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Metabolic polymorphisms as susceptibility markers for lung and oral cavity cancer

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Lung and oral cavity cancers are causally associated with tobacco use. Alcohol is an independent risk factor for oral cavity cancer. Major classes of carcinogens present in tobacco and tobacco smoke are converted into DNA-reactive metabolites by cytochrome P450 (CYP)-related enzymes, several of which display genetic polymorphism. Individual susceptibility to cancer is likely to be modified by the genotype for enzymes involved in the activation or detoxification of carcinogens in tobacco and repair of DNA damage. Molecular epidemiological studies to assess the risk associated with metabolic polymorphisms for cancers of the lung and head and neck have shown that the overall effect of common polymorphisms is moderate in terms of penetrance and relative risk. However, some gene combinations like mutated *CYP1A1/GSTM1*-null genotype seem to predispose the lung and oral cavity of smokers to an even higher risk for cancer or DNA damage, although these results require confirmation in larger well defined studies that take into account the existence of ethnic variations even within the commonly defined groups. Retinoids, isothiocyanates and tea polyphenols have been identified as possible chemopreventive agents for cancers of the lung and oral cavity. While a number of trials have been conducted with retinoids or β-carotene, the results were ambiguous and the causes are still being debated. The possible interaction of chemopreventive agents with metabolic polymorphisms as biomarkers in chemoprevention trials is discussed.

Introduction

Analytical and molecular epidemiology studies have identified two types of population at elevated risk of cancer: (*a*) groups exposed to particular environmental or lifestyle risk factors, and (*b*) carriers of mutated cancer-determining genes that confer a very high cancer risk (Caporaso & Goldstein, 1995). Compared to the highly penetrant polymorphic genes, low-penetrance susceptibility genes (such as those involved in carcinogen metabolism and DNA repair) modestly increase the risk for cancer in exposed individuals, perhaps at low doses of carcinogens (Vineis *et al.*, 1994; Vineis, 1997). An important mechanism by which carcinogens cause DNA mutations is by covalent binding to DNA to form adducts, which if not repaired can result in a mutation when the DNA is copied during cell division. Thus DNA adducts are sensitive markers of exposure and, subject to certain conditions, also of disease risk. However, most carcinogens need to be metabolized by enzymes that may differ between individuals due to either inherited polymorphic DNA sequence variations or up-regulation or down-regulation of genes for metabolic enzymes by other external agents such as diet, alcohol, medication or lifestyle exposures. This entails complex gene–environment and gene–gene interactions. In principle, understanding the molecular basis of disease can facilitate prevention by allowing the identification of individuals who are at increased risk and of susceptible populations so that preventive strategies can be designed for the greatest impact. It is therefore important to identify candidate genes for which biologically plausible mechanisms for effects on cancer risk can be proposed.

Tobacco exposure is the largest single cause of lung and oral cavity cancers (Doll, 1998). Alcohol use is the second major risk factor for the development of oral cancer. The major classes of carcino-

gens from tobacco, polycyclic aromatic hydrocarbons (PAHs) and tobacco-specific nitrosamines, are causally associated with lung and oral cancer (Hoffmann & Hecht, 1990; Hecht, 1999a; McClellan, 1996; Bergen & Caporaso, 1999). Most tobacco carcinogens are converted to DNA-reactive metabolites by oxidative, mainly cytochrome P450-related, enzymes (CYPs) and are further metabolized by phase II enzymes. PAHs are converted into phenolic metabolites; thus benzo[*a*]pyrene (B[a]P) is oxidized to B[a]P-7,8-diol by a CYP-mediated process. Secondary metabolism, mainly involving epoxide hydrolase and other CYP isoforms, leads to formation of the highly reactive (+)-*anti*-B[a]P diolepoxide (BPDE) which is a relatively good substrate for glutathione *S*-transferase (GST) enzymes GSTM1, M2, M3 and P1 (Coles & Ketterer, 1990). Among the tobacco-specific nitrosamines, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine (NNN) are the most important. NNK is a powerful lung carcinogen in all species tested and human exposure levels are comparable to the doses that cause tumours in laboratory animals. The metabolism of NNK includes α-methyl-hydroxylation, α-methylene-hydroxylation and pyridine-*N*-oxidation by CYP-mediated reactions and reduction to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its conjugation as a glucuronide (Hecht, 1999a,b). Tobacco smoke also contains reactive oxygen species and reactive nitrogen species that impose oxidative stress on smokers' tissue. As a consequence, oxidative DNA-base damage has been detected in respiratory tract tissue of smokers, with formation of lipid peroxidation products such as malondialdehyde, crotonaldehyde and *trans*-4-hydroxy-2-nonenal, the last of which can be further epoxidized by CYP-mediated reactions to form promutagenic exocyclic DNA adducts (Chung *et al.*, 1996; Nair *et al.*, 1999a) that could contribute to carcinogenesis in the upper aerodigestive tract (Nath *et al.*, 1998). Chewing of tobacco alone or with betel quid also results in generation of large amounts of reactive oxygen species in the mouth; these and the tobacco-specific nitrosamines are the major genotoxic agents implicated in oral cancer related to chewing (Nair *et al.*, 1996). Since tobacco carcinogens, reactive oxygen species and lipid peroxidation products are likely to be substrates for metabolizing enzymes, the extent of DNA damage and ultimately the cancer risk may be affected by polymorphic CYP and GST enzymes (Brockmöller *et al.*, 1998; Nair *et al.*, 1999b). In many instances, the association between a particular genetic risk factor and cancer appears to be race- or ethnicity-specific. Susceptibility markers that are on the pathway to the development of cancer have often been suggested as appropriate intermediate biomarkers for use in chemoprevention studies. This review focuses on metabolic polymorphisms implicated in susceptibility to lung and oral cancer, with a view to integrating them as biomarkers in chemoprevention strategies.

Polymorphic metabolic genes and cancer risk for oral and lung cancer

Genetic polymorphisms occur at a population frequency of more than 1% and can result in marked inter-individual differences. Numerous alleles that cause defective, qualitatively altered, diminished or enhanced rates of drug metabolism have been identified for many of the phase I and phase II enzymes and underlying molecular mechanisms have been elucidated.

Cytochrome P450

The cytochrome P450 enzymes are a large multigene family and are important in phase I detoxification/activation reactions. In a review of the results of all genotype-based case–control studies published in the 1990s on the effect of genetic variants of CYPs alone or in combination as risk modifiers of tobacco-related cancers (Bartsch *et al.*, 2000), the overall effects of common CYP polymorphisms were found to be modest, with odds ratios ranging from 2 to 10. Extensive studies have been conducted with the aim of identifying risk genotypes for lung cancer, mostly focussing on *CYP1A1*, *CYP2D6* and *CYP2E1*, while studies on oral cavity cancer have appeared more recently.

CYP1A1: The human enzyme CYP1A1 is involved in the activation of major classes of tobacco procarcinogens, such as PAHs and aromatic amines, and is present in many epithelial tissues. About 10% of the Caucasian population has a highly inducible form of this enzyme (termed B[a]P-hydroxylase or previously arylhydrocarbon hydroxylase), which is associated with an

increased risk for bronchial, laryngeal and oral cavity tumours in smokers (Nebert *et al.*, 1996). The induction of *CYP1A1* is initiated by the specific binding of aromatic inducer compounds to the aromatic hydrocarbon (Ah) receptor. An Ah receptor nuclear translocator (*Arnt*) gene is further involved in the *CYP1A1* induction pathway. So far, no relationship has been found between Ah-receptor polymorphism and lung cancer risk (Kawajiri *et al.*, 1995; Micka *et al.*, 1997). Beginning with the pioneering work by Kellerman *et al.* (1973) on B[a]P-hydroxylase inducibility and bronchogenic carcinoma, studies on the association of the genetic polymorphism of *CYP1A1* and cancer started after co-segregation of the CYP1A1 high-inducibility phenotype and polymorphism of the MspI restriction site (Petersen *et al.*, 1991). At the present time, the *CYP1A1* gene is known to contain four important sequence polymorphisms. The *CYP1A1* Ile-Val (m2) mutation in the haem-binding region results in a twofold increase in microsomal enzyme activity and is in complete linkage disequilibrium in Caucasians with the *CYP1A1* *Msp*I (m1) mutation, which has also been associated experimentally with increased catalytic activity (Landi *et al.*, 1994). Although the Ile-Val mutation in the *CYP1A1* allele does not increase activity *in vitro* (Zhang *et al.*, 1996; Persson *et al.*, 1997), it may be linked to other functional polymorphisms, for example in the regulatory region important for *CYP1A1* inducibility. Significant ethnic differences in the frequency of homozygous *CYP1A1* *Msp*I alleles have been observed, and both the *Msp*I and *Val* alleles are rarer in Caucasian than in Japanese populations (Cascorbi *et al.*, 1996a).

Lung cancer: The various *CYP1A1* gene polymorphisms are differentially associated with risk of lung cancer. As the prevalence of both *m1* and *m2* alleles is higher in Japanese, studies have mainly investigated difference in cancer risk between homozygous mutant versus other genotypes, while in other populations, the effect is determined using pooled homo- and heterozygous genotypes. Data on lung cancer among Japanese suggest an increased risk associated with both the *m1* allele (Okada *et al.*, 1994) and *m2* allele (Hayashi *et al.*, 1992) polymorphism; the risk is particularly elevated among light smokers and for development of squamous-cell carcinoma (SCC). In studies on Caucasians, these findings were not confirmed (Hirvonen *et al.*, 1993; Alexandrie *et al.*, 1994; Bouchardy *et al.*, 1997), possibly due to the much lower prevalence of the *m1* allele in Caucasians. However, Xu *et al.* (1996) did show an association for the *m1* allele in Caucasians. An African-American-specific *m3* allele does not seem to confer increased risk for lung cancer overall, but an association with increased risk for adenocarcinoma has been seen (Taioli *et al.*, 1998). A recently described *m4* mutation in close proximity to *m1* has been investigated in one study on Caucasians, and no correlation with lung cancer risk was found (Cascorbi *et al.*, 1996b).

Oral cancer: The CYP1A1 enzyme is present in oral tissue (Romkes *et al.*, 1996), and the link between *CYP1A1* variants and oral cancer risk has been investigated in a number of studies. An overrepresentation of the *CYP1A1* Ile-Val variant occurred among Caucasian patients with oral cancer and this was significantly higher in nonsmokers than smokers (Park *et al.*, 1997). Similarly, an increased prevalence of *CYP1A1* Val-Val variant was found among Japanese patients with head-and-neck cancers and especially those with pharyngeal cancer (Morita *et al.*, 1999). Individuals with the homozygous *CYP1A1* *Msp*I (m1/m1) variant were at significantly increased risk for oral SCC, in particular after exposure to low concentrations of PAHs (Tanimoto *et al.*, 1999) and in combination with *GSTM1*-null, risk was also significantly elevated for various subsites, especially buccal mucosa.

Combined effect of CYP1A1 and GSTM1 genotypes on cancer susceptibility

Lung cancer: Smokers with the exon-7 Ile-Val mutation were found to have more PAH–DNA adducts in their white blood cells than smokers without the variant (Mooney *et al.*, 1997). In lung parenchymal tissue of smokers, levels of BPDE and bulky (PAH)–DNA adducts were positively correlated with CYP1A1 enzyme activity (Alexandrov *et al.*, 1992). Risk of lung cancer, especially SCC, is increased in individuals with a combination of a homozygous rare allele of the *CYP1A1* gene (either *m2* or *m1*) and the null *GSTM1* gene compared with those having other combinations of genotypes in Japanese populations (Hayashi *et al.*,

1992). Subsequently, a remarkably high risk for SCC at low cigarette dose (OR 41, 95% confidence interval (CI) 8.7–193.6) was demonstrated in Japanese (Nakachi *et al.*, 1993). Significant increases were seen in B[a]P adduct levels in lung tissue from smokers with an 'at-risk' genotype, with a multiplicative effect at a lower level of exposure (Bartsch *et al.*, 1995, 1999). BPDE adduct levels in lung and leukocytes of Caucasian smokers were correlated with *CYP1A1* genotype, most strongly in *GSTM1*-deficient smokers (Bartsch, 1996; Rojas *et al.*, 2000). These findings provide a mechanistic basis for the correlation of high-risk genotypes with increased risks for tobacco-related lung cancer, even at low levels of cigarette smoking (Kihara & Noda, 1995). Significantly more *p53* mutations were seen in lung tumours of Japanese smokers having the susceptible *CYP1A1* genotype. Individuals with the combination of *CYP1A1* m2/m2 and *GSTM1*-null genotypes had an eight-fold greater frequency of *p53* mutations than persons with neither genotype (Kawajiri *et al.*, 1996). Operated lung cancer patients with high pulmonary CYP1A1 enzyme inducibility (Bartsch *et al.*, 1990) and/or a high-risk genotype combination (Goto *et al.*, 1996) had shorter survival.

Oral cancer: In Japanese patients, the high-risk combination *CYP1A1* MspI (m1/m1) and *GSTM1*-null genotype conferred a high risk for oral SCC (Sato *et al.*, 1999) and a similar combination increased significantly the risk for cancer of the buccal mucosa and upper gingiva (Tanimoto *et al.*, 1999). In Caucasians, while no effect was reported by several authors (Matthias *et al.*, 1998; Oude Ophius *et al.*, 1998), Park *et al.* (1997) reported a significant effect, especially in non-smoker cases. But in one report, the highest prevalence of *p53* mutation was observed in oral tumours from patients with the *CYP1A1*(Val)/*GSTM1* active genotype (Lazarus *et al.*, 1998).

Overall, there is increasing evidence that individuals with the homozygous *CYP1A1* MspI and Ile-Val genotypes are at higher risk for contracting smoking-associated lung (SCC) and oral cancers, as particularly seen in Asian study populations where these high-risk allele frequencies are 8–18 times higher than in Caucasians (Bartsch *et al.*, 2000). The cancer risk is further increased in carriers of the combined high-risk *CYP1A1*/*GSTM1*-null genotypes.

CYP2D6: *CYP2D6* has received particular attention as a genetic susceptibility factor since the first studies on the link between lung cancer risk and extensive metabolizer (EM) status (Ayesh *et al.*, 1984). The poor metabolizer (PM) phenotype, inherited as an autosomal recessive trait, is due to several defective allelic *CYP2D6* variants, three of which account for more that 90% of all poor metabolizers. An allele associated with 2–12-fold amplification of the *CYP2D6* gene is found in carriers known as 'ultra-rapid metabolizers' (UM) (Daly *et al.*, 1996). Conflicting data exist as to whether *CYP2D6* is expressed in human lung (Raunio *et al.*, 1999). This isozyme can activate the tobacco-specific nitrosamine NNK and also nicotine, but other P450s are more active in this respect. Associations have been found between nicotine dependence and PM phenotype (Boustead *et al.*, 1997) and between UM and smoking addiction (Saarikoski *et al.*, 2000). Higher DNA adduct levels have been reported among homozygous and heterozygous extensive metabolizers compared with people classified as poor metabolizers.

Lung cancer: Among the nine genotyping studies that have been reported, a significant association was found between lung cancer and the EM genotype in three studies, and in one study an association with UM genotype and lung cancer risk was found in African Americans (Bartsch *et al.*, 2000). However, in two meta-analyses, there was no association or one of borderline significance between the EM genotype and increased risk (Christensen *et al.*, 1997; Rostami Hodjegan *et al.*, 1998).

Oral cancer: In Caucasians, a significantly higher frequency of the PM genotype among cases was reported in one study, while the time for lymph node metastasis was shorter in PM compared with EM subjects (Worrall *et al.*, 1998). No effect was found in other studies (Gonzales *et al.*, 1998; Matthias *et al.*, 1998).

Overall, evidence for a role of *CYP2D6* polymorphisms as a risk factor for lung or oral cancer is weak, conflicting and inconclusive.

CYP2E1: The ethanol-inducible *CYP2E1* metabolizes many known procarcinogens, including NNN, NNK and other volatile nitrosamines found in tobacco smoke. Wide inter-individual variation

in expression of the *CYP2E1* gene in humans has been reported, which is possibly due to gene–environment interactions. *CYP2E1* is induced in mice exposed to cigarette smoke by inhalation (Villard *et al.*, 1998). Its regulation involves complex transcriptional and post-transcriptional mechanisms. Several polymorphic alleles occurring at low frequency have been identified and the most studied are the *Rsa*I $G_{-1259}C$ or *Pst*I $C_{-1091}T$ restriction fragment length polymorphisms (RFLP); these appear to be in complete linkage disequilibrium with each other (c1: common and c2: rare allele). Although the primary sequence of the enzyme does not appear to be altered, increased gene transcription has been suggested (Watanabe *et al.*, 1994). A second allele of the *CYP2E1* gene is revealed by a *Dra*I RFLP (C: minor and D: common allele). While in Caucasians no relationship was found between genotype and the activity of this enzyme *in vivo*, in Japanese the presence of the variant c2 alleles resulted in a significant reduction in oral clearance of chlorzoxazone, after adjustment for age and sex. The mean activity in individuals with the c2/c2 genotype was significantly lower than that in individuals with either the homozygous wild-type or the heterozygous genotype. Body weight and dietary factors were the major modulators of inter-individual variation (Le Marchand *et al.*, 1999). As the frequencies of variant alleles are very low in Caucasians and African Americans, the statistical power of these studies was low.

Lung cancer: In a number of studies of Caucasians, no significant association was found, but the wild-type *Dra*I genotype was associated with an increased risk for lung cancer in studies in Japanese (Uematsu *et al.*, 1994), Mexican Americans (Wu *et al.*, 1998) and a mixed Hawaiian population (Le Marchand *et al.*, 1998). More conflicting results have been published concerning the *Rsa*I/*Pst*I mutation. The rare *Pst*I/*Rsa*I c2 allele has been associated with decreased risk for cancer in some studies (Wu *et al.*, 1997; Le Marchand *et al.*, 1998), and in one study the c2 allele frequency was significantly lower among cases than controls (Persson *et al.*, 1993). The homozygous c2 genotype correlated positively with *p53* mutations in Japanese (Oyama *et al.*, 1997a); in a small study of Caucasians, the c2 genotype conferred a significant risk especially for lung adenocarcinoma (El Zein *et al.*, 1997).

Oral cancer : No association between head-and-neck cancer and *CYP2E1* variants was reported in two studies (Matthias *et al.*, 1998; Morita *et al.*, 1999), but a higher prevalence of the c2 allele was reported in non-betel quid-chewing Chinese patients (Hung *et al.*, 1997). As the frequencies of variant alleles are very low in Caucasians and African Americans, the statistical power of the studies was low.

Glutathione S-transferases

The glutathione S-transferase genes (*GST*s) form a superfamily of consisting of four distinct families, named Alpha, Mu, Pi and Theta. The *GSTM1*, *GSTT1* and *GSTP1* genes are polymorphic in humans. *GSTM1* is expressed at high levels in liver but not in lung. *GSTM1* has three alleles: *GSTM1 0/0* (null) is gene deletion in homozygotes, while *GSTM1*A* and *GSTM1*B* differ by a single base in exon 7 and encode enzyme monomers that form active homo- and heterodimeric forms. The prevalence of the *GSTM1*-null genotype shows ethnic differences, with reported ranges of 22–35% in Africans, 38–67% in Caucasians and 33–63% in East Asian populations (Rebbeck, 1997).

Lung cancer: As an illustration of the potential population impact of these genes, it has been estimated that 17% of lung cancers may be attributed to *GSTM1* genotypes (McWilliams *et al.*, 1995). Recent meta-analyses of 19 studies indicate that the *GSTM1*-null genotype confers a modest increased risk of lung cancer, the relative risk among Caucasians being 1.21 (95% CI, 1.06–1.39), and among Asians 1.45 (95% CI, 1.23–1.70). For lung cancer, risk seems to be related particularly to squamous and small cell histologies. Estimates for Asians show higher consistency and risk is greater in Kreyberg I histologies (d'Errico *et al.*, 1999). Ten studies have examined the relationship between *GSTM1* polymorphism and lung cancer risk according to tobacco consumption. In five of the ten studies, the OR was higher among heavy smokers than among light smokers and in the other five studies was higher among light smokers (Stucker *et al.*, 1999). There is evidence that female smokers with the *GSTM1*-null genotype are at higher risk for lung cancer than males (Tang *et al.*, 1998). Although *GSTM1* is not expressed in human lung, GSTM3 activity is found, which seems to be

co-regulated with the *GSTM1* form. Thus, individuals with the nulled *GSTM1* genotype suffer from impaired detoxification of tobacco carcinogens, both qualitatively because of the absence of *GSTM1* in the body and low expression of *GSTM3* in the lung, and quantitatively because of the overall lower GST activity (Nakajima *et al.*, 1995).

Oral cancer: A significant association with the null genotype was seen in some Japanese studies, one at low dose of cigarettes (Sato, 1999) and another in alcohol-drinking cases (Nomura, 2000), while a lack of association has also been reported (Tanimoto *et al.*, 1999). An association was reported among French smokers (Jourenkovo-Mironova *et al.*, 1999b), while other studies among Caucasians have reported a lack of association (Deakin *et al.*, 1996; Park *et al.*, 1997). *GSTM1*-null conferred a significant increased risk for oral leukoplakia among Indian betel-quid chewers, which was further increased in combination with *GSTT1*-null genotypes (Nair *et al.*, 1999b).

GSTT1: Ethnic differences in prevalence exist. The frequency of the *GSTT1*-null genotype varies from 10–18% in Caucasians (Rebbeck, 1997) to 58% in Chinese (Lee *at al.*, 1995). The overall biological effect of this polymorphism is difficult to predict, as the enzyme is involved in both detoxification (monohalomethanes, ethylene oxides) and metabolic activation reactions (methylene chloride).

Lung cancer: A number of studies have shown no association of *GSTT1* with lung cancer risk (Harries *et al.*, 1997; Ryberg *et al.*, 1997). However, a significant association was observed with a concurrent lack of *GSTM1* and *GSTT1* genes and susceptibility to squamous cell lung cancers, although individually neither genotype showed any association (Saarikoski *et al.*, 1998).

Oral cancer: The *GSTT1*-null genotype was shown to increase the risk for oral and pharyngeal cancers among French smokers (Jourenkova-Mironova *et al.*, 1999b), but most other studies did not find an associated risk (Deakin *et al.*, 1996; Mathias *et al.*, 1998; Oude Ophius *et al.*, 1998; Worrall *et al.*, 1998).

GSTP1: Although genetic polymorphisms have been reported, only the exon 5 polymorphism (G to A, valine to isoleucine) in the *GSTP1* gene is linked to changed enzyme activity. The 105Val variant is more active than the 105Ile variant in conjugation of diol epoxides of some PAHs, suggesting that the allele 105Ile variant may be more susceptible to the carcinogenic effects of diol epoxides of PAHs.

Lung cancer: The frequency of the homozygous *GG* genotype was significantly higher in male lung cancer patients (Ryberg *et al.*, 1997), whereas no association was reported in other studies (Butkiewicz & Chorazy, 1999; Harries *et al.*, 1997). The combination of the three risk genotypes of *GSTM3 AA*, *GSTP1* (AG or GG) and *GSTM1*-null conferred an increased risk of lung cancer in heavy smokers (Jourenkova-Mironova *et al.*, 1998).

Oral cancer: The GSTP1 variant genotype conferred an increased risk for oral and/or pharyngeal cancer in a number of studies (Mathias *et al.*, 1998; Jourenkova-Mironovo *et al.*, 1999b; Katoh *et al.*, 1999; Park *et al.*, 1999). In an oral cancer case–control study among ethnic Indians, we observed a statistically significant risk associated with a homozygous variant genotype of the *GSTP1* gene (Nair *et al.*, unpublished).

N-Acetyltransferase

N-Acetyltransferase-1 (*NAT1*) and *N*-acetyltransferase-2 (*NAT2*) genes are polymorphically expressed in a variety of tissues. NAT2 may either detoxify or activate aromatic amines found in tobacco smoke, such as 4-aminobiphenyl (Hengstler *et al.*, 1998). Both phenotypic assays and genotypic assays for NAT2 can be used to classify individuals as rapid or slow acetylators. Genetic variants of the *NAT* genes have been cloned and at least 19 rare alleles for *NAT2* have been detected (Vatsis *et al.*, 1995) but NAT*5, *6 and *7 alleles account for most of the slow-acetylator allele in Caucasian populations, providing high concordance between genotype and phenotype. For NAT1, eight alleles have been identified. The distribution of NAT1 and NAT2 alleles differs widely between racial and ethnic groups.

Lung cancer: Information about the role of NAT enzymes as risk factors for lung cancer is ambiguous. No association with NAT2 slow or rapid geno-

types was seen in a Spanish study (Martinez *et al.*, 1995). In contrast, the homozygous rapid NAT2 genotype was associated with increased risk in a German study (Cascorbi *et al.*, 1996b) and the slow acetylator with an increased risk in a Japanese study (Oyama *et al.*, 1997b). In a Swedish study, an increased risk for NAT2 slow acetylators among never-smokers but an increased risk for rapid acetylators among smokers was seen (Nyberg *et al.*, 1998). A significantly increased risk of developing pulmonary disorders among asbestos-exposed subjects with a combined *GSTM1*-null and *NAT2* slow genotype has also been reported (Hirvonen *et al.*, 1995, 1996). In a meta-analysis of estimates for NAT2 slow acetylators, an OR of 0.96 (95% CI, 0.82–1.10) was computed. A significant association was reported between *NAT1* genotype and lung cancer risk in smokers (Bouchardy *et al.*, 1998; Abdel-Rahman *et al.*, 1998), whereas no association between lung cancer risk and NAT2 was observed. Recently, individuals with *GSTM1*-null and/or NAT2 slow genotypes were reported to be at increased risk of contracting non-operable lung cancer at young age (Hou *et al.*, 2000).

Oral cancer: Only a few studies have been conducted. The NAT1*10 slow metabolizer genotype was associated with oral cancer risk in Japanese, especially in non-smokers, but not the NAT2 rapid-acetylator genotype (Katoh *et al.*, 1998). In another Japanese study, the NAT2 slow-metabolizer genotype predisposed towards higher risk (Morita *et al.*, 1999), reflecting the tendencies observed among Caucasians (Gonzales *et al.*, 1998; Jourenkova-Mironova *et al.*, 1999a).

Other metabolic polymorphisms

There are a number of other metabolic polymorphisms which, given their substrates and functions, could be expected to have some effect on the risk for lung and oral cancers. However, only a few studies testing these hypotheses for some isoforms have appeared.

CYP1A2: CYP1A2 activates many dietary and tobacco procarcinogens, notably aromatic and heterocyclic amines and nitrosamines, and also metabolizes nicotine. In contrast to extrahepatic CYP1A1, CYP1A2 appears to be expressed mainly in the liver and only weakly in the peripheral lung (Mace *et al.*, 1998). Like CYP1A1, CYP1A2 is regulated in part by the Ah-receptor system and induced in humans by a variety of chemicals. The activity of this enzyme can be determined in a non-invasive assay involving measurement of caffeine 3-demethylation. Recently, two genetic polymorphisms of the human *CYP1A2* gene have been identified, one in the 5′ flanking region affecting enzyme inducibility (Nakajima *et al.*, 1999) and another in intron 1 which is associated with high catalytic activity of the enzyme, when subjects are exposed to tobacco smoke (MacLeod *et al.*, 1998; Sachse *et al.*, 1999). About 45% of healthy Caucasians are homozygous for the high-inducibility genotype. A subgroup of smokers had a 1.6-fold increase in caffeine demethylation ratio (ratio of paraxanthine : caffeine in serum) over that in nonsmokers. Interactions between *GSTM1* status and CYP1A2 and CYP1A1 enzyme induction have been observed in smokers: *GSTM1* deficiency was associated not only with increased hepatic CYP1A2 activity in current smokers but also with significantly increased levels of bulky PAH–DNA adducts in the lung parenchyma of smokers and ex-smokers, over that in individuals with wild-type *GSTM1* (Bartsch *et al.*, 1995; Bartsch & Hietanen, 1996). CYP1A2 activity was higher in *GSTM1*-null subjects after exposure to cigarette smoke and heterocyclic amines from cooked meat. Exposed individuals with *CYP1A1* Ile–Val alleles had greater CYP1A2 activity than those with wild-type *CYP1A1* (MacLeod *et al.*, 1997). *GSTM1*-null was associated with higher levels of 4-aminobiphenyl–haemoglobin adducts in smokers (Yu *et al.*, 1995).

CYP1B1: A key enzyme in the production of potentially carcinogenic estrogen metabolites, CYP1B1 also activates many PAH-dihydrodiols, aromatic amines and other groups of procarcinogens. CYP1B1 is also induced by Ah-receptor ligands. Several genetic polymorphisms have been identified (Bailey *et al.*, 1998), but the role of *CYP1B1* in lung/oral cancer has not been investigated in epidemiological studies.

CYP2A6: In humans, CYP2A6 is involved in the metabolism of several carcinogens, mediates 7-hydroxylation of coumarin, a component of cigarette smoke, and activates several nitrosamines in

tobacco smoke, including NNK (Hecht, 1999b; Tiano *et al.*, 1994). The catalytic selectivity of CYP2A6 appears to overlap with that of CYP2E1. The location of CYP2A6 and 2E1 in extrahepatic tissues such as lung, nasal and pharyngeal areas is of interest. Two *CYP2A6* variant alleles have been identified (*2 and *3). The prevalence of the Leu^{160}His variant allele in Caucasians is about 2% and it is associated with lower coumarin 7-hydroxylation activity. A new allele has been described in which exons 5–9 are deleted (Nunoya *et al.*, 1998). Individuals lacking functional CYP2A6 have impaired nicotine metabolism and may thus be protected against tobacco dependence. No association of *CYP2A6**2 with lung cancer was detected in a single report (London *et al.*, 1999).

CYP2C9: The levels of all smoking-related DNA adducts in the larynx were correlated with the presence of P4502C protein, suggesting a role of *CYP2C9* in DNA adduction of PAH-type tobacco carcinogens (Degawa *et al.*, 1994). The level of bulky DNA adducts in normal bronchial tissue of smokers was higher in individuals with the homozygous *CYP2C9**3/*3 genotype (Ozawa *et al.*, 1999). In African Americans and Caucasians, the *2 allele was associated with borderline-increased risk for lung cancer (London *et al.*, 1996, 1997).

CYP2C19: Several defective *CYP2C19* alleles are the basis for the (*S*)-mephenytoin 4′-hydroxylase polymorphism. The most common variant allele, *2, has an aberrant splice site in exon 5 (DeMorais *et al.*, 1994a). The premature stop codon mutant *3 allele has so far been found only in Asians (DeMorais *et al.*, 1994b). There is evidence that CYP2C19 expressed by yeast has a major role in the accumulation of the proximate mutagen B[a]P-7,8-dihydrodiol. A very small study among Japanese patients revealed a significant association of the poor-metabolizer genotype with lung SCC (Tsuneoka *et al.*, 1996).

CYP3A4: CYP3A4 can activate numerous procarcinogenic PAH dihydrodiols, such as B[a]P-dihydrodiol, and also metabolizes NNN (Patten *et al.*, 1997). Whether genetic or solely environmental factors are responsible for the wide variation in human CYP3A4 activity is unknown. Although the three *CYP3A* genes, *3A4*, *3A5*, and *3A7*, are expressed at widely different levels, polymorphism has been found only for *CYP3A4* and *CYP3A5* to date. Several allelic variants of the *CYP3A4* gene have been reported (Peyronneau *et al.*, 1993), but none was apparently related to catalytic activity in the liver samples from which the DNA was derived. No extensive studies on *CYP3A4* polymorphism have been reported.

NQO1: NAD(P)H:quinone oxidoreductase (NQO1) is a flavoprotein that catalyses the reduction of quinones, quinone amines and azo dyes, thereby protecting cells from reactive oxygen species generated from these compounds by the activity of reducing enzymes such as cytochrome P450 reductase. Two alleles have been identified. *NQO1* Pro-Ser, the less common, encodes an inactive protein and is termed null (Rosvold *et al.*, 1995). Significant ethnic variations in the frequency of the variant allele have been reported. The *NQO1* Pro allele is associated with increased risk for lung cancer in non-Hispanic whites and African Americans (Wienke *et al.*, 1997). Allelic variants at *NQO1* have been associated with susceptibility to lung tumours.

Other enzymes: Microsomal epoxide hydrolases (mEH), UDP-glucuronyl transferases (UDPGTs) catalysing glucuronidation of *N*-hydroxyarenes, heterocyclic amines and aromatic dihydrodiols, phenols, quinols and NNK, and myeloperoxidase (MPO) which can activate B[a]P and aromatic amine in cigarette smoke, all display genetic polymorphisms, and would be candidate susceptibility genes to modify the risk for cancers of the lung and oral cavity.

Genetic polymorphism in metabolic enzymes as biomarkers in chemoprevention

As a number of xenobiotic oxidations by cytochrome P450 enzymes are affected by endobiotic chemicals, endobiotic–xenobiotic interactions as well as drug–drug interactions may be of great importance in relation to the chemopreventive, pharmacological and toxicological actions of chemopreventive agents. Three classes of chemopreventive agents, retinoids, tea polyphenols and isothiocyanates, are reviewed in the context of their possible modulation of and interaction with metabolic enzymes, to evaluate the importance of integrating individual metabolic genotype/pheno-

type in designing effective chemopreventive strategies.

Retinoids

Vitamin A, synthetic and naturally occuring retinoids and β-carotene have attracted wide interest as possible chemopreventive agents against lung and oral cavity cancer. β-Carotene is directly absorbed by the intestine and a proportion of it is then converted to retinol. Additionally, β-apo-8´-carotenal, an excentric cleavage product of β-carotene, is a strong inducer of cytochrome P4501A1 and 1A2 enzymes in mice and rats (Gradelet *et al.*, 1996). Such induction of P450 enzymes might occur with high doses of β-carotene supplementation, as reported in ferrets, where the formation of β-apo-8´-carotenal was 2.5 times higher in lung extracts (Wang *et al.*, 1999).

The major established pathway of retinol activation involves mobilization of retinyl esters, reversible conversion of released retinol into retinal and irreversible conversion of retinal into the key functional all-*trans*-retinoic acid. In adult mammalian hepatic tissue, biosynthesis of all-*trans*-retinoic acid is catalysed primarily by ADH and ALDH, but other enzymes including P450 have also been reported to catalyse biosynthesis of all-*trans*-retinoic acid from precursor retinoids (Roos *et al.*, 1998). Rat liver microsomes in the presence of NADPH converted retinol to polar metabolites, including 4-hydroxyretinol (Huang *et al.*, 1999). This activity was also shown in a reconstituted monooxygenase system containing purified forms of rat P450 enzymes including CYP1B1. More recently CYP1A1 has been shown to oxidize retinal to retinoic acid. In human skin, CYP1A1 and CYP1A2 convert all-*trans*- and 9-*cis*-retinoic acid into corresponding isomers. The basal expression of *CYP1A1* and *CYP1A2* can be inhibited by all-*trans*-retinoic acid (Li *et al.*, 1995).

3-Methylcholanthrene(3-MC) and B[a]P can increase all-*trans*-retinoic acid catabolism in human skin or induce local tissue depletion. This is caused primarily by xenobiotic-mediated induction of CYP1A1, which also is involved in inactivation of all-*trans*-retinoic acid to 4-hydroxy-retinoic acid. Competitive inhibitory effects of vitamin A, all-*trans*-retinol, all-*trans*-retinal, all-*trans*-retinoic acid and retinyl palmitate on rat CYP1A1-dependent monooxygenase activity were observed in a reconstituted system containing the microsomal fraction prepared from recombinant *Saccharomyces cerevisiae* cells producing rat CYP1A1 and yeast NADPH-P450 reductase (Inouye et *al.*, 1999). Retinol and retinal decreased the mutagenicity of heterocyclic amines in the Salmonella/reversion assay, behaved as competitive inhibitors of isoquinoline-induced mutagenesis and strongly inhibited CYP1A1- and CYP1A2-dependent monooxygenases activities (Edenharder *et al.*, 1999). Retinol and retinoic acid were strong competitive inhibitors for xenobiotic oxidations catalysed by recombinant human CYP1A1 and CYP2C19 (Yamazaki & Shimada, 1999).

Ethanol and its major oxidative metabolite, acetaldehyde, both inhibit the generation of all-*trans*-retinoic acid (Deltour *et al.*, 1996). Concurrently, the CYP2E1-catalysed oxidation of ethanol can initiate lipid peroxidation via generation of a variety of free radicals. The lipid peroxides thus formed could then be converted via CYP2E1-catalysed reactions to alcohols and aldehydes, including *trans*-4-hydroxy-2-nonenal, that act as potent inhibitors of all-*trans*-retinoic acid synthesis. (Khalighi *et al.*, 1999). Prolonged use of alcohol, drugs or both accelerates the breakdown of retinol through cross-induction of degradative enzymes. There is also competition between ethanol and retinoic acid precursors. Depletion ensues, with associated hepatic and extrahepatic pathology, including carcinogenesis. Ethanol also interferes with the conversion of β-carotene to retinol (Leo & Lieber, 1999). Thus ethanol, while promoting a deficiency of vitamin A, also enhances its toxicity as well as that of β-carotene.

In conclusion, the role of microsomal cytochrome P450 in vitamin A metabolism and maintenance of vitamin A homeostasis should be considered in formulating chemopreventive strategies with retinoids, especially in the presence of exogenous exposure.

Human studies: The prevalence of an array of polymorphic genes was determined in a cohort of male smokers who participated in the α-tocopherol, β-carotene (ATBC) study among a fairly genetically homogeneous Caucasian population in Finland. Unlike *CYP1A1* and *CYP2E1* mutant frequencies, which in keeping with studies among other

Caucasian populations were low, most of the genes studied (including the *GSTM1*-null allele, *NQO1* Ser linked to loss of enzyme activity and *ADH3-2* lower enzyme activity) had a sufficiently high frequency in this population to allow investigation of gene–environment interactions (Woodson *et al.*, 1999a). In a nested case–control study within the ATBC cohort, *GSTM1*-null genotype was not associated with lung cancer risk in male smokers, but may have conferred a higher susceptibility with cumulative tobacco exposure. The association was attenuated by α-tocopherol but not by β-carotene supplementation (Woodson *et al.*, 1999b). One possible mechanism for this interaction may be the reported activation of carcinogens, as β-carotene has been shown to induce several carcinogen-metabolizing enzymes including CYP1A1/2 in ferrets (Wang *et al.*, 1999).

Tea polyphenols

Tea (black, green or oolong) is produced from the tea plant (*Camellia sinensis*) by various processing conditions. Black tea is produced by fermenting the leaves, green tea leaves are not fermented, while oolong teas are semi-fermented followed by a heating process to halt fermentation.

The primary catechins in green tea are epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate. Other polyphenols include flavanols and their glycosides and depsides, such as chlorogenic acid, coumaroylquinic acid and one that is unique to tea, theogallin (3-galloylquinic acid). Also present are quinic acids, carotenoids, trigalloylglucose, lignin, protein, chlorophyll, minerals, caffeine and very small amounts of other methylxanthines such as theophylline, theobromine and theanine. Depending on the amount of oxidation and condensation of catechins, green tea may also contain constituents commonly found in black tea, such as theaflavin, theaflavic acids, volatile compounds and thearubigens (Graham, 1992).

Tea polyphenols, specifically the catechins epigallocatechin-3-gallate, epigallocatechin and epicatechin-3-gallate, which account for 30–40% of the extractable solids of green tea leaves, are believed to mediate many of the cancer-chemopreventive effects. The chemopreventive potential of tea has been extensively reviewed (Yang & Wang, 1993; Weisburger, 1999), but the mode of action is still not clear. There appear to be a whole spectrum of activities with which these tea components interfere. Several plausible mechanisms have been put forward, including inhibition of ultraviolet radiation- and tumour promoter-induced ornithine decarboxylase, cyclo-oxygenase and lipoxygenase activities; antioxidant- and free radical-scavenging activity; enhancement of antioxidant (glutathione peroxidase, catalase and quinone reductase) and phase II (GST) enzyme activities; inhibition of lipid peroxidation; and anti-inflammatory activity. These properties of tea polyphenols make them effective chemopreventive agents against the initiation, promotion and progression stages of multistage carcinogenesis (Katiyar & Mukhtar, 1997). The pharmacokinetic properties of tea polyphenols are largely unknown. Tea catechins are rapidly absorbed and the addition of milk does not impair their bioavailability.

Inhibition of tumorigenesis by tea and tea polyphenols has been demonstrated in several rodent models, for sites including skin, lung, oesophagus, forestomach, duodenum, small intestine, colon and liver (Yang & Wang, 1993; Fujiki, 1999). After oral administration of tea preparations to animals, the activities of glutathione peroxidase, catalase, GST, NADPH-quinone reductase, UDPGT and methoxyresorufin *O*-dealkylase were moderately enhanced. Theaflavins have been shown to have inhibitory action against NNK-induced pulmonary hyperproliferation and tumorigenesis (Yang *et al.*, 1997, 1998). The inhibitory effect of tea on NNK-induced tumorigenesis has been explained in part by increased metabolism of NNK in the rat liver and decreased bioavailability in the lung (Chung *et al.*, 1998). The induction of hepatic cytochrome P450 enzymes such as CYPA2, 1A1, and 2B1 has been described in rats given either green or black tea (Sohn *et al.*, 1994). Caffeine has been identified as the active component in tea responsible for enzyme induction (Chen *et al.*, 1996). Glucuronidation and sulfation of tea polyphenols are the major elimination pathways, and competition among tea polyphenols for glucuronosyltransferase and sulfotransferase could inhibit epigallocatechin-3-gallate elimination. Oral administration of lyophilized green tea to female CD-1 mice stimulated liver microsomal glucuronidation of estrone, estradiol and 4-nitrophenol (Zhu *et al.*, 1998). The effects of green tea

flavonoids on 7-ethoxyresorufin-O-deethylase (CYP1A2), *p*-nitrophenol hydroxylase (CYP2E1), erythromycin-*N*-demethylase (CYP3A) and nifedipine oxidase (CYP3A4) were examined in human liver microsomes. Epicatechin-3-gallate was the most potent inhibitor of 7-ethoxyresorufin-O-deethylase in human liver microsomes. The effect of the green tea flavonoids on 7-ethoxyresorufin-O-deethylase was complex; in addition to inhibition at high concentrations of flavonoid, moderate activation was seen at lower concentrations (Obermeier *et al.*, 1995). Using standardized cell cultures, the green and black tea extracts and tea polyphenols were shown to inhibit B[a]P adduct formation with human DNA and induce GST and quinone reductase (Steele *et al.*, 2000). Activation of the mitogen-activated protein kinase pathway by green tea polyphenols (Lin *et al.*, 1999) might be responsible for the regulation of the antioxidant-responsive element which is believed to mediate the induction of phase II enzymes by many drugs, and may be stimulated by green tea polyphenols in the transcription of phase II detoxifying enzymes (Yu *et al.*, 1997).

Human studies: In contrast to the consistently observed inhibition of tumorigenesis by tea in many animal models, studies concerning the effects of tea on the incidence of human cancers have been inconclusive. Some epidemiological studies on the effect of tea ingestion on cancer risk have suggested an inhibitory effect, others an enhancing effect, and still others a lack of effect (Katiyar & Mukhtar, 1997; Yang, 1999; Goldbohm *et al.*, 1996).

A catechin esterase which converts epigallocatechin-3-gallate to epigallocatechin has been found in saliva. Holding a tea solution in the mouth for a few minutes without swallowing produced high salivary catechin levels, which were two orders of magnitude higher than in plasma. This suggests that slow drinking of green tea could be an effective way to deliver high concentrations of catechins to the oral cavity and oesophagus for prevention studies (Yang *et al.*, 1999). Human oral precancerous mucosal lesions have been reported to respond to tea (Li *et al.*, 1999).

Isothiocyanates

Glucosinolates are stable precursors of isothiocyanates, which are typically present in plants at high concentrations. They are hydrolysed to isothiocyanates by the coexisting but physically segregated enzyme myrosinase, which is released upon wounding (cutting, chewing) plant cells. Isothiocyanates and related substances, indoles, are responsible for the sharp taste in cruciferous (brassica) vegetables (broccoli, cabbage, Brussels sprouts, cauliflower, collards, kale, kohlrabi, mustard greens, rutabaga, turnips, bok choy). In the cruciferous family, the inducer activity is principally due to the highly reactive isothiocyanates (R–N=C=S; mustard oils). More than 20 isothiocyanates have been shown to inhibit the formation of carcinogen-induced tumours of several animal target organs. Of the indole glucosinolates which are predominant in brassica vegetables, glucobrassicin forms an unstable isothiocyanate which degrades into indole-3-carbinol. The isothiocyanate sulforaphane is the most potent naturally occurring inducer of phase II enzymes. A CYP2E1-mediated effect has been associated with the anti-genotoxicity of the broccoli constituent sulforaphane (Barcelo *et al.*, 1996). Young sprouts of broccoli and cauliflower contain much higher levels of glucoraphanin, the glucosinolate precursor of sulforaphane, than do the mature counterparts. Isothiocyanates prevent carcinogenesis in laboratory animals by blocking carcinogen activation or enhancing detoxification. Many isothiocyanates have one or the other type of activity or both in various test systems. Some isothiocyanates are inhibitors of specific cytochrome P450 isozymes in rodent tissues. Numerous isothiocyanates are potent inducers of GST and NADPH quinone reductase. Several effectively inhibit carcinogenesis and their effect can be remarkably specific. While benzyl isothiocyanate (BITC) inhibits lung tumour induction by B[a]P in A/J mice, phenethyl isothiocyanate (PEITC) does not. In contrast, induction of lung tumours by NNK is inhibited by PEITC but not by BITC. PEITC inhibits the metabolic activation of NNK in rat lung and is a selective inhibitor of certain P450 enzymes in rat liver, although it has limited effect on phase II enzymes (Verhoeven *et al.*, 1997; Hecht, 1999c).

Human studies: Several metabolic experiments in humans have consistently shown increased CYP1A2 activity after consumption of cruciferous

vegetables (Verhoeven *et al.*, 1997). A number of studies to test the effect of cruciferous vegetables in humans in the context of metabolic polymorphisms have been reported. Among Chinese in Singapore, urinary isothiocyanate levels were not dependent on either *GSTM1* or *GSTP1* genotypes, but urinary excretion was significantly higher in *GSTT1*-positive than in *GSTT1*-null subjects in the highest tertile of cruciferous vegetable intake. These results suggest the possible presence of inducers of GSTT1 in cruciferous vegetables (Seow *et al.*, 1998). *GSTM1* apparently indirectly plays a role in the induction of CYP1A2 activity. Among weekly consumers of cruciferous vegetables in a study among non-Hispanic whites, *GSTM1*-null individuals showed significantly higher CYP1A2 activity relative to *GSTM1*-non-null individuals. Cruciferous vegetables also induce CYP1A2 activity, and CYP1A2 activity was induced in *GSTM1*-null subjects, suggesting that cruciferous vegetables contain CYP1A2 inducers, which are probably deactivated in the presence of GSTM1 (Probst-Hensch *et al.*, 1998). GSTM1 rapidly conjugates isothiocyanates to GSH, leading to excretion (Zhang *et al.*, 1995). Lin *et al.* (1998) reported that although subjects in the highest quartile of broccoli intake had a low risk of colorectal adenomas, when stratified by the *GSTM1* genotype a protective effect was observed only among subjects with the *GSTM1*-null genotype. Among smoking volunteers, Hecht *et al.* (1995) showed that PEITC (watercress) inhibited the oxidative metabolism of NNK in humans similarly to rodents; 7 of 11 subjects had increased levels of urinary metabolites of NNK (NNAL and NNAL glucuronide). As PEITC can induce UDP glucuronosyltransferase activity and as that is the path of NNK excretion, it would be interesting to study whether polymorphisms in these enzymes would affect NNK detoxification.

Careful consideration should be given in future research to the fact that the plant polyphenols act synergistically in complex ways with other constituents of the plant. These unknown and poorly understood interactions could play a significant role in the anticarcinogenic efficacy of the polyphenol constituents. A lesson can be drawn from pharmaceutical agents available today: isolated extracts of one primary active constituent are generally not without side-effects.

Perspectives: metabolic polymorphisms and chemoprevention in humans

The evaluation of the net effect of chemopreventive agents in real life is difficult, as exposure to many different carcinogens, drugs, dietary factors and endobiotics occurs simultaneously. Several of these use the same metabolic pathways. Induction of a pathway that is protective against one group of compounds may potentiate the toxic or carcinogenic effects of another class or *vice versa*. Although bifunctional inducers such as indole-3-carbinol can be directly beneficial, as shown by the increased metabolism of aflatoxin B_1 to less toxic and carcinogenic metabolites due to induction of P450 enzymes, such a protective effect may not extend to PAHs. Further, prophylaxis based on inhibition of cytochrome P450, known to play a role in primary metabolism of a wide range of dietary and physiological compounds, carries a high risk of inducing adverse responses. A balance between activation and detoxification is the determining factor for the net effect. In contrast to bifunctional inducers (Prochaska & Talalay, 1988) which induce both phase II as well as selected phase I activities, monofunctional inducers elevate phase II metabolism without significantly affecting phase I activity. They appear to be the preferred agents for producing resistance against a wide range of chemical insults without adverse side-effects. The possibility of changed organotropy should also be considered; for example, decreased carcinogen metabolism in the liver could affect peripheral organs such as the lung, or increased metabolism in the liver could affect the kidney or bladder. A detailed understanding of mechanism of action and host genotype/phenotype profile is required before setting up chemoprevention trials.

Molecular epidemiological studies attempting to identify gene–carcinogen interactions and other mechanistic aspects in humans require comprehensive integration of genotype and phenotype biomarkers. IARC (Toniolo *et al.*, 1997; Vineis *et al.*, 1999) proposed that the design and analysis of molecular epidemiological studies should include:

- a clear definition of representative study populations and controls;

- a sample size adequate to provide the necessary statistical power;
- proper documentation (or measurement) of exposure to carcinogens or protective agents;
- avoidance of confounding due to mixed ethnic background of study subjects;
- study only of gene polymorphisms that have been shown to lead to altered phenotypic expression.

Future investigations should include both single and joint risk effects of multiple polymorphism combinations and should assess interaction between susceptibility genes and other endogenous (e.g., hormones) and exogenous (e.g., tobacco smoke, vitamin supplementation) risk factors. For appropriate and rigorous hypothesis testing, these studies will require a reasonable prevalence of relevant alleles. The data generated from the Human Genome Project and the Cancer Gene Anatomy Programme together with gene-chip technology have provided high-throughput means to look at general genetic damage as well as specific genetic changes at the molecular level. Such chips may be designed to evaluate subjects at risk, for example those carrying genetic polymorphisms, for determining appropriate target population for intervention strategies. Knowledge of the prevalence and distribution of common genetic susceptibility factors and the ability to identify susceptible individuals or subgroups will have substantial preventive implications, in particular if more data are collected showing that people with certain 'at-risk' genotypes are more susceptible to low levels of carcinogens. It is conceivable that such subjects could be (i) more easily persuaded to avoid hazardous exposures such as tobacco, (ii) targeted for intensive smoking cessation programmes, (iii) be enrolled in chemoprevention trials and (iv) be involved in cancer screening programmes that would not be appropriate for the general population.

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