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The Relationship between Aryl Hydrocarbon Hydroxylase and Polymorphisms of the CYP1A1 Gene

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We examined the relationship between aryl hydrocarbon hydroxylase (AHH) and the frequency of a *Msp*I mutation in the 3′-flanking region of cytochrome P450 (CYP) 1A1 (MspI polymorphism) and another mutation in exon 7 (Ile-Val polymorphism) in 84 healthy male subjects in Fukuoka, Japan. AHH inducibility (3-methylcholanthrene (MC)-induced AHH activity/non-induced AHH activity) was correlated with the MspI polymorphism ($P<0.0001$) and age class ($P=0.015$), whereas no correlation was found for the Ile-Val polymorphism ($P=0.509$). Age-adjusted AHH inducibility (mean±SE) of the predominant homozygote (genotype A), the heterozygote (genotype B) and a homozygote rare allele (genotype C) genotypes was 4.89±0.36, 4.82±0.29 and 13.61±1.44, respectively. The genotype C showed much higher AHH inducibility than genotypes A and B ($P<0.001$), while no significant difference was observed between genotypes A and B. Non-induced AHH activity was also correlated with these polymorphisms. The AHH activity of a homozygous mutant Val/Val genotype (0.076±0.010 pmol/min/10^6 cells) was significantly higher ($P<0.05$) than that of the wild-type homozygous Ile/Ile (0.044±0.004 pmol/min/10^6 cells) and heterozygous Ile/Val (0.047±0.007 pmol/min/10^6 cells) genotypes. Our study suggests that the genotypes C and Val/Val, which are more frequent in smoking-related lung cancer, are closely related with high AHH inducibility and high non-induced AHH activity, respectively. Thus, the positive relationship between AHH inducibility and lung cancer is supported by our study. If our results are confirmed and the assessment of genotype becomes feasible on a population basis, identification of smokers who have genetically high susceptibility to lung cancer (genotype C or Val/Val) may become important for the prevention of lung cancer.

Key words: Aryl hydrocarbon hydroxylase — MspI polymorphism — Ile-Val polymorphism — CYP1A1 gene

Lung cancer mortality has been increasing rapidly in recent years in Japan, and has exceeded stomach cancer mortality in male Japanese. Although chronic inhalation of cigarette smoke is a major risk factor for the development of lung cancer, it seems important to examine genetic susceptibility to the disease as well. Molecular epidemiology of cancer involves the use of biomarkers of exposure and response in studies of exogenous or endogenous agents and/or host factors that play a role in human cancer etiology. This approach has the potential for identification of susceptible individuals. The individual differences in genetic susceptibility to lung cancer may be accounted for by the activity of the drug-metabolizing enzyme aryl hydrocarbon hydroxylase (AHH).

AHH is a membrane-bound monooxygenase system located in most tissues of the body. In mice, AHH inducibility (AHH activity in 3-methylcholanthrene (MC)-treated mice/AHH activity in olive oil (vehicle)-treated mice) is under the control of the *Ah* locus and certain inbred strains of mice are susceptible to AHH induction by MC treatment (Ah responsive strains), while other strains are not (Ah non-responsive strains). Although a strong correlation was observed between AHH inducibility and tumor incidence in mice,[1,2] the effect of AHH induction on tumorigenesis depends on the effects of the inducer on both metabolic activation and detoxification, the animal model and the carcinogen administered. Thus, the effect of AHH induction on tumorigenesis is complex. Since AHH is considered to be responsible in humans for the activation of benzo[*a*]-pyrene (BP) and other polyaromatic hydrocarbons (PAHs) in cigarette smoke to carcinogens, it may also be important in the causation of lung cancer.

Kellermann *et al.*[3,4] reported that the group of intermediate and high AHH inducibility (MC-induced AHH activity/non-induced AHH activity) appears to have a 15-fold and a 36-fold increased risk for lung cancer, respectively, as compared to the group with low AHH inducibility. Subsequently, Guirgis *et al.*[5] reported higher AHH activity in patients with lung cancer and Trell and his colleagues[6] described an increase in the frequency of intermediate and high AHH inducibility in persons with laryngeal carcinoma. Much effort has been made to determine the relationship between AHH activ-

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ity or inducibility and susceptibility to lung cancer or other forms of cancer in human populations as well, but the results were not very consistent.[7–10] The main reason for this inconsistency may have been difficulty in the measurement of AHH activity in lymphocytes. AHH activity is affected by many internal factors, such as age, gender and race, and external factors, such as smoking, medication and food intake (e.g., coffee intake). In addition, the conditions of cell culture can affect AHH activity. AHH inducibility is accepted as a much better indicator than specific AHH activity because of a high heritability.[11, 12] Comparison of AHH inducibility between healthy controls and lung cancer patients, however, is difficult because of altered AHH activity in cancer patients owing to chemotherapy, the tumor burden and so on.

The human cytochrome P450 (CYP)1A1 is of critical importance for the metabolism of PAHs. Recently, a close correlation between development of lung cancer and a *Msp*I restriction fragment length polymorphism (RFLP) of the 3′-flanking region (MspI polymorphism)[13] and an adenine-to-guanine mutation at residue 462 in exon 7 (Ile-Val polymorphism)[14] of the human CYP1A1 gene were reported. It is possible that nucleotide substitution in exon 7 may change the catalytic activity (non-induced AHH activity). Not only AHH inducibility (regulated by transcriptional factors), but also AHH activity (catalytic activity of already-expressed protein) would seem to be important for susceptibility to lung cancer.

In this study, we examined the correlation of AHH activity and AHH inducibility phenotypes with MspI and Ile-Val polymorphisms of the CYP1A1 gene using peripheral lymphocytes from healthy subjects.

MATERIALS AND METHODS

Chemicals 3-Hydroxybenzo[*a*]pyrene (3-OHBP) was kindly provided by Dr. N. Kinoshita, School of Health Sciences, Kyushu University, Fukuoka. BP was purchased from Sigma Chemical Co. (St. Louis, MO) and recrystallized from methanol solution. Restriction endonuclease *Msp*I and AmpliTaq DNA polymerase were obtained from Takara Shuzo Co. (Kyoto) and Perkin-Elmer Corporation (Norwalk, CT), respectively. All other chemicals and reagents were of the highest quality commercially available.

Subjects and sample collection Eighty-four Japanese healthy male volunteers (employees of Fukuoka prefectural government), aged 20 to 60 (mean 36.8±0.84 years), participated in this study. The subjects gave written informed consent and completed a short questionnaire on recent diet, illness and smoking habit (cigarettes/day). They consisted of both smokers and non-smokers and were not under medication. Heparin (40 IU/ml) was used as an anticoagulant and the blood was generally processed within 2 to 3 h after collection (time of blood collection: 12:00–13:00 p.m.). The blood collection was carried out from October 1992 to December 1992 (winter season).

Separation of lymphocytes A large portion of the blood sample (20–25 ml) was diluted 1:1 with 0.01 *M* phosphate-buffered saline (PBS, pH 6.8). Diluted blood was then layered over one-third volume of Ficoll-Conray solution (specific gravity, 1.077) gradient, and spun at 1500 rpm at room temperature for 30 min. We collected a lymphocyte-rich interphase fraction that formed a white fluffy ring on top of the Ficoll-Conray solution. The lymphocyte-rich fraction was mixed with 10 ml of RPMI-1640 medium (penicillin 100 μg/ml, streptomycin 50 μg/ml and phytohemagglutinin and pokeweed mitogen, both diluted 1:100 with distilled PBS) and spun at 2000 rpm for 10 min to sediment the lymphocytes free of platelets that remained in the supernatant. The lymphocyte pellet was resuspended in 10 ml of RPMI-1640 medium and spun again at 2000 rpm for 10 min. The purified, washed and counted (1×10^6 cells/ml) lymphocytes were resuspended in complete RPMI-1640 medium (containing 20% heat-inactivated fetal bovine serum). Ten ml of cell suspension in a flask was incubated at 37°C for 48 h in an atmosphere of fully humidified air with 5% CO_2.

AHH assay At 48 h, 5 μl of MC in acetone, to give a final concentration of 2.5 μM, was added to the cell culture (to obtain the MC-induced AHH activity). In a control culture (to measure the non-induced AHH activity), the solvent (acetone) alone was added (5 μl/10 ml culture medium). Incubation was continued for an additional 48 h period. The cells from the culture flasks with a viability of 90% or more were harvested, washed twice with 0.05 *M* Tris-HCl buffer supplemented with 0.2 *M* sucrose and 3 m*M* $MgCl_2$, and assayed for AHH activity at 37°C for 50 min with BP as a substrate.[15] We confirmed (unpublished) that this AHH activity assay method is reliable and reproducible.

Detection of MspI and Ile-Val polymorphisms The DNA was isolated from a small portion of the blood sample (about 5 ml). The genotypes of the MspI polymorphism were identified as RFLPs by means of the polymerase chain reaction (PCR).[16] The genotype designated A is a predominant homozygote, in which the MspI site is absent at the 3′-flanking region. A homozygous rare allele was named genotype C, being derived from one base substitution of T with C to form the MspI site. Genotype B is heterozygous for both alleles. The genotyping of Ile-Val polymorphism was based on the presence or absence of a PCR product for two primers with different 3′-terminal bases (A or G) according to the

method of Nakachi *et al.*[17] PCR was performed for 30 cycles of 1 min at 94°C for denaturation, 1 min at 50°C for primer annealing and 1 min at 72°C for primer extension. The other conditions were as described by Hayashi *et al.*[14]

Statistical analysis The analysis of variance was carried out to examine the relationship between the two polymorphisms and AHH activity or AHH inducibility. All the independent variables, age, smoking habit and two polymorphisms, were treated as categorical ones. Age was divided into 3 classes, 20–29, 30–39, and 40+ years old, smoking habits into 2 classes (non-smoker or current smoker), and each of the polymorphisms into 3 classes (MspI polymorphism, genotypes A, B and C; Ile-Val polymorphism, genotypes Ile/Ile, Ile/Val and Val/Val).

All computations were carried out by using the Statistical Analysis System (SAS).[18]

RESULTS

Specific AHH activity and AHH inducibility Table I shows the non-induced and MC-induced AHH activities, and AHH inducibility (MC-induced AHH activity/non-induced AHH activity) in peripheral lymphocytes from 84 male subjects. The reproducibility of AHH activity was confirmed. Non-induced AHH activity ranged from 0.008 to 0.154 pmol/min/10^6 cells with a mean value of 0.048 ± 0.003 pmol/min/10^6 cells. Treatment with MC increased AHH activities from 1.19 to 20.77 fold (mean value: 5.70 ± 0.39 fold) and MC-induced AHH activities reached 0.037–0.812 pmol/min/10^6 cells (mean value: 0.242 ± 0.017 pmol/min 10^6 cells). Very wide ranges of non-induced, MC-induced AHH activities and AHH inducibility were observed. As the distributions of the activities and the inducibility showed skewed patterns, their values were converted into natural logarithms. Then the distributions became close to normal distributions.

Analysis of variance of non-induced AHH activity in relation to selected variables Analysis of variance of non-induced AHH activity in Table II showed that the F value for the model described in "Materials and Methods" was 2.49 ($P=0.024$). The genotypes of the CYP1A1 gene ascribed to the MspI site as identified by RFLPs and PCR were in complete agreement with those obtained by Southern blotting analysis (data not shown). MspI polymorphism ($P=0.009$) was significantly related with non-induced AHH activity. When Ile-Val polymorphism was divided into 2 classes (genotypes Ile/Ile and Ile/Val vs. genotype Val/Val), Ile-Val polymorphism ($P=0.03$) was significantly related with non-induced AHH activity.

AHH activity and AHH inducibility in relation to Ile-Val polymorphism Table III shows that the frequencies of the three genotypes Ile/Ile, Ile/Val and Val/Val were 75.0% (63 of 84), 19.0% (16 of 84) and 5.9% (5 of 84),

Table I. Inducibility[a] of Aryl Hydrocarbon Hydroxylase (AHH) in Lymphocytes from 84 Human Subjects

	Age	Specific AHH activity		AHH inducibility (MC/Non)
		Non-induced	MC-induced	
Mean	36.68	0.048	0.242	5.70
Range	20–52	0.008–0.154	0.037–0.812	1.19–20.77
SE	0.84	0.003	0.017	0.39

a) Values relative to the AHH activity (3-hydroxy BP formed, pmol/min/10^6 cells) of the acetone-treated cells.

Table II. Analysis of Variance of Non-induced AHH Activity

Source	Degree of freedom	Sum of squares	F-value	P	R^2
Model	7	6.10	2.49	0.024	0.190
Error	74	25.94			
Corrected total	81	32.04			
Age class	2	0.203	0.29	0.749	
MspI[a]	2	3.56	5.07	0.009	
Ile-Val[b]	2	1.87	2.67	0.076	
Smoking status	1	0.01	0.03	0.874	

a) MspI polymorphism (genotypes A, B and C).
b) Ile-Val polymorphism (genotypes Ile/Ile, Ile/Val and Val/Val).

Table III. AHH Activity and Inducibility[a] in Relation to Ile-Val Polymorphism

	N (%)	Specific AHH activity		AHH inducibility (MC/Non)
		Non-induced	MC-induced	
Ile/Ile	63 (75.0)	0.044 ± 0.004	0.229 ± 0.020	5.81 ± 0.46
Ile/Val	16 (19.0)	0.047 ± 0.007	0.295 ± 0.041	7.52 ± 1.26
Val/Val	5 (5.9)	0.076 ± 0.010[b]	0.266 ± 0.052	3.51 ± 0.48

a) Adjusted for age.
b) Significantly different from genotypes Ile/Ile and Ile/Val ($P<0.05$).

Table IV. Analysis of Variance of AHH Inducibility in Relation to Age Class, Genotype and Smoking Status

Source	Degree of freedom	Sum of squares	F-value	P	R^2
Model	7	10.49	7.98	<0.0001	0.430
Error	74	13.89			
Corrected total	81	24.38			
Age class	2	1.66	4.43	0.0153	
MspI[a)]	2	7.21	19.20	<0.0001	
Ile-Val[b)]	2	0.26	0.68	0.5092	
Smoking status	1	0.06	0.34	0.5884	

a) MspI polymorphism (genotypes A, B and C).
a) Ile-Val polymorphism (genotypes Ile/Ile, Ile/Val and Val/Val).

Table V. AHH Activities and Inducibility[a)] in Relation to MspI Polymorphism

	N[b)] (%)	Specific AHH activity		AHH inducibility (MC/Non)
		Non-induced	MC-induced	
Genotype A	38 (46.3)	0.051±0.005	0.244±0.027	4.89±0.36
Genotype B	37 (45.1)	0.049±0.005	0.230±0.025	4.82±0.29
Genotype C	7 (8.5)	0.022±0.003[c)]	0.296±0.050	13.61±1.44[d)]

a) Adjusted for age.
b) Because of missing values, 82 subjects were included in the analysis.
c) Significantly different from genotypes A and B ($P<0.0001$).
d) Significantly different from genotypes A and B ($P<0.04$).

respectively. Non-induced AHH activity of genotype Val/Val was significantly higher than those of genotypes Ile/Ile and Ile/Val ($P<0.05$). MC-induced AHH activities and AHH inducibilities among these genotypes did not differ significantly. Values of non-induced AHH activity of the three genotypes (mean±SE) were 0.044±0.004, 0.047±0.007 and 0.076±0.010 pmol/min/10^6 cells, respectively.

Analysis of variance of AHH inducibility in relation to selected variables Analysis of variance of AHH inducibility in Table IV showed that the F value for the model described in "Materials and Methods" was 7.98 ($P<0.0001$). MspI polymorphism ($P<0.0001$) and age class ($P=0.0153$) were significantly related with AHH inducibility, while smoking was not.

AHH activity and AHH inducibility in relation to MspI polymorphism Table V shows the frequencies of the three genotypes and the AHH activity and inducibility for each of them. Adjustment was done for age class only, because the relation between AHH inducibility and smoking status was not significant in this group. The frequencies of genotypes A, B and C were 46.3% (38 of 82), 45.1% (37 of 82) and 8.5% (7 of 82), respectively. Non-induced AHH activity of genotype C was significantly lower than those of genotypes A and B ($P<0.04$). MC-induced AHH activities among these genotypes were similar. The AHH inducibilities of the three genotypes (mean±SE) were 4.89±0.36, 4.82±0.29 and 13.61±1.44, respectively. Age-adjusted AHH inducibility of

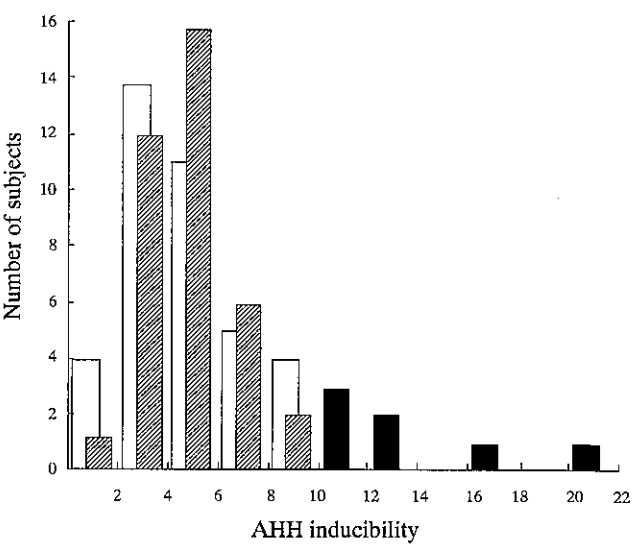

Fig. 1. Distribution of AHH inducibility after age adjustment. □, genotype A; ▨, genotype B; ■, genotype C.

genotype C was much higher than those of the other two genotypes ($P<0.0001$), while no significant difference was observed between genotypes A and B.

Distributions of AHH inducibility Fig. 1 illustrates the distributions of AHH inducibility of the three genotypes after age adjustment. The distributions of genotype A (1.19–9.93) and B (1.81–9.18) were mostly overlapped and in the range of less than 10, while genotype C (10.00–20.77) showed a distinctively higher distribution.

DISCUSSION

BP and other PAHs are suspected to cause cancers, including cancer of the human lung. They occur in cigarette smoke and are formed whenever organic matter is burned with an insufficient supply of oxygen. AHH is a component of the microsomal mixed function oxidase system dependent upon NADPH and CYP. The CYP gene is up-regulated (induced) by PAHs, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and so on. As the inducibility of CYP1A1 increases, so does the metabolism of procarcinogenic PAHs to reactive carcinogenic intermediates; enhanced metabolism often leads to a higher risk of malignancies in mice.[19] In previous studies, high AHH inducibility has also been correlated with an increased risk of bronchogenic carcinoma in comparison with a control group matched for age, sex, and cigarette-smoking history.[3, 12] Despite much effort, this association has not been fully established yet. In our previous study,[20] a very wide range of AHH inducibility was observed and this in turn suggests that varying genetic susceptibility to inducers of the enzyme may exist among the human population. Consequently, toxic/carcinogenic responses to chemicals may vary from person to person. A person with high AHH inducibility may be more sensitive to toxic chemicals than a person with low AHH inducibility. At present, details of the mechanisms of both AHH induction and AHH genetic variations in individuals are unclear. Nevertheless, inherited inducibility of the AHH activity is believed to be an essential factor in carcinogenesis due to the PAHs.[21]

Several CYP1A1 RFLP patterns have been reported in recent years.[13, 22, 25] A close correlation between development of lung cancer and two CYP1A1 polymorphisms (MspI and Ile-Val) was described in Japanese,[17] but was not reproduced in non-Japanese populations.[26–28] This ethnic difference might be due to the allelic frequency in two polymorphisms. For example, the frequency of the genotype C (m2/m2) allele of m2 was 0.332 in a Japanese population,[16] but 0.115 in a Norwegian population.[26] Thus, the homozygous site present in the European Caucasian population (0.115^2) was about ten times less than in the Japanese population (0.332^2), and the Ile-Val polymorphism was less common than the MspI polymorphism.[14, 28] Moreover, the two polymorphisms are closely linked in Japanese and Northern Europeans.[14, 28] The frequency of each genotype in two polymorphisms reported by Japanese reseachers[13, 17] coincided with our result. We could not, however, detect whether these polymorphisms are in genetic disequilibrium with our limited current sample size and somewhat biased sample. Unexpectedly, high AHH inducibility is related to low non-induced AHH activity (Table V). This result was not consistent with the finding that the two polymorphisms are closely linked. We should emphasize again that the object of this study was to determine the relationship between the phenotype of CYP1A1 and the genotype of CYP1A1 using peripheral lymphocytes from healthy controls.

As stated earlier, comparisons of AHH activity between healthy controls and lung cancer patients present problems. AHH activity of lung cancer patients is likely to be distorted by a number of factors. For example, radiation therapy may decrease the lymphocyte count,[29] lymphoblast formation and mitogen response.[30] A linear relationship between AHH inducibility and lymphoblast formation has been suggested.[31] Poor lymphoblast formation in lung cancer patients may lead to underestimation of AHH inducibility. Besides, poor lymphocyte growth in culture may cause difficulty in assessing the AHH inducibility of lung cancer patients.[7] Lymphocytes are not the primary target for the carcinogenic action of PAHs. One of the important questions is whether AHH inducibility in lymphocytes correlates with AHH inducibility in other tissues of the same individuals. Although few data are available on this point, AHH inducibility in lymphocytes from several mouse strains was found to correlate well with that in non-lymphoid tissues from those strains.[32, 33]

If the CYP1A1 induction phenotype could be assessed, this might be helpful in predicting individual risk of cancer and toxicity. However, the determination of the CYP1A1 induction phenotype by enzyme assay is very laborious and cumbersome because the procedure requires more than 20 ml of blood, the isolation and culturing of lymphocytes for 4 days, and a spectrophotofluorometric assay involving hazardous chemicals.[12, 34] On the other hand, the determination of CYP1A1 genotype could easily be done in only a day. It is worthwhile to determine the relationship between the CYP1A1 phenotype and genotype.

In conclusion, although it may be reasonable to assume a close association between high AHH inducibility and genotype C, no report has yet clarified this association.[11] Fortunately, there are many MC high responders among the Japanese. In the present study, we examined the

relationship between the phenotype of CYP1A1 gene and the genotype of CYP1A1 gene in 84 males and found that people who possesses genotype C showed remarkable higher AHH inducibility than those with genotypes A and B. Our result suggests that identification of genotype C smokers, who have high AHH inducibility and are genetically susceptible to lung carcinogenesis, would be helpful for the prevention of lung cancer.

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REFERENCES

1) Kouri, R. E., Salero, R. E. and Whitmire, C. E. Relationships between aryl hydrocarbon hydroxylase inducibility and susceptibility to chemically induced subcutaneous sarcoma in various strains of mice. *J. Natl. Cancer Inst.*, **50**, 363–368 (1973).
2) Kouri, R. E., Ratire, H. and Whitmire, C. E. Genetic control of susceptibility to 3-methylcholanthrene-induced subcutaneous sarcoma. *Int. J. Cancer*, **13**, 714–720 (1974).
3) Kellermann, G., Shaw, C. R. and Luyten-Kellermann, M. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N. Engl. J. Med.*, **289**, 934–937 (1973).
4) Kellermann, G., Luyten-Kellermann, M. and Shaw, C. R. Genetic variation of aryl hydrocarbon hydroxylase in human lymphocytes. *Am. J. Hum. Genet.*, **25**, 327–331 (1973).
5) Guirgis, H. A., Lynch, H. T., Mate, T., Haris, R. E. and Well, I. Aryl hydrocarbon hydroxylase inducibility in lymphocytes from lung cancer patients and normal controls. *Oncology*, **33**, 105–109 (1976).
6) Trell, E., Kordgaard, R., Hood, B., Kittzing, O., Norden, G. and Simonsson, B. G. Aryl hydrocarbon hydroxylase inducibility and laryngeal carcinomas. *Lancet*, **ii**, 140 (1976).
7) Paigen, B., Minowada, J., Gurtoo, H. L., Paigen, K., Parker, N. B., Ward, E., Hayner, N. T., Bross, I .D. J., Boch, F. and Vincent, R. Distribution of aryl hydrocarbon hydroxylase inducibility in cultured human lymphocytes. *Cancer Res.*, **37**, 1829–1837 (1977).
8) Ward, E., Paigen, B., Steenland, K., Vincent, R., Minowada, J., Gurtoo, H. L., Sartori, P. and Havens, M. B. Aryl hydrocarbon hydroxylase in persons with lung or laryngeal cancer. *Int. J. Cancer*, **22**, 384–389 (1978).
9) Lieberman, J. Aryl hydrocarbon hydroxylase in bronchogenic carcinoma. *N. Engl. J. Med.*, **298**, 686–687 (1978).
10) Kärki, N. T., Pokela, R., Nuutinen, L. and Pelkonen, O. Aryl hydrocarbon hydroxylase in lymphocytes and lung tissue from lung cancer patients and controls. *Int. J. Cancer*, **39**, 565–570 (1987).
11) Atlas, S. A., Vesell, E. S. and Nebert, D. W. Genetic control of interindividual variations in the inducibility of aryl hydrocarbon hydroxylase in cultured human lymphocytes. *Cancer Res.*, **36**, 4619–4630 (1976).
12) Paigen, B., Gurtoo, H. L., Minowada, J., Ward, E., Houten, J., Paigen, K., Reilly, A. and Vincent, R. Genetics of aryl hydrocarbon hydroxylase in the human population and its relationship to lung cancer. *In* "Polycyclic Hydrocarbons and Cancer," ed. H. V. Gelboin and P. O. P. Ts'o, Vol. 2, pp. 391–405 (1978). Academic Press, New York.
13) Kawajiri, K., Nakachi, K., Imai, K., Yoshii, A., Shinoda, N. and Watanabe, J. Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P450IA1 gene. *FEBS Lett.*, **263**, 131–133 (1990).
14) Hayashi, S., Watanabe, J., Nakachi, K. and Kawajiri, K. Genetic linkage of lung cancer-associated *Msp*I polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. *J. Biochem.*, **110**, 407–411 (1991).
15) Kiyohara, C., Mohri, N., Hirohata, T., Haraguchi, K. and Masuda, Y. *In vitro* effect of methylsulfonyl polychlorinated biphenyls and 7,8-benzoflavone on aryl hydrocarbon hydroxylase activity in human lymphoblastoid cells. *Pharmacol. Toxicol.*, **66**, 273–276 (1990).
16) Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullius, K. B. and Erlich, H. A. Primer-directed enzymatic amplification of DNA sequencing with a thermostable DNA polymerase. *Science*, **239**, 487–491 (1988).
17) Nakachi, K., Imai, K., Hayashi, S.-I., Watanabe, J. and Kawajiri, K. Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. *Cancer Res.*, **51**, 5177–5180 (1991).
18) SAS Institute Inc. "SAS User's Guide: Statistics, Version 5 Edition" (1985). SAS Institute Inc., Cary, NC.
19) Nebert, D. W. and Jones, J. E. Regulation of the mammalian cytochrome $P_1$450 (CYP1A1) gene. *Int. J. Biochem.*, **21**, 234–252 (1989).
20) Nagayama, J., Kiyohara, C., Masuda, Y. and Kuratsune, M. Aryl hydrocarbon hydroxylase activity in human lymphoblastoid cells by polychlorinated dibenzofuran isomers and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Arch. Toxicol.*, **56**, 230–235 (1985).
21) Nebert, D. W., Benedict, W. F. and Kouri, R. E. Aromatic hydrocarbon produced tumorigenesis and the genetic difference in aryl hydrocarbon hydroxylase. *In* "Chemical Carcinogenesis," ed. P. O. P. Ts'o and J. A. DiPaolo, Part A, pp. 271–288 (1974). Marcel Decker Inc.,

New York.
22) Jaiswal, K. and Nebert, D. W. Two RFLPs associated with the human P_1-450 gene linked to the MPI locus on chromosome 15. *Nucleic Acids Res.*, **14**, 4376 (1986).
23) Haugen, A., Willey, J., Borresen, A. L. and Tefre, T. *Pst*I polymorphism at the human P1450 gene on chromosome 15. *Nucleic Acids Res.*, **18**, 3114 (1990).
24) Bale, A. E., Nebert, D. W. and McBride, O. W. Subchromosomal localization of the dioxin-inducible P1450 locus (CYP1) and description of two RFLPs detected with 3′-P1450 cDNA probe. *Cytogenet. Cell Genet.*, **46**, 574–575 (1987).
25) Spurr, N. K., Gough, A .C., Stevenson, K. and Wolf, C. R. *Msp*-I polymorphism detected with a cDNA probe for the P-450 I family on chromosome 15. *Nucleic Acids Res.*, **15**, 5901 (1987).
26) Tefre, T., Ryberg, D., Haugen, A., Nebert, D. W., Skaung, V., Brøgger, A. and Børresen, A.-L. Human CYP1A1 (cytochrome P1 450) gene: lack of association between the *Msp*I restriction fragment length polymorphism and incidence of lung cancer in a Norwegian population. *Pharmacogenetics*, **1**, 20–58 (1991).
27) Shields, P. G., Sugimura, H., Caporaso, N. E., Petruzzelli, S. F., Bowman, E. D., Trump, B. F., Weston, A. and Harris, C. C. Polycyclic hydrocarbon-DNA adducts and the CYP1A1 RFLP. *Environ. Health Perspect.*, **98**, 191–194 (1992).
28) Hivronen, A., Husgafvel-Pursianin, K., Karjalanen, A., Anttila, S. and 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 Prev.*, **1**, 485–489 (1992).
29) Raben, M., Walach, N., Galili, U. and Schlesinger, M. The effect of radiation therapy on lymphocytes subpopulations in cancer patients. *Cancer*, **37**, 3771–3773 (1976).
30) Prasad, R., Prasad, S., Harrell, J. E., Thornby, J., Liem, J. H., Hudgins, P. H. and Guinn, G. A. Aryl hydrocarbon hydroxylase inducibility and lymphoblast formation in lung cancer patients. *Int. J. Cancer*, **23**, 316–320 (1979).
31) Jett, J. R., Moses, H. L., Branum, E. L., Taylor, W. F. and Fontana, R. S. Benzo(a)pyrene metabolism and blast transformation in peripheral blood mononuclear cells from smoking and nonsmoking population and lung cancer patients. *Cancer*, **41**, 192–200 (1978).
32) Nebert, D. W. and Jensen, N. M. The Ah locus: genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases. *CRC Crit. Rev. Biochem.*, **6**, 401–437 (1979).
33) Alfred, L. J. and Wojdani, A. Effects of 3-methylcholanthrene and benzanthracene on blastogenesis and aryl hydrocarbon hydroxylase induction in splenic lymphocytes from three inbred strains of mice. *Int. J. Immunopharmacol.*, **5**, 123–129 (1983).
34) Jaiswal, A. K., Gonzallez, F. J. and Nebert, D. W. Human P1-450 gene sequence and correlation of mRNA with genetic differences in benzo(a)pyrene metabolism. *Nucleic Acids Res.*, **13**, 4503–4520 (1985).