The Role of Individual Susceptibility in Cancer Burden Related to Environmental Exposure

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Individual susceptibility to cancer may result from several host factors including differences in metabolism, DNA repair, altered expression of protooncogenes and tumor suppressor genes, and nutritional status. Since most carcinogens require metabolic activation before binding to DNA, variations in an individual's metabolic phenotype that have been detected in enzymes involved in activation and detoxification should play an essential role in the development of environmental cancer. This phenotypic metabolic variation has now been related to genetic polymorphisms, and many genes encoding carcinogen-metabolizing enzymes have been identified and cloned. Consequently, allelic variants or genetic defects that give rise to the observed variation and new polymorphisms have been recognized. Development of simple polymerase chain reaction (PCR)-based assays has enabled identification of an individual's genotype for a variety of metabolic polymorphisms. Thus, recent knowledge of the genetic basis for individual metabolic variation has opened new possibilities for studies focusing on increased individual susceptibility to environmentally induced cancer, which are reviewed with special reference to smoking-induced lung cancer. Cancer susceptibility due to chemical exposure is likely to be determined by an individual's phenotype for a number of enzymes (both activating and detoxifying) relevant to that of a single carcinogen or mixtures of carcinogens. Given the number and variability in expression of carcinogen-metabolizing enzymes and the complexity of chemical exposures, assessment of a single polymorphic enzyme (genotype) may not be sufficient. Mutations in the p53 gene are among the most common genetic changes in human cancer. The frequency and type of p53 mutations can act as a fingerprint of carcinogen exposure and may therefore provide information about external etiological agents, intensity of exposure, and host factors affecting the tumorigenesis process. In human lung cancer, p53 mutations (both the mutation pattern and frequency) have been linked with tobacco smoking; the type of mutation most frequently observed is G:C to T:A transversion, a mutation preferentially induced by benzo[*a*]pyrene diol epoxide. An association between the presence of this transversion and the genotype deficient in glutathione *S*-transferase M1-mediated detoxification has been observed in lung cancer. Taken together, these findings suggest that determination of metabolic at risk genotypes in combination with levels of DNA adducts in target (surrogate) tissues and the p53 mutation pattern should allow the identification of susceptible individuals and subgroups in carcinogen-exposed populations. — Environ Health Perspect 104(Suppl 3):569–577 (1996)

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Abbreviations used: AHH, aryl hydrocarbon hydroxylase; AhR, aromatic hydrocarbon receptor; Arnt, ah receptor nuclear translocator; B[*a*]P, benzo[*a*]pyrene; Cyps, cytochrome p450; *EPHX*, gene that encodes mEH; GST, glutathione *S*-transferase; *GSTM1*, glutathione *S*-transferase M1; *GSTT1*, glutathione *S*-transferase T1; mEH, microsomal epoxide hydrolase; MMR, mismatch repair; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PAHs, polycyclic aromatic hydrocarbons; PCR, polymerase chain reaction; RER, replication errors; RFLP, restriction fragment length polymorphism; SCE, sister chromatid exchanges; UGT, uridine diphosphate glucuronosyltransferase; XRE, xenobiotic responsive elements.

Cancer Susceptibility in Environmental Carcinogenesis

Epidemiological studies have estimated that up to 80 to 90% of all cancers are related to environmental factors, tobacco smoke, and diet (*1*). Tobacco use is unquestionably a major causative factor, accounting for about 30% of all cancer cases worldwide, especially lung cancer which is presently the most common malignancy in the world. Individual susceptibility to cancer may result from several host factors including differences in metabolism, DNA repair, altered expression of protooncogenes and tumor suppressor genes, and nutritional status (Figure 1) (*2*). Since most carcinogens require metabolic activation before binding to DNA, individual features of carcinogen metabolism play an essential role in the development of environmental cancer.

Variations in an individual's metabolic phenotype, i.e., phenotypic polymorphism, have been detected in a variety of enzymes involved in activation and detoxification of chemical carcinogens. This phenotypic metabolic variation has now been related to genetic polymorphisms. A growing number of genes encoding carcinogen-metabolizing enzymes have been identified and cloned. Consequently, there is increasing knowledge of the allelic variants or genetic defects that give rise to the observed variation. Development of rather simple new techniques such as polymerase chain reaction (PCR)-based assays has enabled precise identification of an individual's genotype for a variety of metabolic polymorphisms. Also, new polymorphisms have been recognized. Thus, recent knowledge of the genetic basis for individual metabolic variation has opened new possibilities for studies focusing on increased susceptibility to environmental cancer.

Many of the polymorphic genes of carcinogen metabolism show considerable ethnic differences in gene structure and allelic distribution (e.g., rare alleles, gene amplifications, and pseudogenes). Many of the first reports on genetic risk modification were from Japan, and only after several studies among various Caucasian populations has an estimate of allele frequencies, and thus of risk genotypes, been obtained. Remarkable variation in metabolic phenotypes and genotypes has been reported for different ethnic and geographic populations (*3–8*). The strong interethnic variation has been underlined as a major

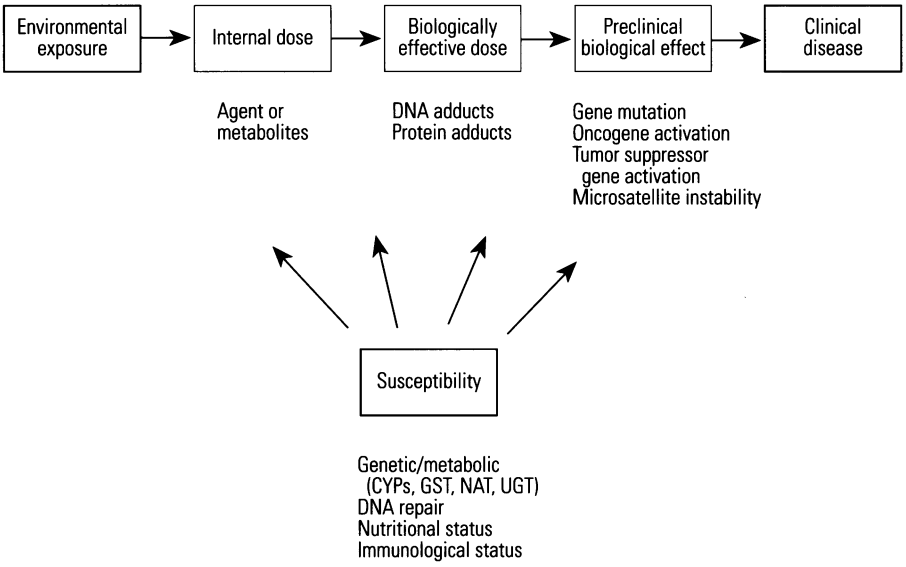

Figure 1. The association of environmental exposure to carcinogens with host factors modifying the susceptibility to adverse health effects. End points to monitor exposure, genetic predisposing alterations, and biological effectiveness of exposure, e.g. adduct formation, are indicated. Adapted from Perera and Santella (*2*).

obstacle for extrapolation of results between different ethnic groups (*4*).

Only a small number of studies have so far been focused on genotyping of the genes involved in the genetic regulation of carcinogen metabolism and on the analyses of combined genotypes in carcinogen metabolism. This review summarizes recent studies in this rapidly expanding field, which mostly concentrate on lung cancer in smokers. The references cited are not exhaustive, and the reader is referred to review articles (*9–11*).

Role of Metabolism and DNA Adducts in Chemical Carcinogenesis

DNA Adducts with Polycyclic Aromatic Hydrocarbons

The majority of human carcinogens do not produce their biological effects per se but require metabolic activation before they can interact with cellular macromolecules. Many compounds are converted to reactive electrophilic metabolites by the oxidative, mainly cytochrome P450-related enzymes (CYPs). A major representative of polycyclic aromatic hydrocarbons (PAHs) is benzo[*a*]pyrene (B[*a*]P), present in tobacco smoke and ambient air in industrialized areas. B[*a*]P is converted into phenolic metabolites such as 3-OH-B[*a*]P and B[*a*]P-7,8-diol, by a CYP-mediated process. Secondary metabolism, mainly involving epoxide hydrolase and another subset of CYP isoforms, leads to the formation of the highly reactive (+)-anti-B[*a*]P diol epoxide. This metabolite has been shown to bind to genomic DNA and activate oncogenes or other critical genes and it is likely to be a causative factor in several types of cancer (*12*). Using a new high-performance liquid chromatography (HPLC) fluorescence assay, the levels of specific (+)-anti B[*a*]P diol epoxide bound to DNA can be quantified through the release of B[*a*]P-tetrols (*13*) both from lung tissue DNA and lymphocyte DNA (*14*).

The formation of smoking-related DNA adducts in human lung tissue may be a good dosimetric exposure marker. Smokers have significantly elevated levels of aromatic or hydrophobic adducts compared with nonsmokers (*15–17*) (Table 1). In some cases it is evident that adduct levels are linearly related to total smoking exposure (*15,16*) but, in the case of lung cancer patients only, the shorter the period of smoking before cancer occurred, the higher the adduct level (*17*). Furthermore, adduct levels are higher in women's lung DNA, when figures are adjusted for smoking exposure, a result that suggests (along with preliminary epidemiological findings) that women are at increased risk of lung cancer from smoking, compared with men. The enhancing effect of smoking on anti-B[*a*]P diol epoxide–DNA levels in peripheral mononuclear cells from coke oven workers has been demonstrated (*18*). The ^{32}P-postlabeling technique gives an estimate of total aromatic adducts. Possible genotype dependence of DNA adducts, whether specific to B[*a*]P or bulky PAH adducts, is being investigated in lung tissue of lung cancer patients.

Tobacco-specific Nitrosamines

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a nicotine-derived tobacco-specific nitrosamine found in cigarette smoke, is a potent pulmonary carcinogen in rodents. NNK and PAHs are believed to be the major carcinogens responsible for lung cancer in smokers. NNK requires metabolic activation to bind to DNA and express its carcinogenic effects. Its metabolism includes α-hydroxylation, pyridine-*N*-oxidation and reduction to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and conjugation of NNAL to its glucuronide. NNAL and the corresponding glucuronide can be detected in human urine and are good exposure indicators of the tobacco-specific nitrosamine NNK.

Table 1. The metabolic and genotype parameters in lung cancer patients according to their smoking habits and *GSTM1* gene status.

Parameter	Smokers (46.6 ± 22.2 pack-years)		Ex-smokers (38.6 ± 24.2 pack-years)		Nonsmokers	
Genotype, *GSTM1*	Wild	Null	Wild	Null	Wild	Null
AHH (nmol/min/mg protein)	1.19 ± 1.33#	0.88 ± 1.26#	0.12 ± 0.15	0.045 ± 0.10*	0.08 ± 0.20	0.27 ± 0.08
Bulky DNA adducts in lung parenchyma (10^{-8} nucleotides)	8.7 ± 4.7#	9.9 ± 6.1#	1.4 ± 0.9	3.4 ± 1.4**	1.6 ± 0.9	1.6 ± 1.0
AUX/17U	9.2 ± 4.2	8.9 ± 3.4	5.4 ± 1.3	5.6 ± 2.7	5.4 ± 1.7	6.7 ± 3.0
17X + 17U/137X	10.8 ± 4.2	17.1 ± 5.3**	14.7 ± 8.4	15.4 ± 6.6	ND	ND

Abbreviations: wild, *GSTM1* positive; null, *GSTM1* negative (gene); AHH, aryl hydrocarbon hydroxylase; AUX, 1-methylxanthine, dimethyluric acid, and 5-acetylamino-6-formylamino-3-methyluracil; 17U, 1,7-dimethyluric acid; 17X, 1,7-dimethylxanthine; 137X, caffeine; ND, no data. Interim results from a collaborative study on Finnish lung cancer patients; $n = 89$. *$p < 0.05$; **$p < 0.01$ as compared to respective *GSTM1*-positive genotype; #$p < 0.01$ as compared to respective *GSTM1* genotype of ex-smokers or nonsmokers.

NNK-derived DNA adducts have only partially been characterized (*19*).

In humans, the balance between toxification and detoxification of NNK may be influenced by the individual's enzymatic capacity. By measuring a urinary metabolite of NNK, NNAL glucuronide, the latter could possibly serve as an index of an individual's activation/detoxification capacity. Because glucuronidation may exert genetic polymorphism (*20*), some smokers with higher glucuronidation capacity measurable by the ratio of NNAL glucuronide/NNAL may partially be protected against the carcinogenicity of NNK.

Endogenous Adducts

The exocyclic DNA adducts ethenodeoxyadenine and ethenodeoxycytosine have been found to be formed by the human carcinogen vinyl chloride and by urethane; they also can be formed from lipid peroxidation products such as *trans*-4-hydroxy-2-nonenal via epoxidation (*21–23*). Etheno adducts thus may serve as a DNA marker of oxidative stress. After development of a highly specific and ultrasensitive assay, these etheno adducts can now be detected with the sensitivity of 4 adducts/10^{10} normal nucleotides (*24*). Recently, a close correlation has been observed between aliphatic epoxide-induced sister chromatid exchanges (SCEs) in cultured human lymphocytes and *GSTT1* polymorphism. The null genotype had higher induced, as well as background, frequencies of SCEs (*25,26*). Because mutagenic epoxides produced from lipid peroxidation products could be substrates for *GSTT1* or *M1*, it is conceivable that the level of etheno–DNA adducts (and the resulting frequency of point mutations) is effected by these polymorphic detoxifying enzymes.

Human Genes Associated with the Metabolism of Carcinogens

CYP1A1

CYP1A1 is well conserved among the xenobiotic-metabolizing enzymes. In human lung tissue of smokers, the level of B[*a*]P diol epoxide adducts and total aromatic DNA adducts were significantly positively correlated with CYP1A1 expression or B[*a*]P-hydroxylase or aryl hydrocarbon hydroxylase (AHH) enzyme activity (*13*). Several studies have indicated an association of the genetic polymorphism of *CYP1A1* and cancer. A co-segregation of the CYP1A1 phenotype and polymorphism of the *Msp*I restriction site in the *CYP1A1* gene was discovered (*27*), but this discovery was challenged later (*28*). Thus the association between the mutant *CYP1A1* alleles and CYP1A1 functional activity is not clear at the moment, but recent studies indicate that variant alleles at the *Msp*I site in exon 7 could result in a more active CYP1A1 enzyme (*29,30*). A significant correlation in a Japanese population between susceptibility to lung cancer and homozygosity for the rare *Msp*I allele was reported by Kawajiri et al. (*31*) and Nakachi et al. (*32*). Another closely linked polymorphism, a point mutation resulting in an amino acid substitution (Ile-Val) in the heme-binding region of the CYP1A1 protein was found by Hayashi et al. (*33*). This genotype results in an altered enzyme activity and was shown to be associated with squamous cell and small cell types of lung cancer (*34*). There are significant ethnic differences in the frequency of *CYP1A1* alleles, and both the m2 and *Val* alleles appear to be rare in Caucasians (*5,35,36*). This requires more follow-up studies involving more cancer patients and controls to unmask the association in Caucasians.

CYP1A2

The CYP1A2 isoform is predominantly expressed in liver and it activates a large number of dietary and environmental procarcinogens. This isoform is expressed in all studied human livers. To date, no genetic polymorphism has been found, but phenotypic polymorphism has been demonstrated using caffeine as a probe drug (*37*).

CYP2A6

In humans, CYP2A6 isoforms are mediators of 7-hydroxylation of coumarin, a component of cigarette smoke, certain alcoholic beverages, and a common constituent of various plants. CYP2A6 is also known to be capable of activating several other nitrosamines present in tobacco smoke and in the diet. The *CYP2A* gene cluster has been recently characterized (*38*). There are three functional genes in the subfamily, i.e., *CYP2A6, CYP2A7,* and *CYP2A12,* and two pseudogenes have been found. Two different variant alleles of the *CYP2A6* gene were identified (*CYP2A6v1* and *CYP2A6v2*). Thus, by developing a PCR-based method to detect *CYP2A6*-poor metabolizers, population studies should be able to assess the role of this polymorphism in tobacco smoke-caused lung cancer risk. This is especially relevant because CYP2A6 mediates the activation of NNK (*39*).

CYP2E1

The ethanol-inducible CYP2E1 metabolizes several known and suspected chemical carcinogens including *N*-nitrosamines. Genetic polymorphisms of the *CYP2E1* gene have been shown to be associated with human cancer. In a Japanese study (*40*), two different alleles for the *CYP2E1* gene were observed with the *Dra*I restriction enzyme. The distribution of the corresponding genotypes among lung cancer cases was significantly different from that among controls, especially the homozygous rare genotype that was absent in the lung cancer group. No difference in the genotype frequencies was found between patients with other cancers and controls (*40*).

Subsequent studies have revealed profound ethnic differences in the frequencies of the polymorphic alleles. For example, in contrast to the Japanese findings, Kato et al. (*41*) studied a group of 128 mostly Caucasian lung cancer patients and found no association between the *Rsa*I genotypes and lung cancer risk. However, a significant association between the defective alleles of the *CYP2E1* gene promoter region (*Rsa*I) and lung cancer risk was shown in a Swedish study (*42*). These contradictory results need to be verified in studies with more statistical power from various ethnic populations.

CYP3A4

The CYP3A4 isoform has been shown to activate numerous important procarcinogens such as B[*a*]P. Although the three different *CYP3A* genes (*3A4, 3A5,* and *3A7*) are expressed at widely varying levels among individuals, polymorphism for only *CYP3A4* and *3A5* has been found to date. Several allelic variants of the *CYP3A4* gene were recently reported by Peyronneau et al. (*43*). Therefore, the distribution of the different alleles in lung cancer patients and controls should be investigated. *CYP3A5,* which is polymorphically expressed in the liver, has been found in human lungs (*44*).

AhR and *Arnt* Genes

The induction of CYP1A1 is initiated by the specific binding of PAH compounds to a soluble intracellular protein, the aromatic hydrocarbon receptor (AhR) (*45*). Hankinson and coworkers (*46*) have recently cloned a gene involved in the CYP1A1 induction pathway, the Ah receptor nuclear translocator (*Arnt*) gene, and

they have identified an *Msp*I restriction fragment length polymorphism (RFLP) in the human gene (*47*). The allele frequencies at the *Arnt* RFLP are 0.62 and 0.38 for the A_1 and A_2 alleles, respectively. The liganded nuclear form of the AhR complex stimulates transcription of the *CYP1A1* gene via interaction with specific DNA sequences, xenobiotic responsive elements (XRE). Hayashi et al. (*48*) found that the expression level of *CYP1A1* was associated with those of AhR and Arnt mRNAs and also that the expression of AhR and Arnt was influenced by cigarette smoking.

Glutathione *S*-transferases

Several carcinogens present in the diet and tobacco smoke are inactivated by glutathione *S*-transferases (GSTs). The known substrates for GSTs in cigarette smoke are those derived in bioactivation from PAH. The most studied carcinogenic PAH diol epoxide, B[*a*]P 7,8-diol-9,10-epoxide, is a relatively good substrate for many forms such as *GSTM1, M2,* and *M3,* and better still with *GSTP1* (*49*).

The genetic polymorphism of the *GSTM1* gene that encodes the glutathione *S*-transferase M1 enzyme is a result of a homozygous deletion of the entire *GSTM1* gene locus (*50*). The *GSTM1* gene locus contains three alleles, i.e., the *GSTM1A* and *GSTM1B* alleles, which differ by a single amino acid, and a deficient *GSTM1* null allele. About 50% of the Caucasian population inherits two deficient alleles (i.e., they are homozygous for the null allele of the gene) and are thus devoid of GSTM1 activity. The *GSTM1* null genotype frequency has been reported to show marked ethnic variation (*51*). Individuals lacking GSTM1 could be at a greater risk for developing lung cancer due to deficient detoxification processes; this notion is supported by recent studies (*52*). In persons who lack the *GSTM1* gene, activation of carcinogens in tobacco smoke (e.g., B[*a*]P) appears to be increased, while the efficacy of detoxification is limited both qualitatively (absence of GSTM1-1 enzyme and low expression of GSTM3-3 enzyme) and quantitatively (low overall GST activity). This was confirmed by biochemical studies (Table 1). The metabolic activity (AHH activity, level of bulky PAH–DNA adducts in lung parenchyma) was measured in Finnish lung cancer patients divided according to their smoking habits and *GSTM1* genotype (*9*). AHH activity was highest in smokers, independent of the *GSTM1* genotype; also, the amount of DNA adducts was highest in smokers (Table 1). When smokers and ex-smokers were grouped according to *GSTM1* gene status, smokers with a nulled *GSTM1* gene had about 10% more bulky PAH–DNA adducts in lung parenchyma, whereas ex-smokers with this gene defect had a 2.4-fold excess of these DNA lesions. An independent study (*17*) also reported an excess of individuals with *GSTM1* deficiency with high adduct levels in their lung tissue among male lung cancer patients. In our study (Table 1), one other parameter was determined: cytochrome P4501A2-catalyzed activity measurable in the urine by the use of caffeine as a probe drug (*53*). Kadlubar et al. (*53*) compared the ratio of 1,7-dimethylxanthine + 1,7-dimethyluric acid/caffeine (17X + 17U/137X) and the ratio of 1-methylxanthine + 5-acetylamino-6-formylamino-3 methyluracil/1,7-dimethylxanthine + 1-dimethyluric acid, showing that the former parameter is a better indicator of this enzyme activity. Our study showed a significant difference ($p < 0.01$) in this parameter, best representing *CYP1A2* activity between *GSTM1* positive (wild type) and mutated (null) gene in smokers (Table 1). This suggests a clustering of metabolic parameters leading to increased adduct formation, although in this study only about 10% more adducts were found in this group (null *GSTM1*) of smokers. Alternatively or additionally, the *GSTM1* gene status may profoundly affect the metabolism and excretion of caffeine metabolites, thus altering the ratio of 17X + 17U/137X.

The first epidemiological studies appeared to confirm a relationship between *GSTM1* deficiency and lung cancer risk (*54,55*). There are, however, several putative confounding factors that are known to affect the phenotype such as environmental exposures, nutrition, and differences in smoking habits (*56*). In recent genotyping studies using PCR assays (*51*), no association has been found between null genotype and lung adenocarcinoma, but a tendency for an association between the *GSTM1* genotype and squamous cell carcinoma has been reported (*57–59*).

GSTT1

Recently a GST null phenotype unrelated to the *GSTM1* was described for the glutathione-dependent detoxification of naturally occurring monohalomethanes. In human erythrocytes the monohalomethanes are detoxified by conjugation with glutathione (*60–62*). About 60 to 80% of the human population is able to carry out this metabolic reaction, whereas the remainder is unable to do so (*61*). Further characterization of this phenotype showed that glutathione conjugation of the industrially used chemicals dichloromethane and ethylene oxide (which is also a metabolic product of ethylene in animals and humans) could only be catalyzed by blood samples from the conjugator population (*63*). However, positive conjugator status is not necessarily beneficial because conjugation of monohalomethanes and ethylene oxide is detoxifying, whereas conjugation of dichloromethane yields a mutagenic metabolite (*64*). Given that monohalomethanes, ethylene oxide, and dichloromethane and other man-made alkyl halides have wide industrial uses, any polymorphic locus that may be involved in their metabolism would have epidemiological interest. In studies on smoking-related cancers, *GSTT1* polymorphism is of particular interest because monohalomethanes are present in tobacco smoke (*65*).

EPHX

Human microsomal epoxide hydrolase (mEH) is an important biotransformation enzyme that metabolizes reactive epoxide intermediates to more water-soluble *trans*-dihydrodiol derivatives (*66,67*). Substrates for the enzyme include epoxides of environmental toxins such as the carcinogenic PAHs, aromatic amines, and benzene (*67–69*). Frequently, the metabolism of epoxide-containing compounds by mEH results in the production of inherently less reactive and less toxic intermediates (*66,70*). However, in certain instances, notably in concert with oxidative metabolism by the cytochrome P450s, hydrolysis of particular PAH epoxides by mEH can lead to the formation of highly electrophilic and mutagenic diol epoxides (*67,69*). The gene encoding mEH (*EPHX*) is inducible by certain chemicals. Recently certain allelic variants of the *EPHX* gene, encoding different combinations of amino acid residues at positions 113 and 139 in mEH protein, were shown to directly influence enzyme activity, possibly by affecting protein stability (*71*). Therefore, it is reasonable to postulate that individuals with specific allelic combinations, especially at homozygous state, may be at differential risk for the ability to metabolize reactive epoxides efficiently.

Uridine Diphosphate Glucuronosyltransferases

The uridine diphosphate glucuronosyltransferases (UGTs) comprise a family of

isoforms. It is known that at least four cDNA-expressed human hepatic UGTs hydroxylate glucuronidase derivatives of the model carcinogens B[*a*]P and 2-acetylaminofluorene (*72*). Recently, there has been considerable progress in the molecular genetics of this enzyme family (*73*). Nine human cDNAs have been cloned to date, and they have been classified into two families, UGT1 and UGT2, on the basis of the similarity of their deduced amino acid sequences (*74*). Evidence for variability in the general population has been obtained, but no genetic polymorphism of the *UGT* genes has so far been observed (*75*). The data based on phenotyping analysis of NNK metabolites, NNAL and NNAL glucuronides, in the urine of smokers suggest the presence of polymorphism in UGT (*20*).

Susceptible Genotypes and Genetic Alterations in Lung Cancer

Genotypes and Lung Cancer Risk

Cancer susceptibility due to chemical exposure is likely to be determined by an individual's phenotype for a number of enzymes, both activating and detoxifying, relevant to that of single carcinogens or mixtures of carcinogens. Given the number and variability in expression of carcinogen-metabolizing enzymes now identified and the complexity of chemical exposures, assessment of a single polymorphic enzyme or genotype may not be sufficient. Several recent reports have evaluated effects of combinations of the risk genotypes on cancer susceptibility. Hayashi et al. (*34*) described a 5.8-fold relative risk for all lung cancer types and a 9.1-fold relative risk for squamous cell carcinoma in Japanese individuals who were homozygous both for the *CYP1A1 Val* and *GSTM1* null risk alleles. In a recent study by Nakachi et al. (*32*), 85 patients with squamous cell lung carcinoma were genotyped for *CYP1A1* and *GSTM1* alleles. Individuals with the susceptible *CYP1A1 Msp*I genotype combined with deficient GSTM1 were at a remarkably high risk of developing the carcinoma with an odds ratio of 16. The risk was even higher in individuals who had the other susceptible genotype of *CYP1A1* (*Val/Val*) combined with *GSTM1* null genotype (OR = 41). The great differences in these odds ratios suggest that the *CYP1A1* allelic defects leading to the risk genotypes affect the function of the *CYP1A1* gene in a distinct way. These findings are consistent with the notion that some procarcinogens in cigarette smoke are activated by CYP1A1 and inactivated by GSTM1 enzymes. The rare occurrence of the *CYP1A1 Msp*I m2 allele in the Caucasian population (*35,36*) precludes any conclusions about the extent of risk modification by the genotype homozygous for both *CYP1A1* m2 and *GSTM1* null alleles in lung cancer in Caucasian populations and thus has to be clarified further.

DNA Adducts and Cancer Susceptibility Genes

A number of studies have tried to relate metabolic phenotype or, more recently, genotype to cancer risk. These efforts are presently extended to studies on various other biomarkers of cancer such as markers of exposure and (early) effects that included DNA adducts, urinary mutagenicity, cytogenetic damage, and p53 mutations.

In a genotyping study, no correlation was found between the homozygous risk *Msp*I genotype or amino acid replacement genotype of *CYP1A1* and DNA adducts in smokers (*5*); in a phenotype study, however, a correlation between aromatic lung DNA adducts and CYP1A1 activity among smokers was observed (*13,76*). Unexpectedly an association of the *CYP1A1* genotype heterozygous for the rare risk allele (m1/m2) with low adduct levels in white blood cell DNA was observed among chimney sweeps in Sweden (*77*). In the same study, an increased level of adducts was detected in the *GSTM1* null individuals. Also, a Norwegian report has indicated that mutations detected in the tumor suppressor gene p53 in lung tumors from *GSTM1* null patients who smoked were more frequently the type experimentally known to be caused by (+)-anti-B[*a*]P diol epoxide (*78*).

Genetic Alterations

The tumor suppressor gene p53 encodes a nuclear protein that has several biological functions including cell cycle control and DNA repair and replication. This protein has been suggested to have a role in early response to cellular DNA damage. Mutations in the p53 gene are among the most common genetic changes in human cancer and are found in more than 50% of all cancers. p53 mutations have also been shown to have important clinical implications. The frequency and type of p53 mutations can act as a fingerprint of carcinogen exposure and may provide information about external etiological agents and internal factors affecting the tumorigenesis process (*79,80*). In human lung cancer, p53 mutations (both the mutation pattern and frequency) have been linked with tobacco smoking (*78,81–83*). The type of mutation most frequently observed in lung cancer is G:C to T:A transversion (*80*), a type of mutation preferentially induced in experimental systems by (+)-anti-B[*a*]P diol epoxide. Furthermore, an association between the genotype deficient in GSTM1-mediated detoxification and presence of G:C to T:A transversions has been observed in lung cancer patients (*78*). These findings suggest that investigation of p53 mutation patterns in relation to metabolic at-risk genotypes and levels of DNA adducts in lung tissue will provide valuable information for understanding mechanisms of pulmonary carcinogenesis.

Alterations in microsatellite sequences (simple sequence repeats) of the human genome were originally observed in sporadic and hereditary forms of colon cancer (*84,85*). Since the discovery, a variety of human tumors including small cell and non-small cell lung cancer have been found to contain similar instability of microsatellite sequences (*86–88*). Somatic and germline mutations of the mismatch repair (MMR) genes have been found in the patients with tumors showing replication errors (RER) (*89,90*). The present data suggest that, although many sporadic tumors have mutations in MMR genes, microsatellite instability has been observed in many tumors without such mutations and is therefore probably due to other alterations (*91*). Similarly, it has been shown that various forms of genetic instability are increased in frequency in cells that lack a normal p53 gene; as a consequence, additional genetic alterations may result (*92*). Studies on somatic microsatellite instability in lung cancer in relation to p53 mutations and possible polymorphisms in mismatch repair genes should open new approaches to identify high-risk subjects.

Chromosomal Mapping of Loci Affecting Predisposition to Lung Cancer

Besides genes affecting carcinogen metabolism, other genes appear to affect inherited predisposition to lung cancer (*93,94*). On mouse chromosome 8, an important locus affecting inherited predisposition to lung cancer in a region homologous to the human 12p12 has recently been mapped (*94,95*). Sellers et al. (*96*) suggested that, in humans, the pattern of lung cancer is

best explained by Mendelian co-dominant inheritance of a single autosomal locus which is expressed only in the presence of tobacco smoke and that influences the age at onset of lung cancer. However, lung cancers do not show familial clustering of cases, indicating a possible low penetrance or a multigenic nature of the lung cancer predisposition trait. Even if rare pedigrees of lung cancer could be identified, genetic linkage study in these pedigrees would be very difficult to carry out, considering the poor prognosis of lung cancer. Nevertheless, genetic linkage studies may also be performed in affected sibling pairs (*97*), and this may represent a feasible alternative approach to identify chromosomal locations of lung cancer susceptibility genes in humans. Genetic linkage studies have constituted an important approach to study the genetics of diseases through the identification of the number and chromosomal location of loci affecting a disease (*98,99*). The chromosomal mapping of a disease loci allowed the subsequent cloning of disease genes whose germ-line mutations cause inherited predisposition to common tumor types (colon carcinoma, breast cancer, etc.). Thus, genetic linkage studies in lung cancer pairs of siblings and of second degree relatives may facilitate finding the chromosomal location of loci affecting inherited predisposition to lung cancer.

Perspectives

Recent knowledge of the genetic basis for individual metabolic variation has opened new possibilities for studies focusing on increased individual susceptibility to environmentally induced cancer, and the development of simple PCR-based assays has enabled the identification of an individual's genotype for a variety of metabolic polymorphisms. Cancer susceptibility due to chemical exposure is likely to be determined by an individual's phenotype for a number of enzymes, both activating and detoxifying, relevant to that of a single carcinogen or mixtures of carcinogens. Given the number and variability in expression of carcinogen-metabolizing enzymes and the complexity of chemical exposures, assessment of a single polymorphic enzyme (genotype) may not be sufficient, and the establishment of a risk profile for each individual or subgroup seems to be required. Mutations in the p53 gene are among the most common genetic changes in human cancer. The frequency and type of p53 mutations can act as a fingerprint of carcinogen exposure and may therefore provide information on external etiological agents, intensity of exposure, and host factors affecting the tumorigenesis process. Given the rapid advances in methodology, the determination of metabolic at-risk genotypes in combination with levels of DNA adducts in target (surrogate) tissues and p53 mutation patterns should allow identification of susceptible individuals/subgroups in carcinogen-exposed populations. Once identified, these high-risk subjects might be persuaded more easily to stop their (smoking) habits or to avoid hazardous exposure, or in the case of smokers, it might be possible to offer an intensive or personalized smoking cessation program.

REFERENCES

1. Doll R, Peto R. The causes of cancer. Quantitative estimates of avoidable risks of cancer in the United States today. Oxford:Oxford University Press, 1981.
2. Perera FP, Santella R. Carcinogenesis. In: Molecular Epidemiology, Principle and Practices (Schulte PA, Perera FP, eds). San Diego:Academic Press, 1993;277–300.
3. Butler MA, Lang NP, Young JF, Caporaso NE, Vineis P, Hayes RB, Teitel CH, Massengill JP, Lawsen MF, Kadlubar FF. Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. Pharmacogenetics 2:116–127 (1992).
4. Ingelman-Sundberg M, Johansson I, Persson I, Yue Q-Y, Dahl ML, Bertilsson L, Sjöqvist F. Genetic polymorphism of cytochromes P450: interethnic differences and relationship to incidence of lung cancer. Pharmacogenetics 2:264–271 (1992).
5. Shields PG, Sugimura H, Caporaso NE, Petruzzelli SF, Bowman ED, Trump BF, Weston A, Harris CC. Polycyclic aromatic hydrocarbon–DNA adducts and the CYP1A1 restriction fragment length polymorphism. Environ Health Perspect 98:191–194 (1992).
6. Bell DA, Taylor JA, Butler MA, Stephens E, Wiest J, Brubaker LH, Kadlubar FF, Lucier GW. Genotype/phenotype discordance for human arylamine N-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. Carcinogenesis 14:1689–1692 (1993).
7. Bertilsson L, Dahl M-L, Sjöqvist F, Åberg-Wistedt A, Humble M, Johansson I, Lundqvist E, Ingelman-Sundberg M. Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine [Letter]. Lancet 341:63 (1993).
8. Lin HJ, Han C-Y, Lin BK, Hardy S. Slow acetylator mutations in the human polymorphic *N*-acetyltransferase gene in 786 Asians, blacks, Hispanics and whites: application to metabolic epidemiology. Am J Hum Genet 52:827–834 (1993).
9. Bartsch H, Rojas M, Alexandrov K, Camus A-M, Castegnaro M, Malaveille C, Anttila S, Hirvonen K, Husgafvel-Pursiainen K, Hietanen E, Vainio H. Metabolic polymorphism affecting DNA binding and excretion of carcinogens in humans. Pharmacogenetics 5:S84–S90 (1995).
10. Kaderlik KR, Kadlubar FF. Metabolic polymorphisms and carcinogen–DNA adduct formation in human populations. Pharmacogenetics 5:S108–S117 (1995).
11. Rannug A, Alexandrie A-K, Petersson I, Ingelman-Sundberg M. Genetic polymorphism of cytochromes P450 1A1, 2D6 and 2E1: regulation and toxicological significance. J Occup Environ Med 37:25–36 (1995).
12. Pelkonen O, Nebert DW. Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. Pharmacol Rev 34:189–222 (1982).
13. Alexandrov K, Rojas M, Geneste O, Castegnaro M, Camus AM, Petruzzelli S, Giuntini C, Bartsch H. An improved fluorometric assay for dosimetry of benzo(*a*)pyrene diol-epoxide-DNA adducts in smoker's lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. Cancer Res 52:6248–6253 (1992).
14. Rojas M, Alexandrov K, van Schooten F-J, Hillebrand M, Kriek E, Bartsch H. Validation of a new fluorometric assay for benzo(*a*)pyrene diolepoxide–DNA adducts in human white blood cells: comparisons with ^{32}P-postlabelling and ELISA. Carcinogenesis 15:557–560 (1994).
15. Phillips DH, Hewer A, Martin CN, Garner RC, King MM. Correlation of DNA adduct levels in human lung with cigarette smoking. Nature 336:790–792 (1988).
16. Phillips DH, Schoket B, Hewer A, Bailey E, Kostic S, Vincze I. Influence of cigarette smoking on the levels of DNA adducts in human bronchial epithelium and white blood cells. Int J Cancer 46:569–575 (1990).
17. Ryberg D, Hewer A, Phillips DH, Haugen A. Different susceptibility to smoking-induced DNA damage among male and female lung cancer patients. Cancer Res 54:5801–5803 (1994).

18. Rojas M, Alexandrov K, Auburtin G, Wastiaux-Denamur A, Mayer L, Mahieu B, Sebastien P, Bartsch H. Anti-benzo[*a*]pyrene diol epoxide–DNA adduct levels in peripheral mononuclear cells from coke oven workers and the enhancing effect of smoking. Carcinogenesis 16:1373–1376 (1995).
19. Hoffmann D, Hecht SS. Advances in tobacco carcinogenesis. In: Chemical Carcinogenesis and Mutagenesis I (Cooper CS, Grover PL, eds). Berlin, Heidelberg:Springer, 1990;63–103.
20. Hecht SS, Carmella SG, Murphy SE, Idris AM, Richie JP Jr, Hoffmann D. Recent studies on tobacco-specific nitrosamines [Abstract]. In: Proceedings of the XVI International Cancer Congress, 30 October–5 November 1994, New Delhi, India. New Delhi:Allied Publishers Ltd., 1994;222.
21. Sodum RS, Chung F.-L. 1,N^2-Ethenodeoxyguanosine as a potential marker for DNA adduct formation by *trans*-4-hydroxy-2-nonenal. Cancer Res 48:320–323 (1988).
22. Bartsch H, Barbin A, Marion M-I, Nair J, Guichard Y. Formation, detection, and role in carcinogenesis of ethenobases in DNA. Drug Metab Rev 26:349–369 (1994).
23. El Ghissassi F, Barbin A, Nair J, Bartsch H. Formation of 1,N^6-ethenoadenine and 3,N^4-ethenocytosine by lipid peroxidation products and nucleic acid bases. Chem Res Toxicol 8:278–283 (1995).
24. Nair J, Barbin A, Bartsch, H. 1,N^6-ethenodeoxyadenosine and 3,N^4-ethenodeoxycytidine in liver DNA from humans and untreated rodents detected by immunoaffinity/^{32}P-postlabelling. Carcinogenesis 16:613–617 (1995).
25. Norppa H, Hirvonen A, Järventaus H, Uusküla M, Tasa G, Ojajärvi A, Sorsa M. Role of *GSTT1* and *GSTM1* genotypes in determining individual sensitivity to sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes. Carcinogenesis 16:1261–1264 (1995).
26. Wiencke JK, Pemble S, Ketterer B, Kelsey KT. Gene deletion of glutathione *S*-transferase θ: correlation with induced genetic damage and potential role in endogenous mutagenesis. Cancer Epidemiol Biomarkers Prev 4:253–259 (1995).
27. Petersen DD, McKinney CE, Ikeya K, Smith HH, Bale AE, McBride OW, Nebert DW. Human *CYP1A1* gene: cosegregation of the enzyme inducibility phenotype and an RFLP. Am J Hum Genet 48:720–725 (1990).
28. Wedlund PJ, Kimura S, Gonzalez FJ, Nebert DW. 1462V mutation in the human *CYP1A1* gene: lack of correlation with either the *Msp* I 1.9 kb (M_2) allele or CYP1A1 inducibility in a three-generation family of east Mediterranean descent. Pharmacogenetics 4:21–26 (1994).
29. Crofts F, Taioli E, Trachman J, Cosma GN, Currie D, Toniolo P, Garte SI. Functional significance of different human *CYP1A1* genotypes. Carcinogenesis 15:2961–2963 (1994).
30. Landi MT, Bertazzi PA, Shields PG, Clark G, Lucier GW, Garte SJ, Cosma G, Caporaso NE. Association between *CYP1A1* genotype, mRNA expression and enzymatic activity in humans. Pharmacokinetics 4:242–246 (1994).
31. 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 263:131–133 (1990).
32. 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 53:2994–2999 (1993).
33. Hayashi SI, Watanabe J, Nakachi K, 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).
34. Hayashi S, Watanabe J, Kawajiri K. High susceptibility to lung cancer analyzed in terms of combined genotypes of *P450IA1* and mu-class glutathione *S*-transferase genes. Jpn J Cancer Res 83:866–870 (1992).
35. Tefre T, Ryberg D, Haugen A, Nebert DW, Skaug V, Brogger A, Borresen AL. Human *CYP1A1* (cytochrome P1450) gene: lack of association between the *Msp*I restriction fragment length polymorphism and incidence of lung cancer in a Norwegian population. Pharmacogenetics 1:20–25 (1991).
36. Hirvonen A, Husgafvel-Pursiainen K, 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 1:485–489 (1992).
37. Kalow W, Tang BK. The use of caffeine for enzyme assays: a critical appraisal. Clin Pharmacol Ther 53:503–514 (1993).
38. Fernandez-Salquero P, Gonzalez FJ. The *CYP2A* gene family: species differences, regulation, catalytic activities and role in carcinogenesis. Pharmacogenetics 5:S123–128 (1995).
39. Tiano HF, Wang R-L, Hosokawa M, Crespi C, Tindall KR, Langenbach R. Human CYP2A6 activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK): mutational specificity in the *gpt* gene of AS52 cells. Carcinogenesis 15:2859–2866 (1994).
40. 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 82:254–256 (1991).
41. Kato S, Shields PG, Caporaso NE, Hoover RN, Trump BF, Sugimura H, Weston A, Harris CC. Cytochrome *P450IIE1* genetic polymorphisms, racial variation, and lung cancer risk. Cancer Res 52:6712–6715 (1992).
42. Persson I, Johansson I, Bergling H, Dahl ML, Seidegård J, Rylander R, Rannung A, Högberg J, Ingelman-Sundberg M. Genetic polymorphism of cytochrome *P4502E1* in a Swedish population. Relationship to incidence of lung cancer. FEBS Lett 319:207–211 (1993).
43. Peyronneau MA, Renaud JP, Jaouen M, Urban P, Cullin C, Pompon D, Mansuy D. Expression in yeast of three allelic cDNAs coding for human liver P-450 3A4. Different stabilities, binding properties and catalytic activities of the yeast-produced enzymes. Eur J Biochem 218:355–361 (1993).
44. Kivistö KT, Griese E-U, Fritz P, Linder A, Hakkola J, Raunio H, Beaune P, Kroemer HK. Expression of cytochrome P4503A enzymes in human lung: a combined RT-PCR and immunohistochemical analysis of normal tissue and lung tumours. Naunyn-Schmiederberg's Arch Pharmacol 353:207–212 (1996).
45. Landers JP, Bunce NJ. The Ah receptor and the mechanism in dioxin toxicity. Biochem J 276:273–287 (1991).
46. Hoffman EC, Reyes H, Chu FF, Sander F, Conley LH, Brooks BA, Hankinson O. Cloning of a factor required for the activity of the Ah (dioxin) receptor. Science 252:954–958 (1991).
47. Johnson BS, Brooks BA, Reyes H, Hoffman EC, Hankinson O. An *Msp*I RFLP in the human *ARNT* gene, encoding a subunit of the nuclear form of the Ah (dioxin) receptor. Human Mol Genet 1:351 (1992).
48. Hayashi SI, Watanabe J, Nakachi K, Eguchi H, Gotoh O, Kawajiri K. Interindividual difference in expression of human Ah receptor and related P450 genes. Carcinogenesis 15:801–806 (1994).
49. Coles B, Ketterer B. The role of glutathione and glutathione transferase in chemical carcinogenesis. CRC Crit Rev Biochem Mol Biol 25:47–70 (1990).
50. Seidegård J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on *trans*-stilben oxide are due to a gene deletion. Proc Natl Acad Sci USA 85:7293–7297 (1988).
51. Bell DA, Thompson CL, Taylor J, Miller CR, Perera F, Hsieh LL, Lucier GW. Genetic monitoring of human polymorphic cancer susceptibility genes by polymerase chain reaction: application to glutathione transferase μ. Environ Health Perspect 98:113–117 (1992).
52. Nakajima T, Elovaara E, Anttila S, Hirvonen A, Camus A-M, Hayes JD, Ketterer B, Vainio H. Expression and polymorphism of glutathione *S*-transferase in human lungs: risk factors in smoking-related lung cancer. Carcinogenesis 16:707–711 (1995).

53. Kadlubar FF, Butler MA, Kaderlik KR, Chou H-C, Lang NP. Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. Environ Health Perspect 98:69–74 (1992).
54. Seidegård J, Pero RW, Miller DG, Beattie EJ. A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. Carcinogenesis 7:751–753 (1986).
55. Seidegård J, Pero RW, Markowitz MM, Roush G, Miller GD, Beattie EJ. Isozyme(s) of glutathione transferase (class mu) as a marker for the susceptibility to lung cancer: a follow up study. Carcinogenesis 11:33–36 (1990).
56. Ketterer B, Harris JM, Talaska G, Meyer DJ, Pemble SE, Taylor JB, Lang NP, Kadlubar FF. The glutathione *S*-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. Environ Health Perspect 98:87–94 (1992).
57. Zhong S, Howie AF, Ketterer B, Taylor J, Hayes JD, Beckett GJ, Wathen CG, Wolf CR, Spurr NK. Glutathione *S*-transferase mu locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. Carcinogenesis 12:1533–1537 (1991).
58. Brockmöller J, Kerb R, Drakoulis N, Nitz M, Roots I. Genotype and phenotype of glutathione *S*-transferase class μ isoenzymes μ and ψ in lung cancer patients and controls. Cancer Res 53:1004–1011 (1993).
59. Hirvonen A, Husgafvel-Pursiainen K, Anttila S, Vainio H. The *GSTM1* null genotype as a potential risk modifier for squamous cell carcinoma of the lung. Carcinogenesis 14:1479–1481 (1993).
60. Redford-Ellis M, Govenlock AH. Studies on the reaction of chloromethane with human blood. Acta Pharmacol Toxicol 30:36–48 (1971).
61. Peter H, Deutschmann S, Reichel C, Hallier E. Metabolism of methyl chloride by human erythrocytes. Arch Toxicol 63:351–355 (1989).
62. Hallier E, Deutschmann S, Reichel C, Bolt HM, Peter H. A comparative investigation of the metabolism of methyl bromide and methyl iodide in human erythrocytes. Int Arch Occup Environ Health 62:221–225 (1990).
63. Thier R, Föst U, Deutschmann S, Schröder KR, Westphal G, Hallier E, Peter H. Distribution of methylene chloride in human blood. Arch Toxicol 14:254–258 (1991).
64. Thier R, Taylor JB, Pemble SE, Griffith-Humphreys W, Persmark M, Ketterer B, Guengerich FP. Expression of mammalian glutathione S-transferase 5-5 in *Salmonella typhimurium* TA 1535 leads to base-pair mutations upon exposure to dihalomethanes. Proc Natl Acad Sci 90:8576–8580 (1993).
65. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB. Human glutathione *S*-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300:271–276 (1994).
66. Guengerich FP. Epoxide hydrolase: properties and metabolic roles. Rev Biochem Toxicol 4:5–30 (1982).
67. Jerina DM. Metabolism of aromatic hydrocarbons by the cytochrome P-450 system and epoxide hydrolase. Drug Metab Dispos 11:1–4 (1983).
68. Gonzalez FJ, Samore M, McQuiddy P, Kasper CB. Effects of 2-acetaminofluorene and *N*-hydroxy-2-acetaminofluorene on the cellular levels of epoxide hydrolase, cytochrome p-450b, and NADPH-cytochrome c (P-450) oxidoreductase messenger ribonucleic acids. J Biol Chem 257:11032–11036 (1982).
69. Wood AW, Chang RL, Levin W, Yagi H, Thakker DR, van Bladderen PJ, Jerina DM, Conney AH. Mutagenicity of the enantiomer of the diastomeric bay-region benz(*a*)anthracene 3,4-diol-1,2-epoxides in bacterial and mammalian cells. Cancer Res 43:5821–5825 (1983).
70. Seidegård J, DePierre JW. Microsomal epoxide hydrolase: properties, regulation and function. Biochim Biophys Acta 695:251–270 (1983).
71. Hasset C, Aicher L, Sidhu JS, Omiecinski CJ. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression *in vitro* of amino acid variants. Hum Mol Genet 3:421–428 (1994).
72. Jin C-J, Miners JO, Burchell B, Mackenzie PI. The glucuronidation of hydroxylated metabolites of benzo(*a*)pyrene and 2-acetylaminofluorene by cDNA-expressed human UDP-glucuronosyltransferases. Carcinogenesis 14:2637–2639 (1993).
73. Owens TS, Ritter JK. The novel bilirubin/phenol UDP-glucuronosyltransferase *UGT1* gene locus: implications for multiple familial hyperbilirubinaemia phenotypes. Pharmacogenetics 2:93–108 (1992).
74. Burchell B, Nebert DW, Nelson DR, Bock KW, Iyanagi T, Jansen PLM, Lancet D, Mulder GJ, Chowdhury JR, Siest G, Tephy T, Mackenzie P. The UDP-glucuronosyltransferase gene family: suggested nomenclature based on evolutionary divergence. DNA Cell Biol 10:487–494 (1991).
75. Daly AK, Cholerton S, Gregory W, Idle JR. Metabolic polymorphisms. Pharmacol Ther 57:129–160 (1993).
76. Anttila S, Vainio H, Hietanen E, Camus A-M, Malaveille C, Brun G, Husgafvel-Pursiainen K, Heikkilä L, Karjalainen A, Bartsch H. Immunohistochemical detection of pulmonary cytochrome P450IA and metabolic activities associated with P450IA1 and P450IA2 isozymes in lung cancer patients. Environ Health Perspect 98:179–182 (1992).
77. Ichiba M, Hagmar L, Rannug A, Högstedt B, Alexandrie A-K, Hemminki K. Aromatic DNA adducts, micronuclei and genetic polymorphism for CYP1A1 and GST1 in chimney sweeps. Carcinogenesis 15:1347–1352 (1994).
78. Ryberg D, Kure E, Lystad S, Skaug V, Stangeland L, Mercy I, Børresen A-L, Haugen A. p53-mutations in lung tumors. Relationship to putative susceptibility markers for cancer. Cancer Res 54:1551–1555 (1994).
79. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 253:9–53 (1991).
80. Harris C, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 329:1318–1327 (1993).
81. Suzuki H, Takahashi T, Kuroishi T, Suyama M, Ariyoshi Y, Takahashi T, Ueda R. p53 mutations in non-small cell lung cancer in Japan: association between mutations and smoking. Cancer Res 52:734–736 (1992).
82. Ridanpää M, Karjalainen A, Anttila S, Vainio H, Husgafvel-Pursiainen K. Genetic alterations in p53 and K-*ras* in lung cancer in relation to histopathology of the tumor and smoking history of the patient. Int J Oncol 5:1109–1117 (1994).
83. Husgafvel-Pursiainen K, Ridanpää M, Anttila S, Vainio H. p53 and *ras* gene mutations in lung cancer: implications for smoking and occupational exposures. J Occup Environ Med 37:69–75 (1995).
84. Ionov YM, Peinado A, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequencies reveal a new mechanism for colonic carcinogenesis. Nature 363:558–561 (1993).
85. Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin J-P, Järvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B, de la Chapelle A. Clues to the pathogenesis of familial colorectal cancer. Science 260:812–816 (1993).
86. Wooster R, Cleton-Jansen A-M, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BAJ, von Deimling A, Wiestler OD, Cornelisse CJ, Devilee P, Stratton MR. Instability of short tandem repeats (microsatellites) in human cancers. Nature Genet 6:152–156 (1994).
87. Merlo A, Mabry M, Gabrielson E, Vollmer R, Baylin SB, Sidransky D. Frequent microsatellite instability in primary small cell lung cancer. Cancer Res 54:2098–2101 (1994).
88. Shridhar V, Siegfried J, Hunt J, del Mar Alonso M, Smith DI. Genetic instability of microsatellite sequences in many non-small cell lung carcinomas. Cancer Res 54:2084–2087 (1994).
89. Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell 75:1027–1038 (1993).
90. Bronner,C.E. Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, Tannergard P, Bollag RJ, Godwin AR, Ward DC,

Nordenskjold M, Fishel R, Kolodner R, Liskay RM. Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. Nature 368:258–261 (1994).
91. Liu B, Nicolaides NC, Markowitz S, Willson JKV, Parsons RE, Jen J, Papadopoulos N, Peltomäki P, de la Chapelle A, Hamilton SR, Kinzler KW, Vogelstein B. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nature Genet 9:48–55 (1995).
92. Harris C. p53: at the crossroads of molecular carcinogenesis and risk assessment. Science 262:1980–1981 (1993).
93. Malkinson AM. The genetic basis of susceptibility to lung tumors in mice. Toxicology 54:241–271 (1989).
94. Dragani TA, Manenti G, Plerotti MA. Genetics of murine lung tumors. Adv Cancer Res 67:83–119 (1995).
95. Gariboldi M, Manenti G, Canzian F, Falvella FS, Radice T, Pierotti MA, Binelli G, Della Porta G, Dragani TA. A major susceptibility locus to murine lung carcinogenesis maps on chromsome 6. Nature Genet 3:132–138 (1993).
96. Sellers TA, Potter JD, Bailey-Wilson JE, Rich SS, Rothschild H, Elston RC. Lung cancer detection and prevention: evidence for an interaction between smoking and genetic predisposition. Cancer Res 82:2694–2697 (1992).
97. Lander ES, Schork NJ. Genetic dissection of complex traits. Science 285:2037–2048 (1994).
98. Knudson AG. All in the (cancer) family. Nature Genet 5:103–104 (1993).
99. Knudson AG. Antioncogenes and human cancer. Proc Natl Acad Sci USA 90:10914–10921 (1993).