was obtained. The subgroup of squamous cell cancer showed a significant overrepresentation of the m2 allele (odds ratio = 2.51, $P = 0.05$). Male and female patients showed no statistically significant difference in the m2 distribution. Lung cancer patients were also stratified according to smoking history and compared to the whole reference group (Table 6). It appears that the above overrepresentation of m2 is based mainly on the nonsmoker group (odds ratio = 7.52, $P = 0.006$).

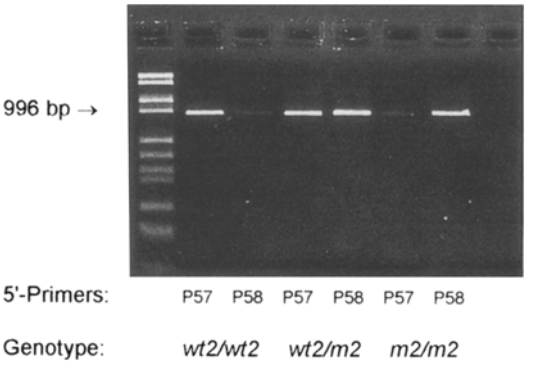

Fig. 3. Agarose gel electrophoresis of allele-specific polymerase chain reaction for detection of the 4889 A to G transition in exon 7 (m2). 5' Primers P57 (wt2-specific) and P58 (m2 specific) are used together with 3' primer P72. Oligonucleotide sequences are given in Table 3. *Left*, oligonucleotide standard

A list of all 17 lung cancer patients carrying the m2 mutation is given in Table 7. It is remarkable that blood group A is rare (frequency = 0.12), and that blood group B tends to be overrepresented (frequency = 0.41) in this particular subgroup (frequency of A in the German population = 0.42, B = 0.137 [19]). However, the number of cases is too small for statistical evaluation, and AB0 blood group frequencies were not target parameters of our study.

Common evaluation of m1 and m2

To test to the degree to which m2 (the rarer trait) is linked to m1, Table 4 provides a frequency pattern

Table 6. Allele frequency of the m2 mutation at position 4889 in exon 7 of *CYP1A1* in lung cancer patients in relation to the reference group, listed by histological classification and smoking status

		Alleles				Odds ratio m2:wt2	95% confidence limits	*P*
		m2		wt2				
	n	*n*	%	*n*	%			
Lung cancer	142	19	6.7	265	93.3	2.16	0.96–5.11	0.033
Squamous cell carcinoma	52	8	7.7	96	92.3	2.51	0.85–7.05	0.050
Large cell carcinoma	23	3	6.5	43	93.5	2.10	0.36–8.36	NS
Small cell carcinoma	26	3	5.8	49	94.2	1.84	0.32–7.29	NS
Adenocarcinoma	34	5	7.4	63	92.6	2.39	0.63–7.75	NS
Mixed and others	7	0	0.0	14	100.0	0.00	0.00–10.5	NS
Nonsmokers	10	4	20.0	16	80.0	7.52	1.56–28.9	0.006
Moderate smokers	9	1	5.9	17	94.1	1.77	0.04–13.5	NS
Smokers >10 PY	123	14	5.7	232	94.3	1.82	0.75–4.50	NS
Reference	171	11	3.2	331	96.8	1.00	–	

Table 7. Characteristics of the 17 out of 142 lung cancer patients carrying the m2 mutation in *CYP1A1*

No.	Age (years)	Sex	AB0-blood-group	Rh	Smoking (PY)	Occupation	Histology	Allele configuration	
								6235 nt	4889 nt
m2 mutation in absence of m1 ($n=8$)									
076	78	M	B	+	30	Bricklayer	Squamous cell carcinoma	wt1/wt1	wt2/m2
077	59	M	0	+	48	Businessman	Squamous cell carcinoma	wt1/wt1	wt2/m2
078	78	M	0	+	25	Businessman	Squamous cell carcinoma	wt1/wt1	wt2/m2
216	53	M	A	ND	24	Mechanic	Large cell carcinoma	wt1/wt1	wt2/m2
032	74	M	B	+	10	Engineer	Adenocarcinoma	wt1/wt1	wt2/m2
120	70	F	0	–	0	Turner	Adenocarcinoma	wt1/wt1	wt2/m2
238	87	F	B	+	0	Doctor's assistant	Adenocarcinoma	wt1/wt1	wt2/m2
257	63	F	0	+	50	Worker	Adenocarcinoma	wt1/wt1	wt2/m2
m2 mutation in presence of m1 ($n=9$)									
003	70	M	A	+	15	Shop assistant	Squamous cell carcinoma	wt1/m1	wt2/m2
020	69	M	0	–	25	Engineer	Squamous cell carcinoma	wt1/m1	wt2/m2
022	71	M	B	+	25	Policeman	Squamous cell carcinoma	wt1/m1	wt2/m2
208	58	M	0	–	63	Worker	Squamous cell carcinoma	wt1/m1	wt2/m2
002	74	M	B	+	50	Crane driver	Squamous cell carcinoma	m1/m1	wt2/m2
008	80	M	0	+	0	Laboratory assistant	Large cell carcinoma	wt1/m1	m2/m2
201	73	F	0	+	108	Driver	Large cell carcinoma	wt1/m2	wt2/m2
269	62	F	B	ND	84	Secretary	Small cell carcinoma	wt1/m1	m2/m2
035	73	M	B	+	55	Businessman	Adenocarcinoma	wt1/m1	wt2/m2

ND, Not determined

of all allelic conformations with m1 and wt1 compared to m2 and wt2. Among the reference patients 11 had an m2 allele (all were heterozygotes); 10 of these were linked to wt1/m1, and only one individual with wt2/m2 had an wt1/wt1 constellation. Thus, a close (about 90%) though not strict linkage exists of m2 to m1.

In contrast, among lung cancer patients there were 15 carriers of wt2/m2; 8 of these had the genotype wt1/wt1, and only 6 were linked to the wt1/m1 genotype and 1 to m1/m1. The odds ratio of the genetic constellation (wt2/m2, wt1/wt1) in lung cancer and reference patients compared with all remaining genotypes was 10.15 (95% confidence limits 1.33–453, $P = 0.009$). Thus, in lung cancer a strikingly high number of individuals with the m2 allele were found in whom no m1 allele existed. Even when the two lung cancer patients with m2/m2 and the single individual with m1/m1 were included in the evaluation, there was an odds ratio of 8.89 (95% confidence limits = 0.83–433, $P = 0.04$), calculated on the base of the eight cases not linked to m1 versus nine cases linked, compared to one reference patient not linked versus ten

reference patients linked. The eight and nine cases of lung cancer with absence or presence of m1, respectively, are casuistically listed in Table 7.

Estimation of gene frequencies for m1/m1 and m2/m2

The group of reference patients should fulfill the preconditions for the application of the Hardy-Weinberg law. Frequency $f_{m1/m1}$ was calculated as 0.58% and $f_{m2/m2}$ as 0.11%; thus it is conceivable that no such genotypes were found among the 171 reference patients. The frequency of individuals with both mutations being homozygous (m1/m1, m2/m2) should be lower than 0.11% as the linkage of m2 to m1 is not obligatory. Among the 142 lung cancer patients one individual was homozygous m1/m1 (0.70%; Table 4). This frequency had 95% confidence limits of 0.02–3.91%. Two lung cancer patients presented with homozygous m2/m2 (1.41%, 95% confidence limits = 0.17–5.00%), a frequency 13-fold higher than estimated for the reference group.

Discussion

Lack of association of m1 to lung cancer in Caucasians

The allele frequency of m1 in our German reference group (7.3%) was in the range of findings in a Norwegian [28], Finnish [8], and a North American [25] population, the latter consisting of Caucasian and black individuals but differed clearly from an about fourfold higher frequency of the m1 allele in the Japanese (33.2%) [16]. Previous studies reported have divergent results on the correlation of mutation m1 and the occurrence of lung cancer. In the Japanese population a significantly higher frequency of m1 alleles was observed in lung cancer patients than in controls (odds ratio = 1.36) [16], while no significant association between m1 alleles and lung cancer was established in our study among the Caucasian population (odds ratio = 1.17).

In addition, the Japanese study [16] showed a distinct association of the m1 frequency to squamous cell cancer (odds ratio 1.75, $P = 0.004$); in the German population there was a similar trend which, however, lacked statistical significance (Table 5). Similar to our study, no association of lung cancer and frequency of m1 alleles was detected in the Norwegian [28] or Finnish [8] study. Due to the rarity of the m1 trait among Caucasians, a statistical confirmation of the lack of an association is difficult to obtain. High numbers would be required to keep the type II error at a low level of $\beta = 0.10$. Moreover, one must consider a fundamental difference: among Japanese patients with lung cancer there were 21.2% homozygous carriers of m1/m1, whereas in our cancer group this frequency was only 0.70% ($n = 1$). Thus, the conclusions derived from European studies rely predominantly on the evaluation of heterozygous carriers of wt1/m1. Carriers of m1/m1 and wt1/m1 may have a different cancer risk.

Mutation m2 as risk factor for lung cancer

The point mutation m2 at position 4889 in exon 7 of *CYP1A1*, first described by Hayashi et al. [7], was found to be significantly more frequent in lung cancer patients than in controls (odds ratio = 2.16; $P = 0.033$; Table 6). The allelic distribution in this group was not in the Hardy-Weinberg equilibrium, indicating a special risk for certain allelic combinations. Especially patients with squamous cell carcinoma displayed this mutation much more frequently (odds ratio = 2.51; $P = 0.05$) than expected from the reference group. Similar observations to ours have been reported by Kawajiri et al. [13] for a Japanese population, and to our knowledge no data are available in other ethnic groups. Comparing the genotype distributions between lung cancer patients and a healthy population, they found that individuals with homozygous m2 showed about threefold higher risk to lung cancer than those of another genotype. Due to the low frequency in the Caucasian population only two individuals with homozygous m2 were detected among lung cancer patients and none in the reference group. The frequency of m2/m2 in the reference patients was therefore estimated by the Hardy-Weinberg equation and amounted to $f_{m2/m2} = 0.0011$. According to this, homozygous m2 carriers may possess a 13-fold risk for lung cancer. When heterozygous individuals are included in the risk appraisal, the odds ratio of 1.98 still indicates increased risk, although with marginal statistical significance. Altogether, these data suggest that mutation m2 in exon 7 is indeed a susceptibility factor for lung cancer also in the Caucasians. As already stated for m1, this conclusion is based mainly on heterozygous individuals, whereas in Japan homozygous carriers of m2 are frequent [7, 13].

Genetic linkage between m1 and m2

The wt2/m2 allele conformation was coincident in 10 of 11 individuals with the wt1/m1 allele confor-

mation in the controls. Such a close, although not obligatory, linkage was also observed in Japan [7]. It is assumed that the linkage is predominantly on the same DNA strand, but this has not been confirmed so far for each patient. Interestingly, in lung cancer patients singular A to G transitions (m2) were found in 8 of 17 cases without linkage to the T to C transition (m1). This overrepresentation of single m2 without linkage to m1 may be a special risk constellation. The genetic combination (wt2/m2, wt1/wt1) was highly overrepresented in lung cancer (odds ratio = 10, 15, P = 0.009).

Mechanistic considerations

The question arises as to how *CYP1A1* is regulated, and how an increased m2 frequency compared to the reference group is associated with lung cancer. The impact of *CYP1A1* polymorphism for development of bronchogenic cancer is often explained as enhanced inducibility, leading to higher enzyme activities to activate precarcinogens [5, 14]. Possibly, the m1 mutation in the noncoding 3' flanking region is a marker for alterations on regulatory regions of *CYP1A1* at the 5' side. Indeed, Peterson et al. [18] demonstrated a relatively higher degree of inducibility in cultured lymphocytes from persons with wt1/m1 compared to wt1/wt1. On the other hand, the isoleucine to valine replacement (m2) in exon 7 seems to increase enzymatic activity, as shown for the ability to metabolically activate benzo(a)pyrene to mutagenic products [9, 13]. Higher arylamine hydrocarbon hydroxylase activity was obtained with the m2 form of CYP1A1 which was cloned into yeast cells [13]. Therefore, m1 might define a marker for alterations on regulatory sites, whereas m2 represents a more active CYP1A1 form. In both cases the activation of precarcinogens to ultimate carcinogens may be enhanced. As no suitable substrate is available for phenotyping CYP1A1 in humans, the significance of findings in yeast cell systems should be confirmed in systems more close to man, for example, primary human cell cultures.

This study demonstrates a significant overrepresentation of m2 in lung cancer patients but not of m1. Since nonsmokers with bronchogenic carcinomas carry the m2 allele much more frequently (odds ratio = 7.52; Table 6), it may be speculated that homozygous m2/m2 carriers have a notably increased risk at low exposure to precarcinogenic compounds such as contained in cigarette smoke and may be in the very special constellation of passive smoking. Similarly, Kawajiri et al. [13] showed for homozygous carriers of the m1 allele an increased susceptibility to lung cancer for moderate or nonsmokers (total consumption of more than 3×10^5 cigarettes). However, proposed linkages to polymorphisms of the Ah locus encoding the Ah receptor, responsible for the inducibility of a battery of at least six genes of phase I and phase II enzymes [17], are still uncovered. Therefore more data are required to yield information on the association between inducibility and polymorphisms of *CYP1A1*.

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