Polymorphisms of Xenobiotic-Metabolizing Enzymes and Susceptibility to Cancer

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The variation in individual responses to exogenous agents is exceptionally wide. It is because of this large diversity of responsiveness that risk factors to environmentally induced diseases have been difficult to pinpoint, particularly at low exposure levels. Opportunities now exist for studies of host factors in cancer or other diseases in which an environmental component can be presumed. Many of the studies have shown an elevated disease proneness for individuals carrying the potential at-risk alleles of metabolic genes, but a number of controversial results have also been reported. This article is an overview of the data published to date on metabolic genotypes related to individual susceptibility to cancer. — *Environ Health Perspect* 107(Suppl 1): 37–47 (1999). *http://ehpnet1.niehs.nih.gov/docs/1999/Suppl-1/37-47hirvonen/abstract.html*

Key words: *CYP1A1, CYP2D6, CYP2E1, GSTM1, GSTM3, GSTP1, GSTT1, NAT1, NAT2,* genetic polymorphisms

People living in industrialized countries are exposed extensively to chemicals that cause mutations, cancer, and birth defects. It is well established that, e.g., lung carcinogenesis in humans is caused mainly by cigarette smoking. However, not all smokers develop pulmonary cancers. Cancer-causing chemicals, or chemical carcinogens, require metabolic activation to react with cellular macromolecules. Mutations in genes encoding enzymes or proteins involved in cellular control such as oncogenes and tumor-suppressor genes result in uncontrolled cell growth and cancer (*1,2*). Steps required for the carcinogenesis process include: *a*) metabolic activation of a carcinogen by cellular xenobiotic-metabolizing enzymes, *b*) binding of the active metabolite to DNA to produce a DNA adduct, *c*) faulty repair of the adduct to produce a gene mutation, *d*) cell replication to fix the mutation to the genome, and *e*) progression to a full neoplasm of the replicating cell containing the mutated genes. This progression is often accompanied by further genetic alterations in other cell-cycle control genes that occur through gene mutations, gene rearrangements, and gene/chromosome deletion (*3,4*). The overall process can occupy a major portion of the lifespan of an individual.

The paradigm for mechanism of action of chemical carcinogens has been well established in model cell culture and animal systems, and studies in humans appear to support the possibility that most cancers are initiated by chemical/dietary exposures and proceed through various stages of preneoplastic lesions consisting of partially transformed cells to full metastatic cancers (*1*). In rodent models, the progression stage can be enhanced by treatment with tumor promoters, which themselves do not necessarily exhibit the properties of carcinogens (*5*). These chemicals are thought to mediate cell proliferations that fix the mutation in the genome. Another class of chemicals called nongenotoxic carcinogens has been described in rodent model systems (*6–8*). These agents are not metabolically activated to genotoxic derivatives but presumably alter cell-cycle control. Many nongenotoxic carcinogens are also tumor promoters. However, their mechanisms of action are not presently known.

It is widely held that humans differ in their susceptibilities to cancer. Certain individuals may be more susceptible, whereas others are more resistant to cancer. This may be due to a number of factors including health, nutritional status, and gender. From what is known about the mechanism of action of carcinogens, it is thought that genetic background could play a significant role. The obvious candidate genes are those encoding the xenobiotic-metabolizing enzymes (XMEs) that activate or inactivate carcinogens (*9,10*). Variable levels of expression of these enzymes could result in increased or decreased carcinogen activation. In fact, it is well established that genetic differences occur in expression of the XMEs. Scientists have been aware of genetically based differences in sensitivity to therapeutically used drugs for more than 30 years. This knowledge led to a field known as pharmacogenetics (*11*). Historically, this term was used to describe genetic differences in drug metabolism, but the field later expanded into the area of cancer susceptibility (*12*).

XME Polymorphisms and Cancer Susceptibility

Cytochromes P450s

The cytochrome P450 (CYP)-dependent monooxygenases represent the first line of defense against toxic lipophilic chemicals because they catalyze reactions involving incorporation of an atom of molecular oxygen into the substrate (*13*). The resulting increase in hydrophilicity facilitates further metabolic processing and excretion. Unfortunately, certain chemicals are activated to their ultimate carcinogenic form rather than being detoxified. Most carcinogen activation occurs through generation of epoxides or *N*-hydroxy intermediates that are further metabolized by transferases.

The main CYPs in humans that metabolize carcinogens are CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1, CYP3A4, and CYP3A5 (*14*). These enzymes have specificities for various classes of carcinogens and genetic polymorphism has been identified for most of them (*13–16*). CYPs are most extensively expressed in the liver although their levels of expression vary depending on the P450 form (*17*). These interindividual differences in expression may be due to the genetic polymorphisms or the extent of induction. Certain forms are also expressed in lung, gastrointestinal tract, kidney, and larynx/nasopharangeal tissue. In nonhepatic epithelial tissues, activation of carcinogens probably occurs directly in the cells being transformed although arylamines and heterocyclic amines are partially activated in the liver and transported to extrahepatic target sites where they undergo full activation.

Manuscript received at *EHP* 8 July 1998; accepted 28 September 1998.

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Abbreviations used: AHH, aryl hydrocarbon hydroxylase; AHR, aryl hydrocarbon receptor; BaP, benzo[*a*]pyrene; CYP, cytochrome P450; EPHX, mEH gene; GST, glutathione *S*-transferase; mEH, microsomal epoxide hydrolase; MPO, myeloperoxidase; NAT, *N*-acetyltransferase; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; PM, poor metabolizer; RFLP, restriction fragment length polymorphism; UM, ultrarapid metabolizer; XME, xenobiotic-metabolizing enzyme.

Expression of certain forms of CYP depends on their induction. Induction of *CYP1* genes is mediated by the transcription factor called the aryl hydrocarbon receptor (AHR), a member of a small family of proteins called the basic-helix-loop-helix transcription factors (*18,19*).

The first CYP polymorphism was identified for CYP2D6 based on the occurrence of adverse drug reactions to the cardiovascular drugs debrisoquine and sparteine and aptly termed the debrisoquine/sparteine polymorphism (*20*). Individuals who are metabolically competent are referred to as extensive metabolizers, and those who are incapable of metabolism of these drugs are poor metabolizers (PMs). Over 40 drugs are known to be substrates for CYP2D6 (*20*). This polymorphism exhibits marked ethnic differences in its frequency; 5 to 10% of Caucasians but < 1% of Asians lack expression of active enzyme because of deficient *CYP2D6* alleles. More than 10 partially or totally inactive variant alleles of *CYP2D6* have been characterized (*21,22*).

The most common defective *CYP2D6* allele among Caucasians is *CYP2D6*4*, which is characterized by a base substitution in the splice site at the intron 3/exon 4 boundary that leads to a frameshift (*21,22*). This allele was previously called *CYP2D6B* and accounts for more than 70% of all the inactivating alleles in Caucasian populations. Another variant allele, *CYP2D6*3* (previously called *CYP2D6A*), consists of a single base pair deletion in the coding sequence in exon 5 and also causes a frameshift. This allele accounts for approximately 5% of the alleles and leads to a loss of CYP2D6 enzyme activity (*21,22*). The third loss of enzyme activity (~10–15% of the inactivating alleles) is caused by the deletion of the entire *CYP2D6* gene (*CYP2D6*5*, previously called *CYP2D6D*). By analyzing these three polymorphic sites, it is possible to identify at least 95% of European PMs (*23,24*). More recently an allele representing amplification/duplication of the gene (*CYP2D6*2XN*) has been described (*25*). Individuals who inherit more than two copies of the *CYP2D6* gene have been found to have very high CYP2D6 enzyme activity and consequently are designated ultrarapid metabolizers [UMs; (*26*)]. The frequency of the duplicated allele seems to vary widely between populations of different ethnic origins. About 1% of the Swedish, Germans, Chinese, and black Zimbabweans are UMs (*27–30*). Among Spaniards, however, the frequency is 7% (*31*), and a very high prevalence has been observed among Saudi Arabians [21%; (*32*)] and Ethiopians [29%; (*33*)].

Many studies have been conducted, with conflicting results, on the potential association between polymorphic expression of CYP2D6 and the incidence of various types of cancer (*34,35*). However, the combined results of several studies in various parts of the world suggest a significant but small decrease in risk of lung cancer for individuals with the *CYP2D6* PM genotype (*36*). In keeping with this, an excess risk of lung cancer was recently associated with high CYP2D6 activity in heavy smokers only, a finding that may partly explain the inconsistent findings (*37*).

The *CYP1A* gene family has two members: *CYP1A1*, which is predominantly expressed in extrahepatic tissues such as the lung, and *CYP1A2*, which is concentrated in the liver (*22*). *CYP1A1* and *CYP1A2* have overlapping catalytic activity and are both thought to play an important role in carcinogen activation. *CYP1A1* is involved, e.g., in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs) to their carcinogenic metabolites in the lung (*22*). As an example, *CYP1A1*-dependent aryl hydrocarbon hydroxylase (AHH) activities in human lung tissue (microsomes) correlate with activation of benzo[*a*]pyrene 7,8-diol to the ultimate carcinogen (*38,39*). Furthermore, the AHH activities correlated with the benzo[*a*]pyrene 7,8-diol-9,10-epoxide (BaPDE) DNA adduct levels in human lung tissue (*40*).

Interindividual variations in the *CYP1A1*-mediated AHH activity appear to have an as yet unknown genetic basis. Using mitogen-stimulated peripheral blood mononuclear cells, Kellerman and coworkers (*41*) observed a trimodal distribution of AHH induction consistent with a codominant inheritance at a single genetic locus segregating for a more common allele conferring low inducibility and a rarer allele conferring high inducibility. At a later time two closely linked genetic polymorphisms were detected within the *CYP1A1* gene. The first polymorphism detected was a point mutation in the 3′ flanking region of the gene, a restriction fragment length polymorphism (RFLP) detected by *Msp*I restriction enzyme (*42*). Another polymorphic site was found to be located in exon 7, where a nucleotide substitution causes an Ile-to-Val amino acid change in the heme-binding region of the enzyme (*43*). Both the *CYP1A1 Msp*1 and Ile/Val variant alleles are much more prevalent in Asians than in Caucasians. More recently a third polymorphism has been reported in exon 7 (*44*). However, the effects of these genetic polymorphims on CYP1A1 enzyme activity thus far have remained obscure (*44–47*).

The expression of CYP1A1 is regulated by the cytoplasmic AHR, together with AHR nuclear translocator and several other regulatory proteins (*48,49*). Because no clear correlations have been observed between *CYP1A1* allelic variants and lung cancer incidence in Caucasians, it has been suggested that variations in susceptibility to lung cancer may in fact be attributed to polymorphisms in these genes affecting the CYP1A1 inducibility rather than the *CYP1A1* gene itself.

Subsequent to the report suggesting that the extent of inducibility of CYP1A1 was increased in lymphocytes from lung cancer patients compared to controls (*41*), a number of attempts were made to confirm these findings [reviewed by d'Errico et al. (*50*)]. Strong correlations between lung cancer risk and homozygosity for the *CYP1A1* variant alleles have been reported in several Japanese studies (*42,43,51,52*). However, although a similar association was also reported in an American population (*53*), no such association was found in Europeans (*54–58*). Recent reports that suggest an association between increased risk for breast cancer (*59*) and endometrial cancer (*60*) among Caucasian females also remain unconfirmed.

CYP1A2 metabolizes aflatoxin B_1, various heterocyclic and aromatic amines, and certain nitroaromatic amines (*61*). No genetic polymorphism has yet been characterized in the *CYP1A2* gene, but considerable individual variations have been reported both in the level of expression in the human liver (*62*) and in the rate of metabolism of CYP1A2 substrates, including aromatic amines (*61,63,64*). *CYP1A2* polymorphism, therefore, may well be an important modifier of individual susceptibility to environmentally induced cancers.

CYP2A6 is subject to genetic polymorphism that is detected by an inability of certain people to carry out the 7-hydroxylation of coumarin (*65–68*). Only three variant alleles have been found that encode inactive *CYP2A6* (null alleles) (66,69). It has recently been suggested that individuals carrying the *CYP2A6*-null alleles are less susceptible to develop

tobacco-related cancers because they have decreased risks of becoming addicted to smoking (*70*). Moreover, if they do become dependent, they seem to smoke less than those without impaired nicotine metabolism. Because tobacco smoke contains nitrosamines that can be activated to carcinogens by CYP2A6, these individuals may also be less efficient in activating the tobacco smoke-derived procarcinogens.

In addition to CYP1A1 and CYP1A2, CYP2C9 also appears to play a role in the oxidative metabolism of benzo[*a*]pyrene (BaP). Allelic variants of *CYP2C9* with functional repercussions have been identified (*71*). Recently a slight increased risk of lung cancer was associated with *CYP2C9*2*, which is the most common variant allele in Caucasians (*72*), but contradictory findings have also been reported (*73*).

Inactive *CYP2C19* alleles result in poor metabolism of *S*-mephenytoin, which has been shown to be more prevalent in Asians than in Caucasians (*74*). The latter have approximately 1 to 2% PMs, whereas the former have up to 25% PMs. Interestingly, this is opposite for the findings on *CYP2D6* polymorphism. The *CYP2C19* polymorphism is thought to be of little clinical significance because of the large therapeutic indices of the drugs currently in use that are metabolized by CYP2C19.

Several base changes distinguishable by RFLP analyses have been found in *CYP2E1* gene (*75–79*). Although these polymorphisms do not appear to alter the primary sequence of the enzyme, an effect on gene transcription has been suggested (*80*). However, no correlation has been found between the variant alleles of *CYP2E1* and its expression *in vitro* or *in vivo* (*81–87*). In a Japanese study, individuals homozygous for the variant *Dra*I alleles of *CYP2E1* were reported to have decreased lung cancer risk, especially individuals with high cumulative smoking doses (*88,89*). This genotype was found less frequently in the Finnish than in the Japanese population (*90*). Moreover, no differences were observed in the frequency of this genotype between lung cancer patients and controls, a finding that agreed with Swedish observations (*91*). Also, the variant *Rsa*I allele was extremely rare among Scandinavians (*90,91*). However, a Swedish study suggested that homozygosity for the *Rsa*I allele poses an increased risk of lung cancer (*91*), whereas a Taiwanese study suggested that this allele was associated with increased risk of nasopharyngeal carcinoma (*92*).

Epoxide Hydrolase

Microsomal epoxide hydrolase (mEH) is an enzyme involved in the first-pass metabolism of highly reactive epoxide intermediates. It catalyzes, with broad substrate specificity, the conversion to less toxic *trans*-dihydrodiols of highly reactive, cytotoxic arene oxides and aliphatic epoxides (*93*). The enzyme acts coordinately with, for example, CYP1A1 and CYP1A2 to inactivate deleterious polycyclic hydrocarbon oxides and epoxides. Further epoxidation of the diol group can convert inactive diols to highly toxic, mutagenic, and carcinogenic polycyclic hydrocarbon diol epoxides (*94*). Thus, epoxide hydrolase exhibits the same dual role of procarcinogen detoxification and activation found in some CYPs and, consequently may also play an important role in epoxide toxicity.

The mEH enzyme is expressed in all tissues thus far examined, with highest levels in the liver, kidney, and testis, and 10- to 100-fold lower levels in the lung and lymphocytes (*95–97*). Within cells, mEH is localized mainly to the endoplasmic reticulum where it can transiently associate with the CYP mixed-function oxygenase system (*98*). Endogenous substrates for mEH have not been readily identified. However, the high degree of mEH structural conservation between several mammalian species and apparent ubiquitous tissue expression imply that mEH has an important role in cellular metabolism (*96*).

Interindividual differences in mEH activity ranging from several- to 40-fold have been reported in various human tissue types (*96*). The molecular basis for variation in mEH activity has not yet been characterized completely. Genetic polymorphisms have, however, been identified within exons 3 and 4 of the mEH gene (*EPHX*) (*99,100*), which results in His_{113}-Tyr and Arg_{139}His amino acid substitutions, respectively. *In vitro* expression analyses indicated that the corresponding mEH activities decrease approximately 40% (Tyr_{113}) or increase by at least 25% (His_{139}). The activity level observed in the presence of both variations approximates that observed for the wild-type genotype (*100*). Recently a genetic variation in the 5′ flanking sequence of *EPHX* was observed. This may be an additional contributing factor to the range of functional mEH expression existing in human populations (*101*).

Data from the few studies addressing a possible association between *EPHX* polymorphisms and cancer support a dual role for the mEH in the carcinogenic process. It has been suggested that the *EPHX* His_{113} variant allele increases the risk of aflatoxin-associated hepatocarcinoma (*102*) but decreases the risk of ovarian cancer (*103*). With regard to lung cancer, no significant association was found to the *EPHX* genotypes (*104*).

Glutathione *S*-Transferases

Among the detoxification systems, the glutathione *S*-transferases (GSTs) play a critical role in providing protection against electrophiles and products of oxidative stress (*105*). GSTs are a superfamily of enzymes that have broad and overlapping substrate specificities. Four families of cytosolic soluble GSTs have so far been identified in humans and are referred to as alpha, mu, pi, and theta (*105*). The known substrates for GSTs in cigarette smoke are those derived from in bioactivation from PAHs, namely, PAH diolepoxides. The most studied carcinogenic PAH diolepoxide, BaPDE, is a good substrate for many GST isoforms like GSTM2, GSTM3, and especially for GSTM1 and GSTP1 (105,106). In general, class mu enzymes show highest activities with most epoxides.

To date, genetic polymorphism has been found in four of the *GST* genes. One of these is *GSTM1*, which is expressed in only about half of Caucasians because of a homozygous deletion (null genotype) of the gene in the other half (*107*). In addition to the null genotype, two functional alleles denoted as *GSTM1*A* and *GSTM1*B* have been described. These alleles differ by a base substitution ($C_{534}G$) in the latter, which has not been shown to affect GSTM1 activity.

In several recent studies an increased risk of cancer has been observed among *GSTM1* null smokers, but several conflicting reports also exist (*50,108–110*). In light of the compiled data it has been estimated that 17% of both lung cancers (*110*) and bladder cancers (*111*) may be attributable to *GSTM1* genotypes. Although these values provide only a crude measure of the potential population impact of these genes, they suggest that *GSTM1* deficiency could contribute to a substantial incidence of cancer at the population level. In contrast, at the individual level the risk associated with the *GSTM1* null genotype may be smaller than has been anticipated.

GSTM3 is one of the most abundant GSTs in human lungs (*112–114*). As a deviation from the wild-type *GSTM3*A* allele, the variant allele *GSTM3*B* carries a

deletion of three base pairs in intron 6, which results in the generation of a recognition sequence for the YYI transcription factor. The functional consequence of this is still unclear, but both negative and positive regulatory effects have been suggested (*112,115*).

People with low expression of GSTM3 were previously observed to be at increased risk of developing adenocarcinoma of the lung (*114*). Recent genotyping studies indicate that individuals who are homozygous or heterozygous for the *GSTM3*B* alleles have lower risk of cancers of the larynx (*116*) and lung (*117,118*) than individuals with the homozygous wild-type genotype.

The third polymorphic *GST* gene *GSTP1* encodes an isoform that is known to metabolize many carcinogenic compounds, among them BaPDE. Given that GSTP1 is the most abundant GST isoform in the lungs (*113*), it is thought to be of particular importance in the detoxification of inhaled carcinogens. Two variant alleles, *GSTP1*B* and *GSTP1*C*, have been detected in addition to the wild-type allele *GSTP1*A*. *GSTP1*B* has an $A_{313}G$ transition in exon 5, causing $Ile_{104}Val$ amino acid change. In addition to this base substitution, *GSTP1*C* allele has a $C_{341}T$ transition, resulting in a $Ala_{113}Val$ amino acid change. Both of the affected codons are in the electrophile-binding site of the GSTP1 enzyme (*119*). Compared to *GSTP1*A*, proteins encoded by *GSTP1*B* and *GSTP1*C* have been shown to have decreased enzyme activity when expressed in *Escherichia coli* (*119, 120*). Individuals homozygous for the *GSTP1*B* alleles have been suggested to detoxify the ultimate carcinogen of BaP, i.e., (+)-*anti*-BaPDE, more efficiently than heterozygotes or wild-type homozygotes (*121*). Hence, they could also be less susceptible to the carcinogenic effects of BaP.

In a recent study, a 3-fold increased risk of bladder and testicular cancer was observed for individuals homozygous for the *GSTP1* low-activity alleles (*GSTP1*B* and *GSTP1*C* alleles not differentiated) compared to controls (*122*). A similar association was also reported for cancers of the larynx (*123*) and lung (*124*), followed by both supporting and contrasting findings (*73,118,125,126*).

A deletion polymorphism similar to that observed for *GSTM1* has also been discovered for the *GSTT1* gene (*127*). The prevalence of *GSTT1* null individuals shows a wide variation among ethnically different populations; in Caucasians the prevalence is 10 to 20% (108). *GSTT1* participates in detoxification of potentially carcinogenic monohalomethanes (*128*) and reactive epoxide metabolites of butadiene (*129,130*), both of which are constituents of tobacco smoke. The *GSTT1* null genotype has been associated with increased risk of lung (*131*) and larynx cancers (*132*), but like the *GSTM1* null genotype, controversial reports also exist (*125,133–135*).

Because different GST isoenzymes have overlapping substrate specificities (*105*), deficiencies of GST isozymes may be compensated for by other isoforms and use of alternative metabolic pathways. This may be one reason for the abundance of controversial data on *GST* polymorphisms and cancer proneness (*136*).

N-Acetyltransferases

N-Acetylation polymorphism causes individual variations in biotransformation of various xenobiotics with primary aromatic amine or hydrazine structures (*137,138*). The NAT2 (*139*), which was until recently thought to be the only polymorphic *N*-acetyltransferase (NAT), is responsible for the well-known inherited interindividual variation in the ability to acetylate substrates such as the arylamine drugs procainamide and sulfamethazine, the arylamine carcinogen benzidine, and some hydrazine drugs such as isoniazid and hydralazine (*137,138*). Recently another human *N*-acetyltransferase, NAT1 (*138*), which is widely expressed in tissues (*140*) and cultured cells (*141*), has also been found to be polymorphic (*142*).

These findings may be of great clinical and toxicologic importance because certain chemicals may be *N*-acetylated to a significant degree by both NAT1 and NAT2. These include the carcinogenic aromatic amines 2-aminofluorene, benzidine, 4-aminophenyl, 4,4-dichloroaniline, and 2-naphthylamine (*143–148*), and the cancer chemotherapeutic agent dinaline (4-amino-*N*-[2′-aminophenyl] benzamide) (*149*). They are encoded at two distinct loci on chromosome 8p21.3-23.1 along with *NATP*, a pseudogene that does not encode a functional protein (*150*). The new nomenclature of *NAT1* and *NAT2* alleles used henceforth in this review is based on the consolidated classification system of Vatsis et al. (*151*).

Seven *NAT1* alleles in human populations have been reported in the literature (*150*). The *NAT1*4* allele is denoted as the wild type. A prominent change in one of the variants (*NAT1*10*), which has an alteration of the consensus polyadenylation signal (*142*), was recently reported to be associated with both higher NAT1 activity in bladder and colon tissue and DNA adduct levels in the colon tissues (*152,153*). Given that *NAT1* has been reported to be primarily responsible for the NAT activity in the human uroepithelium (*154*), these findings are of special interest in studies on bladder cancer risk. The association between the *NAT1*10* allele and NAT1 activity *in vivo* has not been confirmed in subsequent studies. This may be partly explained by previous misclassifications of a recently described *NAT1*14* allele having $G_{560}A$ base substitution ($Arg_{187}Gln$) in combination with the $T_{1088}A$ and $C_{1095}A$ substitutions present in *NAT1*10* allele. This allele produces a defective NAT1 protein, which leads to functional impairment in the metabolism of NAT1-selective substrates both *in vitro* and *in vivo* (*150*). In the *NAT1*3* allele only the latter substitution is present in contrast to the wild-type *NAT1*4* allele, whereas in the *NAT1*11* allele, several changes are found in addition. Recently an allele (*NAT1*17*) was reported that was suggested to differ from the *NAT1*11* allele in that it also has a $G_{445}A$ base substitution ($Val_{149}Ile$). Subsequently, however, researchers have agreed that *NAT1*11* also contains this substitution and that the *NAT1*17* designation will be used for some future new alleles (*155*). Consequently, it is now thought that the previous findings that the $Val_{149}Ile$ amino acid change correlates with increased *N*-acetylation activity (*156*) applies to the *NAT1*11* allele. In the *NAT1*15* allele, $C_{559}T$ substitution ($Arg_{187}Stop$) results in truncated protein and total loss of NAT1 activity. The functional repercussions of two additional variants, *NAT1*5* and *NAT1*16*, remain to be determined (*150*).

With regard to the *NAT2* gene, in addition to the wild-type allele *NAT2*4*, at least 23 different *NAT2* mutations have been found to date [for additional references, see Grant et al. (*150*)]. Seven of the nine observed nucleotide transitions lead to amino acid changes, whereas the remaining two base substitutions exert no influence on the amino acid sequence (*150*). Several allelic variants of *NAT2* reportedly result from certain combinations of these nine base substitutions. Rapid acetylators have at least one wild-type *NAT2*4* allele, whereas slow acetylators have inherited two slow acetylation-associated alleles.

Investigators have reported a wide range of values for acetylation activity in

different groups (*157*). From the few population studies currently completed on *NAT1*, it appears that the *NAT1* putative fast acetylator alleles are found in frequencies ranging from 15 to 25% in Caucasians to 50% in Asians; *NAT1*4* and *NAT1*10* are the most prevalent alleles in Caucasians (*158–160*). The predominance of the putative *NAT1* slow acetylator status-associated genotype (homozygous or heterozygous for *NAT1*10*) has been reported to be about 70% among British Caucasians (*158*), 61% among French Caucasians (*160*), and 50% among an American population consisting of Caucasians, African Americans, and Latinos (*159*).

The frequencies of *NAT2* slow acetylator alleles range from 5% in Japan to 90% in Egypt (*150,161*). The predominance of the *NAT2* slow acetylator genotype has been reported to be about 60% among Germans (*162,163*), 53% among American Caucasians (*163*), 63% among Poles (*164*), and 50% among Finns (*165*). In contrast, in the Japanese or Chinese populations, the rapid genotype is largely overrepresented (92 and 80%, respectively) (*166,167*).

Previous phenotyping studies as well as subsequent genotyping studies have suggested a modifying role for *NAT* genotypes in all major cancer sites. Two main types of biologic mechanisms could explain these findings (*168*). First, CYP-mediated *N*-hydroxylation of arylamines yields electrophilic intermediates that are inactivated by conjugation with glucuronide or acetylation by NATs (*161, 169*). In urinary bladder carcinogenesis, *N*-acetylation of arylamines is a competing pathway for *N*-oxidation. The unconjugated *N*-hydroxy metabolites can enter the circulation, undergo renal filtration, and be transported to the urinary bladder (*170*). A number of previous phenotyping studies provided evidence that the NAT2 slow acetylator phenotype is a significant risk factor for the occurrence of bladder cancer, particularly for individuals occupationally exposed to arylamines. Subsequent genotyping studies supported the important role of NAT2 slow acetylation status as a risk factor for arylamine-induced bladder cancer (*168,171,172*). There is, however, also the possibility that slow acetylators survive longer than rapid acetylators in patients with bladder cancer (*173*). Recent data suggest that a prominent variant allele of *NAT1* (*NAT1*10*) associated with increased enzyme activity is also a risk factor for smoking-related bladder cancer (*174*).

Another area of research is based on the hypothesis that fast acetylators are at increased risk for cancers at sites other than the bladder because of the activation of procarcinogens such as heterocyclic amines. Exposure to heterocyclic amines is fairly common; these potent mutagens and rodent carcinogens are formed when meat and fish are cooked at household temperatures. The heterocyclic amines are poor substrates for *N*-acetylation in human liver, but they readily undergo hepatic *N*-oxidation and subsequent *N*-glucuronidation, which results in conjugated *N*-hydroxy metabolites that can be transported to the colonic lumen (*175*). In colonic mucosa, the *N*-hydroxy derivatives are good substrates for *O*-acetylation, which results in reactive *N*-acetoxyarylamines capable of forming covalent DNA adducts (*170*). The association between the *NAT1* fast acetylator trait and colorectal tumors could be due to enhanced *O*-acetylation of aromatic amines in cigarette smoke or to heterocyclic amines in cooked meat because both smoking and high intake of red meat have previously been associated with colorectal cancer (*176,177*). The role of NAT1 activity is less clear if heterocyclic amines are the aromatic amine compounds of primary relevance to human colorectal cancer. Some data indicate that among the acetyltransferases, NAT2 is more important than NAT1 for bioactivation of heterocyclic amines *in vitro* (*178–181*).

Several previous phenotyping studies (*168*) suggest that rapid acetylators are at higher risk to develop cancer of the colon. Several recent genotyping studies have reached a similar conclusion (*168*). Moreover, preliminary data suggest that the *NAT1*10* allele is also a risk factor in smoking-related colon cancer (*158,182*).

The *N*-acetylation phenotype also has been widely studied in relation to susceptibility to breast and lung cancer. Several case–control studies compared the prevalence of the slow acetylator phenotype in breast cancer patients with the prevalence found in controls; their outcomes were mixed (*168*). Similarly, a recent genotyping study indicated an increased risk of breast cancer for slow NAT2 acetylators who smoked 20 or more cigarettes per day (*183*). However, two subsequent studies provided little evidence of an association between the *NAT2* genotypes and breast cancer (*184,185*).

Other studies have evaluated the utility of acetylation as an indicator of risk for pulmonary malignancies and liver cancer. A set of four phenotyping studies yielded inconclusive results about the potential association between the NAT2 acetylator status and lung cancer risk [for a review, see Hirvonen (*168*). Subsequent genotyping studies also did not give any conclusive evidence (*186–188*). However, the potential role of *NAT* genotypes as modifiers of individual responses to environmental agents is supported in three recent studies that found that the *NAT2* slow acetylator genotype posed an increased risk of mesothelioma (*189*) and hepatocellular carcinoma (*190*), whereas the *NAT1* high-activity allele increased risk of smoking-related lung cancer (*160*).

It is possible that *N*-acetylation is an important detoxification step in environmental exposures. The combination of the *NAT1* and *NAT2* susceptible genotypes possibly is a particularly unfavorable genotype composition in arylamine exposures. In support of this possibility, Bell et al. (*158*) recently observed that the association between increased risk of colorectal cancer and the fast *NAT1* acetylator allele (*NAT1*10*) was most apparent among fast *NAT2* acetylators. Moreover, this genotype combination together with high red meat intake caused a remarkably increased risk of colon cancer (*182*). Further addressing the potential importance of individual acetylation capacity, Badawi et al. (*153*) found that the carcinogenic DNA adduct levels in the mucosa of the urinary bladder were highest in arylamine-exposed individuals who had inherited both the slow *NAT2* acetylator genotype and the rapid *NAT1* acetylation-associated (*NAT1*10*) allele.

NAD(P)H:Quinone Oxidoreductase

NAD(P)H:quinone oxidoreductase reduces quinones to dihydroquinones, a reaction considered to be critical in the detoxification of these highly reactive metabolites (*191*). It is an important enzyme in both activation and detoxification pathways known to protect against the carcinogenicity and mutagenicity of quinone compounds and their metabolites and to activate procarcinogenic compounds (*192*). A polymorphic allele of the human *NQO1* gene, with an amino acid change causing low catalytic activity (*193–195*), recently was associated with increased susceptibility to malignancies such as colon and lung cancer (*195–198*).

Other Potentially Relevant Xenobiotic-Metabolizing Enzymes

A number of polymorphic metabolic enzymes other than those previously

mentioned exist that may also be important in individual variations of susceptibility to cancer. Myeloperoxidase (MPO) is an enzyme found primarily in the lysosomes of neutrophils. Exposure to a variety of pulmonary insults such as cigarette smoke stimulates recruitment of neutrophils into human lung tissue (*199*) and local release of MPO (*200,201*). MPO activates carcinogens such as BaP and aromatic amines in tobacco smoke (*170,202*). An allelic variant with a G to A base substitution in the promoter region of the *MPO* gene recently has been shown to result in reduced gene transcription. Homozygotes for the variant allele recently have been suggested to be less susceptible to lung cancer (*203*).

Sulfotransferases, which exist as a superfamily, can participate in the metabolic activation of arylamine and heterocyclic amine carcinogens (*204*).

The uridine diphosphate (UDP)-glycosyltransferases (UGTs) conjugate active metabolites of carcinogens and multiple forms are expressed in liver and extrahepatic tissues (*205–207*). UGTs can also participate in the metabolism of arylamines and heterocyclic amines. Although genetic defects in a form of UGT that conjugates bilirubin have been described, genetic differences in their expression have not been demonstrated (*208*).

The flavin-containing monooxygenases (FMOs) are a superfamily of xenobiotic-metabolizing enzymes that oxidize numerous nucleophilic compounds (*209,210*). These enzymes primarily carry out the inactivation of drugs and do not activate the common classes of carcinogens (*209*). A low-frequency polymorphism was found in FMO A1. This gives rise to a condition called trimethylaminurea or Fish Odor Syndrome, which is due to an individual's inability to carry out the *N*-oxidation of tertiary aliphatic amines found in foodstuffs (*210*).

The serum paraoxonase/acetylesterase catalyzes the hydrolysis of organophosphate pesticides such as paraoxon, carbamates, and carboxylic acid esters. It also hydrolyzes mustard gas and Sarin. A genetic polymorphism resulting in a high-activity and a low-activity allele has also been found in this enzyme (*211,212*).

Future Directions

It is anticipated that rapid advances will be made in methodology to determine potential metabolic at-risk genotypes. These advances may include less invasive collection methods for test samples (e.g., buccal cell and urinary cell samples), automated DNA extraction combined with robotic sample handling, and high-density oligonucleotide array-based genetic test methods. At present, many research laboratories are conducting association studies and contradictory reports are emerging inpthe literature. Several sources of potential bias exist that partly account for these divergent findings, usually an initial small study showing a positive association. This raises the important issue of power calculations in planning subsequent studies. High profile reporting to the public of results of studies that may ultimately turn out to be erroneous is also problematic in this context. Also, there recently has been debate about publication bias—selective publishing of only positive associations.

If the potential biases mentioned above are carefully controlled, genetic screening studies may in the near future help us identify susceptible individuals and subgroups in environmentally exposed populations. Companies offer gene tests to individuals and employers. As long as this testing is not scientifically and ethically above reproach, it can benefit only companies selling the tests. There is an urgency to address several important ethical questions with regard to societal and public health. For instance, should insurance companies and employers be allowed to use genetic testing to discriminate against people based on their genotypes? Although such testing undoubtedly would be beneficial if used to ensure that the workplace is safe for everyone, including the most sensitive individuals, it might also be used for denial of employment, health insurance coverage, or life insurance policies. Social and ethical problems encountered in using genetic susceptibility information must be anticipated and rational schemes devised to circumvent the potential misuse of our abilities to identify at-risk individuals.

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