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Assessment of cancer susceptibility in humans by use of genetic polymorphisms in carcinogen metabolism

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Prevention is an important and effective measure for reducing death caused by cancer. Thus information on individual susceptibility to cancer is valuable in suggesting high risk individuals to avoid intake of carcinogenic substances and receive frequent physical screening. To this end, polymorphisms found within cytochrome P450 (*CYP*) genes implicated in the metabolism of procarcinogens are expected to be good genetic targets in assessing human cancer susceptibility. We have found polymorphisms in the *CYP2E1* and *CYP1A1* genes associated with lung cancer susceptibility, though there were some discrepancies from observations made by other investigators. Discrepancies among investigators from different regions, however, are very common in these pharmacogenetic studies. We present an explanation for these discrepancies, difficulties associated with prediction of relative risk of individuals, and future directions.

Introduction

Prevention stands as ultimate strategy to conquer cancer. Information of individual susceptibility to cancer with a predictive value may lead to identification of high risk individuals, thereby decreasing possible development of cancer by recommending them to reduce their chance of exposures to carcinogenic substances (reviewed in Caporaso *et al.*, 1991; Nebert, 1991). From the underlying basis that carcinogenic substances and viral agents are believed to cause most cancers (chemicals causing approximately 70% of the cases), the disease could be prevented by avoiding exposure to carcinogenic substances and viral agents (reviewed in Doll & Peto, 1981). To this end, pharmacogenetics, which is a field study of variability in individual responses to chemicals as a consequence of differences in individual genetic background, is an attractive resource rapidly gaining general acceptance. Indeed, polymorphic response to number of chemicals has been reported to date, and at least some of them fit criteria reasonably well as determinants for susceptibility to certain cancers (reviewed in Caporaso *et al.*, 1991; Nebert, 1991). Pharmacogenetics mainly uses two technologically different approaches, which are not mutually exclusive but rather complementary. One is assaying individual metabolic capacity for certain drugs and the other is directly genotyping gene(s) encoding enzymes involved in chemical metabolisms. The former approach, usually more laborious, expensive, and above all, possibly harmful to patients, gives a plausible biological basis for the relevance of the polymorphism and cancer susceptibility. However, it is confounded by other factors, such as age, sex, season, life style, treatment, etc. The genotyping approach is less laborious, less expensive, less potentially harmful, and relatively less confounding, although the biological basis for relevance of the polymorphism and cancer susceptibility is less apparent. It is also confounded by polymorphisms not having a causative role for determining cancer susceptibility, and may simply be linked by chance to a *bona fide* determinant for cancer susceptibility by linkage disequilibrium. Therefore, the establishment of reliable, inexpensive, simple, harmless, and rapid methods which can be applied by massive screening to determine individual relative risk of developing cancer is a goal to be aimed at.

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Importance of prevention in reducing cancer

Although various advances in cancer therapy have made a significant portion of cancers curable, cancer still remains as the leading life-threatening disease among advanced nations, with treatment consuming tremendous resources (Coleman *et al.*, 1993). Additionally, effective therapy is still limited in certain types of cancers. At present, approximately half of cancer patients are likely to die and half are likely to recover from the disease. Two thirds of the latter half may recover by surgical therapy, one fifth by radiotherapy, and the rest by chemotherapy (Fig. 1). Advances in radiotherapy are limited since its effectiveness is largely dependent on the site of the cancer. Combination therapy is also decreasing because of its ineffectiveness in many cases. Thus, the realization of reducing cancer death is dependent on increasing the recovery rate from surgical therapy, chemotherapy (including gene therapy), on reducing the incidence of cancer, or on developing totally different strategies for therapy (Fig. 1). In each category, pharmacogenetics is useful. Regarding chemotherapy, careful re-examination of human responses to drugs, one aspect of pharmacogenetics, will lead us to understand variability in individual responses to anti-cancer drugs, subsequently allowing physicians to determine appropriate dosage of the drugs for each patient. For surgical therapy, successful cure rates will be improved by increasing early diagnosis as a consequence of recommending careful and frequent screening, especially among high risk groups. Of course, the ultimate goal will be to reduce cancer incidence by preventing cancer. Since about 70% of the cancer causing agents are believed to be chemical substances (Doll & Peto, 1981), avoiding exposure to carcinogenic substances plays a central role in prevention, although the use of antagonistic drugs for prevention may also be considered in extremely high risk groups (Greenwald, 1994). Nevertheless, absolute exclusion of carcinogenic substances is impossible. Therefore, the solution is to identify high risk individuals and to recommend them to alter their occupations, life style, etc. to avoid unnecessary exposure to substances to which they are sensitive (reviewed in Nebert, 1991). The most striking case to date is the slow *N*-acetylator phenotype, which is a prevalent polymorphism comprising more than 50% of the population of the Western world. This phenotype is weakly associated with bladder carcinoma (OR ranging from 1–1.7 depending on researchers, reviewed in Caporaso *et al.*, 1991). However, in chemical workers with bladder carcinoma, virtually all were found to be of the slow *N*-acetylator phenotype (Cartwright *et al.*, 1982), suggesting that alteration of occupational exposure to lessen contact with such chemicals might be a beneficial preventive measure for these high risk groups. Prevention is all the more important because patients recovered from cancers are much more prone to develop secondary cancer (not metastasis), for the patient may be inherently more susceptible to cancer.

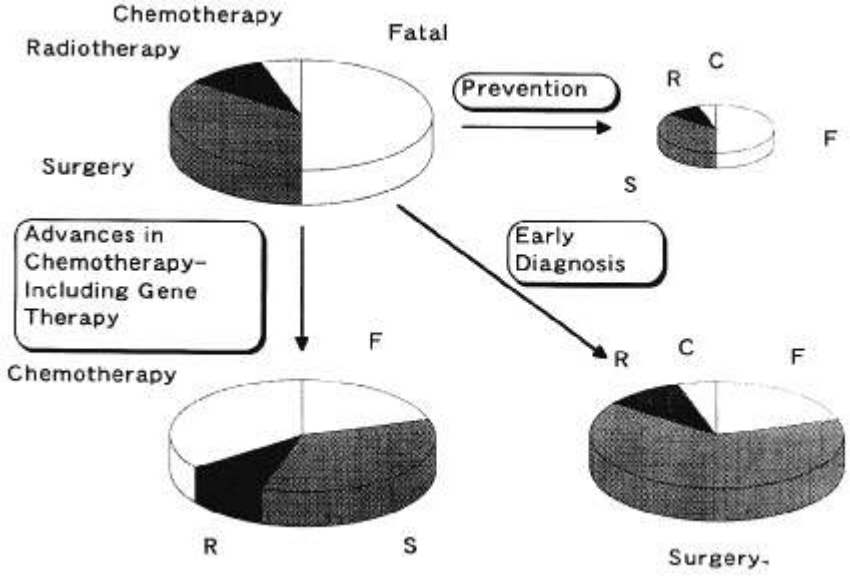

Fig. 1. Future directions for reducing cancer death. General fate of cancer patients is shown according to publications in Japan, although proportions are highly variable depending on hospitals. Absolute proportion, however, is not important for the sake of the discussion in the text. Note that the pie chart on top right corner is reduced in size to show reduction in cancer incidence. F, fatal; S, surgery; R, radiotherapy; C, chemotherapy.

Table 1. CYP2E1 *Dra* I RFLP and lung cancer susceptibility

Region		*CC* (%)	*CD* (%)	*DD* (%)	*Total*	*Reference*
Japan (Miyagi)	Control	11 (14.5)	22 (28.9)	43 (56.6)	76	Uematsu *et al.*, 1994
	Lung cancer	2 (2.2)	42 (46.2)	47 (51.6)	91	
	Smoking history < 20 pyr	1 (4.0)	16 (64.0)	8 (32.0)	25	
	Smoking history > 20 pyr	1 (1.9)	21 (38.9)	32 (59.3)	54	
Finland	Control	1 (0.8)	24 (19.8)	96 (79.3)	121	Hirvonen *et al.*, 1993
	Lung cancer	2 (2.0)	14 (13.9)	85 (84.2)	101	
Sweden	Control	2 (1.0)	38 (18.4)	166 (80.6)	206	Persson *et al.*, 1993
	Lung cancer	0 (0.0)	33 (17.1)	160 (82.9)	193	

Finally, development of such methods to identify individual cancer susceptibility and use of the information in preventive strategies will enable us to reduce the cost of treatment as well as cancer death.

Mutations and polymorphisms determining cancer susceptibility

The existence of a certain population susceptibility to cancer is most pronounced in the hereditary predisposition to cancer (reviewed by White, 1992). The best example is familial retinoblastoma, a rare tumour of the retina occurring one out of 20 000 births in Caucasians, in which more than 90% of affected individuals develop an average of six independent tumours in their eyes (reviewed in Zacksenhaus *et al.*, 1993). Since they are at a great disadvantage for survival, they do not constitute a major part of the population (Ramel, 1992). Thus, these genetic alterations in the *RB1* gene are called mutations, for the abnormalities are easily distinguishable from the normal allele. However, a less obvious predisposition to cancer (i.e. very low penetrance), is more frequently observed as late onset cancer gathering in close relatives. Ordinarily cancer is a late onset disease occurring after the reproductive period, therefore, a weak cancer susceptibility phenotype does not serve as a selective pressure against survival of the species and the cancer susceptibility allele would be considered neutral in terms of survival of the species (Kimura, 1981). Although, these weakly susceptible phenotypes are not a great risk factor for an individual, they may be widely distributed in a population and the total risk for the population would be large, particularly in societies with a significant aged population. Such alterations are called risk-associated factors and in many cases it is not possible to easily distinguish between normal and abnormal alleles. These low penetrance genes are most likely to show multi-gene inheritance and are not suitable subjects for ordinary positional cloning or candidate gene methods. Nevertheless, such genes involved in the metabolism of carcinogens (reviewed in Kawajiri & Fujii-Kuriyama, 1991; Kawajiri *et al.*, 1993) and DNA repair systems (reviewed in Weeda *et al.*, 1993; Service, 1994), which are not essential for survival but may affect susceptibility to cancer are good candidate genes to start with. Methodology is straightforward: 1) choose candidate genes; 2) search for polymorphisms within them; 3) identify polymorphisms which are frequent among patients and infrequent among the normal population; and 4) find a biologically plausible explanation.

We will describe our efforts using such a strategy, in the identification of putative cancer susceptibility polymorphisms in genes involved in P450 systems, namely *CYP2E1* and *CYP1A1*, which are known to activate procarcinogens into ultimate carcinogens.

CYP2E1 polymorphisms and lung cancer susceptibility

CYP2E1 is an enzyme involved in the oxidation of ethanol and carcinogenic activation of low molecular weight nitrosamines existing in diets, cigarette smoke and air (Gonzalez, 1988; Guengerich *et al.*, 1991). Among restriction fragment length polymorphisms (RFLP), *Taq* I, *Dra* I, *Rsa* I, two *Msp* I and *Pst* I, found within *CYP2E1*, we previously reported that the *Dra* I RFLP is associated with lung cancer (Uematsu *et al.*, 1991). The *Dra* I RFLP, located within intron 6 of the *CYP2E1* gene, was detected by amplifying a 950 bp DNA fragment encompassing the *Dra* I RFLP. Distribution of the C allele, an allele without the *Dra* I site and allele D, an allele with the *Dra* I site, was studied among cancer patients and normal controls

(Table 1). The distribution among patients with cancer of digestive tract origin was not significantly different from that among normal controls. The distribution of heterozygote C/D alleles among lung cancer patients, however, was significantly more frequent than that of normal controls, giving an OR of 2.1 (Uematsu *et al.*, 1991, 1994). Moreover, this tendency decreased in the group of patients with greater than 20 pack-years of smoking history (Uematsu *et al.*, 1994), probably due to over-exposure to carcinogenic substances overriding the genetic component. This implies that high risk individuals need to avoid even a small amount of the susceptible carcinogens compelling vigorous restrictions in their life style, thus reducing the value of this polymorphism in the prevention of cancer. Furthermore, the odds ratio of 2.1 is usually not sufficiently compelling to recommend restrictions in life style. Therefore, although the result gives a hope for such a strategy in the prevention of cancer, at the same time, it demands us to identify new polymorphisms of more practical use. In addition, inconsistent observations have also been reported by other researchers (Table 1, references are given in the Table), which will be described in the next section. Incidentally, the tendency can be interpreted that the OR increased in the group of patients with less than 20 pack-years of smoking history because of exposure to, as yet unidentified, carcinogenic substances not contained in cigarette smoke.

Finally, *CYP2E1* mRNA expression was elevated in xenografted liver from patients heterozygous for the RFLP (Uematsu *et al.*, 1994). This observation hardly gives a biologically plausible explanation for the association of the RFLP and lung cancer, necessitating further investigations.

CYP1A1 polymorphisms and lung cancer susceptibility

CYP1A1 is the best studied P450 among P450s involved in activation of procarcinogens (Gonzalez, 1988; Guengerich, 1992). CYP1A1 is induced by aryl hydrocarbons (Ah), such as tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene, benzo[a]-pyrene, and it activates benzo[a]pyrene which is a major carcinogenic substance in cigarette smoke. Human lung cancer, one of the most problematic cancers today, is known to be highly dependent on exposure to procarcinogens abundant in cigarette smoke (IARC, Tobacco smoking. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 1984: **38**, 221–228). It is also known that DNA adducts appear as a consequence of the induction of CYP1A1 by smoking (Phillips *et al.*, 1988; McLemore *et al.*, 1990). Thus identification of the risk associated with polymorphisms of Ah-metabolizing enzymes, such as CYP1A1 and GSTM1 (Nakachi *et al.*, 1993), is invaluable for identifying preventive measures, and especially a polymorphism of practical use which can be applied to mass epidemiological screening. The association of such a polymorphism has been implicated since positives correlation between CYP1A1 inducibility and smoking related lung cancer susceptibility has been documented for decades (Kellermann *et al.*, 1973a, b), although it still remains controversial (reviewed in Caporaso *et al.*, 1991; Ingelman *et al.*, 1992).

We started with reexamining the reported *Msp* I RFLP, which resides in the 3′-flanking region of the *CYP1A1* gene. This *Msp* I RFLP is reported to be associated with lung cancer, especially in the case of Kreyberg I type lung cancers, which are highly associated with smoking history (Kawajiri *et al.*, 1990). Distribution of recessive m2/m2 homozygotes (allele with and without the *Msp* I site, designated m1 and m2, respectively) is elevated in lung cancer, especially in Kreyberg I type lung cancer, giving an OR of 3.21 (Table 2). Analogous to the CYP2E1 described earlier, sub-classification of this group by amount of cigarette consumption has revealed that being homozygous for m2/m2 contributes to a further elevation of the OR to 7.31 in the low consumption group, however, this elevation of OR drops to 1.13 in the high consumption group (Nakachi *et al.*, 1991). This effect of genetic constitution being overridden by exposure to a carcinogen, also reduces the value of this determinant in practical use. In addition, the *Msp* I RFLP is in close linkage with the Ile/Val polymorphism, the residue 462 polymorphism in the heme binding region of *CYP1A1* (the m1 allele being closely linked to *CYP1A1* (Ile) and the m2 allele to *CYP1A1* (Val) in Japanese). CYP1A1(Val) is claimed to have elevated aryl hydrocarbon hydroxylase activity compared with CYP1A1(Ile), explaining the association of the *Msp* I RFLP to lung cancer susceptibility. This seems hardly a plausible explanation because the heterozygote individuals are not at elevated risk (Nakachi *et al.*, 1991). One would expect the m1/m1 homozygote and m1/m2 heterozygote to exhibit different ORs depending on cigarette consumption.

Therefore, we performed a confirmatory study in the hope of identifying putative susceptibility determinants closely linked to the *Msp* I RFLP in Japanese that also play a causative role. Contrary to our first expectation, we obtained the opposite result: m1/m1 homozygotes were significantly prevalent in lung cancer patients in Miyagi, Japan (Table 2). Therefore, we are still continuing to investigate the RFLP as well as searching for a new polymorphism of practical value.

Regarding this discrepancy, more than one group has reported a lack of association between the polymorphisms and lung cancer (Table 2, references are given in the Table). Discrepancies among investigators from different regions are quite common in this type of research, implying that these neutral genes, in terms of survival of the species, will fluctuate among populations (Kimura, 1981; Ramel, 1992). Indeed there are very few reports that can be generalized for many races (reviewed in Caporaso *et al.*, 1991). Therefore, it is possible that regional genetic variations exist even within Japanese populations of the Miyagi prefecture and the Saitama prefecture, since the Japanese are very reluctant to move – in contrast to the general belief that they are a homogeneous population. Only a *bona fide* risk determinant that also plays a causative role in cancer susceptibility can evade this problem. In the cases where no association has been found, it may also be explained by the result being obscured by a stronger susceptibility determining allele – since a relatively subtle susceptibility determinant may be masked by a stronger susceptibility determinant.

Ah receptor polymorphisms and lung cancer susceptibility

There is also a possibility that genes involved in the induction of CYP1A1 are relevant to lung cancer susceptibility. The mechanism of CYP1A1 induction is well-documented (reviewed in Hankinson, 1993; Nebert *et al.*, 1993; Swanson & Bradfield, 1993). Ah receptor (AhR) by binding to Ah, releases hsp90 complexed with AhR, thereby allowing AhR to form a ternary complex with the AhR nuclear translocator (ARNT). This ternary complex in turn binds to the xenobiotic responsive element (XRE) which is present several times in the regulatory region of the *CYP1A1* gene, triggering induction of expression (Hines *et al.*, 1988; Kubota *et al.*, 1991). Furthermore, mouse strains are classified into two groups, one highly sensitive to Ah insult, developing cancer of the lung and the skin, the other not (Kouri *et al.*, 1973; Watanabe *et al.*, 1975; Nebert & Jones, 1989). This strain difference is now known to be governed by four polymorphic alleles of AhR determined by a difference in the mobility of the protein through SDS-polyacrylamide gel (Poland & Glover, 1990; Swanson & Bradfield, 1993). By comparing the structure and TCDD binding properties of the AhR of a sensitive strain C57B6 and an insensitive strain DBA/2J, Fujii-Kuriyama and his colleagues have shown that the polymorphism is the determinant of the elevated binding constant between AhR and its ligand TCDD, thereby elevating cancer susceptibility in the sensitive strain. AhR is a ligand-dependent receptor expressed in various tissues and developmental stages and belongs to the *PAS* gene superfamily (which includes *per*,

Table 2. CYP1A1 *Msp* I RFLP and lung cancer susceptibility

Region		*m1/m1* (%)	*m1/m2* (%)	*m2/m2* (%)	*Total*	*Reference*
Japan	Control	166 (44.3)	169 (45.1)	40 (10.7)	375	Nakachi *et al.*, 1991
(Saitama)	Lung cancer	61 (40.4)	58 (38.4)	32 (21.2)	151	
	Squamous cell Carcinoma	19 (33.3)	23 (40.4)	15 (26.3)	57	
	Adenocarcinoma	30 (50.0)	22 (36.7)	8 (13.3)	60	
Japan	Control	66 (51.2)	47 (36.4)	16 (12.4)	129	Kihara *et al.*,
(Kanagawa)	Lung cancer*	16 (45.7)	13 (37.1)	6 (17.1)	35	personal communication
Japan	Control	11 (25.6)	24 (55.8)	8 (18.6)	43	Ikawa *et al.*,
(Miyagi)	Lung cancer	35 (49.3)	26 (36.6)	10 (14.1)	71	unpublished result
USA	Control	43 (76.8)	11 (19.6)	2 (3.6)	56	Shields *et al.*, 1993
	Lung cancer	33 (68.8)	12 (25.0)	3 (6.3)	48	
Finland	Control	95 (78.5)	24 (19.8)	2 (1.7)	121	Hirvonen *et al.*, 1992
	Lung cancer	65 (74.7)	22 (25.3)	0 (0.0)	87	
Norway	Control	167 (78.8)	43 (20.3)	2 (0.9)	212	Tefre *et al.*, 1991
	Lung cancer	172 (77.8)	47 (21.3)	2 (0.9)	221	

*Small cell carcinoma only

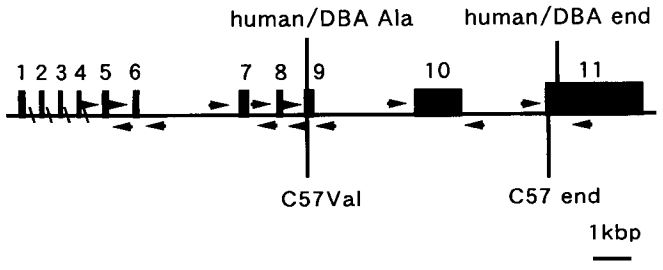

Fig. 2. Structure of the human *AHR* gene. The human *AHR* gene is shown schematically. Polymorphisms speculated to elevate TCDD binding affinity are shown below, and to reduce the affinity are shown over the gene. Solid boxes: exons; solid lines: introns; arrows: primers synthesized for PCR amplification.

AhR/ARNT, *sim*), whose other members are believed to function in the control of circadian rhythm and neural development (Swanson & Bradfield, 1993). This suggests that AhR should not solely exist for the binding of foreign ligands such as TCDD for induction of *CYP1A1*. Thus, a polymorphism impairing TCDD binding without impairing biological activity essential for AhR is possible, explaining the hyper-polymorphic allele in the mouse.

We have, therefore, sought to find a similar polymorphism within the human *AHR* gene, since it is possible that AhR polymorphism may also act as a *bona fide* cancer susceptibility determinant that plays a causative role in humans. We have started with the isolation of the human gene encoding AhR from a human cosmid library and determined the sequence surrounding the exons (Fig. 2). Although, we are still in the process of searching for polymorphisms using primers shown in Fig. 2 for polymerase chain reaction (PCR) amplification of the gene, so far we have identified two polymorphisms within intron 10. However, we have not yet been able to identify the polymorphisms residing in exons. In addition, we have not been able to identify the same polymorphisms found in the C57B6 and the DBA/2J strain of the mouse (Fig. 2).

Future directions

Assessing human susceptibility to cancer for prevention and early diagnosis may be a powerful tool in reducing cancer deaths and the financial burden of treatments. For this purpose, although it may take a long time as mentioned earlier, it is important to find a *bona fide* cancer susceptibility polymorphism which at the same time plays a causative role, enabling application of the polymorphism to mass screening in many races. Moreover, the number of factors influencing susceptibility may be large (multi-gene inheritance) and the number of candidate genes, polymorphisms and DNA samples to be analysed will grow enormously. Therefore, a carefully designed system is required which allows both the analyses of hundreds of DNA samples, and the efficient search for new polymorphisms to handle as many genes as possible (reviewed in Cotton, 1993). Finally, the susceptibility information thus obtained should only be used to give suggestion of high risk individuals, not to make obligations against their will and absolutely not for other purposes.

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References

Caporaso N, Landi MT, Vineis P. Relevance of metabolic polymorphisms to human carcinogenesis: evaluation of epidemiologic evidence. *Pharmacogenetics* 1991: **1**, 4–19.

Cartwright RA, Glashan RW, Rogers HJ, Ahmad RA, Barham HD, Higgins E, Kahn MA. Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. *Lancet* 1982: **2**, 842–845.

Coleman MP, Esteve J, Damiecki P, Arslan A, Renard H. Trends in cancer incidence and mortality. *IARC Sci Publ* 1993: **1993**, 1–806.

Cotton RG. Current methods of mutation detection. *Mutat Res* 1993: **285**, 125–144.

Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J Natl Cancer Inst* 1981: **66**, 1191–1308.

Gonzalez FJ. The molecular biology of cytochrome P450s *Pharmacol Rev* 1988: **40**, 243–288.

Greenwald P. Experience from clinical trials in cancer prevention. *Ann Med* 1994: **26**, 73–80.

Guengerich FP. Metabolic activation of carcinogens. *Pharmacol Ther* 1992: **54**, 17–61.

Guengerich FP, Kim DH, Iwasaki M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* 1991: **4**, 168–179.

Hankinson O. Research on the aryl hydrocarbon (dioxin) receptor is primed to take off. *Arch Biochem Biophys* 1993: **300**, 1–5.

Hines RN, Mathis JM, Jacob CS. Identification of multiple regulat-

ory elements on the human cytochrome P450IA1 gene. *Carcinogenesis* 1988: **9**, 1599–1605.

Hirvonen A, Husgafvel PK, Karjalainen A, Anttila S, Vainio H. Point-mutational *Msp* I 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* 1992: **1**, 485–489.

Hirvonen A, Husgafvel PK, Anttila S, Karjalainen A, Vainio H. The human *CYP2E1* gene and lung cancer: *Dra* I and *Rsa* I restriction fragment length polymorphisms in a Finnish study population. *Carcinogenesis* 1993: **14**, 85–88.

Ingelman SM, Johansson I, Persson I, Yue QY, Dahl ML, Bertilsson L, Sjoqvist F. Genetic polymorphism of cytochromes P450: interethnic differences and relationship to incidence of lung cancer. *Pharmacogenetics* 1992: **2**, 264–271.

Kawajiri K, Fujii-Kuriyama Y. P450 and human cancer. *Jpn J Cancer Res* 1991: **82**, 1325–1335.

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 Lett* 1990: **263**, 131–133.

Kawajiri K, Nakachi K, Imai K, Watanabe J, Hayashi S. The *CYP1A1* gene and cancer susceptibility. *Crit Rev Oncol Hematol* 1993: **14**, 77–87.

Kellermann G, Luyten KM, Shaw CR. Genetic variation of aryl hydrocarbon hydroxylase in human lymphocytes. *Am J Hum Genet* 1973a: **25**, 327–331.

Kellermann G, Shaw CR, Luyten KM. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N Engl J Med* 1973b: **289**, 934–937.

Kimura M. Possibility of extensive neutral evolution under stabilizing selection with special reference to nonrandom usage of synonymous codons. *Proc Natl Acad Sci USA* 1981: **78**, 5773–5777.

Kouri RE, Salerno RA, Whitmire CE. Relationships between aryl hydrocarbon hydroxylase inducibility and sensitivity to chemically induced subcutaneous sarcomas in various strains of mice. *J Natl Cancer Inst* 1973: **50**, 363–368.

Kubota M, Sogawa K, Kaizu Y, Sawaya T, Watanabe J, Kawajiri K, Gotoh O, Fujii-Kuriyama Y. Xenobiotic responsive element in the 5′-upstream region of the human *P-450c* gene. *J. Biochem Tokyo* 1991: **110**, 232–236.

McLemore TL, Adelberg S, Liu MC, McMahon NA, Yu SJ, Hubbard WC, Czerwinski M, Wood TG, Storeng R, Lubet RA, *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 Cancer Inst* 1990: **82**, 1333–1339.

Nakachi K, Imai K, Hayashi S, Watanabe J, Kawajiri K. Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. *Cancer Res* 1991: **51**, 5177–5180.

Nakachi K, Imai K, Hayashi S, Kawajiri K. Polymorphisms of the *CYP1A1* and glutathione *S*-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Res* 1993: **53**, 2994–2999.

Nebert DW. Role of genetics and drug metabolism in human cancer risk. *Mutat Res* 1991: **247**, 267–281.

Nebert DW, Jones JE. Regulation of the mammalian cytochrome P1-450 (*CYP1A1*) gene. *Int J Biochem* 1989: **21**, 243–252.

Nebert DW, Puga A, Vasiliou V. Role of the Ah receptor and the dioxin-inducible [*Ah*] gene battery in toxicity, cancer, and signal transduction. *Ann NY Acad Sci* 1993: **685**, 624–640.

Persson I, Johansson I, Bergling H, Dahl ML, Seidegard J, Rylander R, Rannug A, Hogberg J, Sundberg MI. Genetic polymorphism of cytochrome P4502E1 in a Swedish population. Relationship to incidence of lung cancer. *FEBS Lett* 1993: **319**, 207–211.

Phillips DH, Hewer A, Martin CN, Garner RC, King MM. Correlation of DNA adduct levels in human lung with cigarette smoking. *Nature* 1988: **336**, 790–792.

Poland A, Glover E. Characterization and strain distribution pattern of the murine Ah receptor specified by the Ahd and Ahb-3 alleles. *Mol Pharmacol* 1990: **38**, 306–312.

Ramel C. Evolutionary aspects of human cancer. *Pharmacogenetics* 1992: **2**, 344–349.

Service RF. Stalking the start of colon cancer. *Science* 1994: **263**, 1559–1560.

Shields PG, Caporaso NE, Falk RT, Sugimura H, Trivers GE, Trump BF, Hoover RN, Weston A, Harris CC. Lung cancer, race, and a *CYP1A1* genetic polymorphism. *Cancer Epidemiol Biomarkers Prev* 1993: **2**, 481–485.

Swanson HI, Bradfield CA. The AH-receptor: genetics, structure and function. *Pharmacogenetics* 1993: **3**, 213–230.

Tefre T, Ryberg D, Haugen A, Nebert DW, Skaug V, Brogger A, Borresen AL. Human *CYP1A1* (cytochrome P(1)450) gene: lack of association between the *Msp* I restriction fragment length polymorphism and incidence of lung cancer in a Norwegian population. *Pharmacogenetics* 1991: **1**, 20–25.

Uematsu F, Kikuchi H, Motomiya M, Abe T, Sagami I, Ohmachi T, Wakui A, Kanamaru R, Watanabe M. Association between restriction fragment length polymorphism of the human cytochrome *P450IIE1* gene and susceptibility to lung cancer. *Jpn J Cancer Res* 1991: **82**, 254–256.

Uematsu F, Ikawa S, Kikuchi H, Sagami I, Kanamaru R, Abe T, Satoh K, Motomiya M, Watanabe M. Restriction fragment length polymorphism of the human *CYP2E1* (cytochrome-*P450IIE1*) gene and susceptibility to lung cancer – possible relevance to low smoking exposure. *Pharmacogenetics* 1994: **4**, 58–63.

Watanabe M, Watanabe K, Konno K, Sato H. Genetic differences in the induction of aryl hydrocarbon hydroxylase and benzo(a)pyrene carcinogenesis in C3H/He and DBA/2 strains of mice. *Gann* 1975: **66**, 217–226.

Weeda G, Hoeijmakers JH, Bootsma D. Genes controlling nucleotide excision repair in eukaryotic cells. *Bioessays* 1993: **15**, 249–258.

White R. Inherited cancer genes. *Curr Opin Genet Dev* 1992: **2**, 53–57.

Zacksenhaus E, Bremner R, Jiang Z, Gill RM, Muncaster M, Sopta M, Phillips RA, Gallie BL. Unraveling the function of the retinoblastoma gene. *Adv Cancer Res* 1993: **61**, 115–141.