SYMPOSIUM OVERVIEW

Genetic Polymorphisms in Human Drug Metabolic Enzymes

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Results obtained from both epidemiologic studies and experimental animal model systems have shown a wide range of phenotypic variation in the ability of individuals to metabolize drugs and environmental chemicals. Several studies have noted correlations between specific metabolic phenotypes and the incidence of disease, suggesting that certain allelic forms of drug metabolic enzymes can render the individual either more sensitive or resistant to the toxic or therapeutic effects of exogenous drugs and chemicals. While some of this variation can be attributed to different environmental exposures, it has become clear that genetic factors also play an important role in determining the response of the individual organism to exogenous agents. Recent advances in molecular biological techniques have begun to allow scientists to correlate observed phenotypic differences with the actual differences in genetic sequence at the gene level. This has allowed a correlation between gene structure and function and provided a mechanistic basis to explain the interaction between the individual organism's genetic background and its response to environmental exposures. This symposium will thus focus on recent advances linking genetic polymorphisms in both Phase I and Phase II metabolic enzymes in the human population to the response to environmental toxicants.

Studies conducted over the past 3 or 4 decades have demonstrated a wide degree of variability in the metabolism of environmental chemicals in both experimental animal models and in the human population. The degree of phenotypic variation includes both quantitative as well as qualitative differences in the individual organism's metabolism of a variety of chemical agents. This variability in metabolism can have both beneficial and deleterious effects as correlations, both positive and negative, between specific metabolic phenotypes and disease incidence have been reported by several laboratories. These results have suggested that certain allelic forms of specific drug metabolic enzymes can confer either resistance or enhance sensitivity to the toxic or therapeutic effects of exogenous drugs and chemicals. Some of these differences in drug metabolism have been identified as resulting from differential exposure to environmental agents, which can induce the levels of expression of various drug metabolic enzymes (Conney, 1967, 1982; Gonzalez, 1988). However, it has become clear that genetic factors also play an important role in determining individual response to environmental exposures (Nebert, 1991; Idle, 1991). Indeed, several studies have noted differences in the metabolic capacity for a variety of drugs and chemicals between different ethnic groups that cannot be accounted for by socioeconomic or environmental conditions.

While classic enzymology and immunological approaches cannot be used to attribute specific metabolic phenotypes to particular allelic forms of the enzymes, molecular biological approaches have been employed in recent years in an attempt to link alterations in phenotype with actual differences in genetic sequence. These studies have allowed scientists to correlate directly gene structure and function and provide for a mechanistic basis to explain the interaction between the individual organism's genetic background and its response to environmental exposures. This symposium will thus focus on recent advances linking genetic polymorphisms in both Phase I and Phase II enzymes with specific metabolic phenotypes in the human population. This will include two talks on Phase I enzymes, in particular the ADH2
gene and members of the \textit{CYP2C} subfamily, as well as two presentations on the Phase II enzymes, \textit{N}-acetyltransferases (NATs)\textsuperscript{2} and glutathione \textit{S}-transferases (GSTs).

The first speaker, Dr. Gail McCarver, will discuss how allelic differences in the alcohol dehydrogenase gene, \textit{ADH2}, can modulate the interaction between intrauterine exposure to ethanol, ethanol metabolism, and toxic effects in the developing organism. Dr. Douglas Bell will highlight recent advances in his laboratory linking specific allelic forms of \textit{NAT} 1 with the risk for colorectal cancer and will discuss the potential for interaction between the \textit{NAT1} and \textit{NAT2} genes. Dr. David Eaton, the cochair for this session, will review the extensive literature on the four GST genes in determining susceptibility and protection from cancer in several organ systems. The last speaker, Dr. Joyce Goldstein, will discuss her laboratory’s recent studies demonstrating that allelic forms of both the \textit{CYP2C19} and the \textit{2C9} genes show markedly different catalytic activity with commonly prescribed drugs, including barbiturates, antidepressants, anti-anxiety drugs, \textit{\beta}-blockers, and anticoagulants.

\textbf{ALCOHOL DEHYDROGENASE: GENES, ETHANOL (ETOH) METABOLISM, AND INTRAUTERINE ETOH EXPOSURE RISK (D. G. McCarver)}

Support for pharmacogenetic differences in ethanol metabolism as determinants of susceptibility to alcohol related birth defects includes multiple animal studies showing the importance of variation in blood alcohol levels (Bonthius \textit{et al}, 1988; Goodlett \textit{et al}, 1990; Chernoff, 1980) and epide-miologic human studies demonstrating ethnic differences in susceptibility (Sokol \textit{et al}, 1980, 1986). For example, African Americans are at increased risk for adverse offspring outcome compared to Caucasians, even when ethanol intake during pregnancy is statistically controlled (Sokol \textit{et al}, 1980, 1986). Adverse outcomes following intrauterine ethanol exposure range from the full fetal alcohol syndrome to effects of varying severity, such as growth retardation, isolated structural abnormalities, or neurobehavioral deficits (Streissguth \textit{et al}, 1980; Golden \textit{et al}, 1982; Abel \textit{et al}, 1985; Sokol \textit{et al}, 1986).

Ethanol is oxidized to acetaldehyde by two enzyme systems, alcohol dehydrogenase, or ADH, and the microsomal ethanol oxidizing system (MEOS). Acetaldehyde is then oxidized predominantly by aldehyde dehydrogenase (ALDH) to acetate. Genetic variation has been described for each of the involved enzyme systems. The oxidation of ethanol to acetaldehyde is the rate determining step, and many investigators believe ADH to be the more important enzyme (Mezey, 1976). Eight ADH genetic loci have been identified, at least two of which, \textit{ADH2} and \textit{ADH3}, are known to be polymorphic (Jornvall and Hoog, 1995). The possible alleles at the \textit{ADH3} polymorphic locus encode enzymes which have relatively similar kinetic constants (Mezey, 1976). In contrast, the kinetic constants of the \textit{\beta} isoenzymes which are encoded at the polymorphic \textit{ADH2} locus vary by orders of magnitude (Table 1) (Bosron \textit{et al}, 1983a; Burnell \textit{et al}, 1989; Bosron and Li, 1986; Ehrig \textit{et al}, 1990). Furthermore, the \textit{ADH2*3} allele which encodes the enzyme with the greatest disparity is unique to the African American population, occurring with an allelic frequency of 15\% (Bosron \textit{et al}, 1983b; Bosron and Li, 1987). Multiple genetic DNA polymorphisms have been described for CYP2E1, the predominant enzyme in MEOS, but none have been shown to affect in vivo enzyme activity. The null variant of ALDH, which is associated with decreased elimination of acetaldehyde, does not occur in the African American population. Thus, of the possible known genetic determinants of ethanol metabolism, the polymorphism at the \textit{ADH2} locus is most likely to affect outcome in the African American population.

If the \textit{ADH2} polymorphism impacts susceptibility to alcohol related birth defects by increasing variation in ethanol concentrations, substantial variation in ethanol metabolism among African American women would be expected. Furthermore, differences in \textit{ADH2} genotype should be significantly associated with that metabolic variation. Both observations recently have been made in studies of ethanol disposition in postpartum women (McCarver, unpublished data). In this study, women were enrolled using a stratified recruitment design based on previously determined \textit{ADH2} genotype and alcohol intake. Using this stratification, about half of the study population had at least one \textit{ADH2*3} allele and about half had alcohol intakes of more than 7 drinks per week. Within this selected sample population, the ethanol apparent \textit{V}_{\text{max}} varied about 5-fold and the apparent \textit{K}_{m} varied about 100-fold. Both \textit{ADH2*3} genotype and the amount of ethanol intake in the previous 72 h were associated with significant differences in ethanol metabolism (model \(p = 0.01\), multivariate analysis of variance). The presence of the \textit{ADH2*3} allele was associated with greater \textit{K}_{m} values and greater \textit{V}_{\text{max}} values (\(p < 0.01\)), which is consistent with what would be predicted from \textit{in vitro} data (Table 1). Controlling for genotype, increasing ethanol intake was associated with greater apparent \textit{V}_{\text{max}} values. The outcome of the offspring of the women is currently being evaluated.

In a larger population study designed to evaluate both maternal and offspring \textit{ADH2} genotype as a determinant of risk from intrauterine alcohol exposure, the \textit{ADH2*3} allele

\textsuperscript{2} Abbreviations used ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; BPDE, benzo[a]pyrene-7,8-dihydriodiol-9,10-epoxide; CI, confidence interval; EM, extensive metabolizer; ETOH, ethanol; GST, glutathione transferase; MDI, mental developmental index; MEOS, microsomal ethanol oxidizing system; NAT, \textit{N}-acetyltransferase; OR, odds ratio; PAH, polycyclic hydrocarbon; PM, poor metabolizer; TSO, \textit{trans}-stilbene oxide.
TABLE 1
Kinetic Characteristics of the ADH2 Isozymes Present in the African American Population

<table>
<thead>
<tr>
<th>ADH genotype</th>
<th>ADH isozyme</th>
<th>(K_m) (mM ethanol)</th>
<th>(V_{max}) (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ADH^2*1)</td>
<td>(\beta_1)</td>
<td>0.05</td>
<td>9</td>
</tr>
<tr>
<td>(ADH^2*3)</td>
<td>(\beta_2)</td>
<td>36</td>
<td>300</td>
</tr>
</tbody>
</table>

appeared to be protective (McCarver et al., in press). In this study, mother–infant pairs \((N = 243\) pairs) were selected for recruitment during pregnancy based on maternal ethanol intake during the periconceptional period and maternal \(ADH2\) genotype. About half of the women had at least one \(ADH2^*3\) allele and about a third were classified as heavy drinkers during the periconceptional period, defined as drinking more than 1 standard drink a day. About half of the infants had at least one \(ADH2^*3\) allele. For all statistical analyses, multiple confounding variables were tested, including maternal socioeconomic status, education, other children in the home, presence of smoking, as well as number of cigarettes and illicit substance use.

Drinking during pregnancy was associated with lower mental developmental index (MDI) scores on the Bayley Scales of Infant Development (Streissguth et al., 1980; Golden et al., 1982); however, this was secondary to the effect of alcohol exposure on the infants whose mothers did not have an \(ADH2^*3\) allele (Fig. 1, left). Those infants had a mean MDI score of about 102. In contrast, alcohol exposed offspring whose mothers had an \(ADH2^*3\) allele had MDI scores that were similar in distribution to nondrinking women. The impact of offspring \(ADH2\) genotype was similar. Infants without an \(ADH2^*3\) allele whose mothers consumed alcohol during pregnancy did significantly worse on neurobehavioral testing than alcohol-exposed offspring with an \(ADH2^*3\) allele (Fig. 1, right). The later group of infants had developmental scores similar to infants of nondrinking women. These findings were confirmed with analysis of variance testing in which all potential confounders were included. The strongest predictor of lower developmental scores was the three-way interaction between maternal drinking at the first prenatal visit, the absence of a maternal \(ADH2^*3\) gene, and the presence of an offspring \(ADH2^*3\) allele \((p < 0.01)\).

The presence of the \(ADH2^*3\) allele also appeared to be protective when intrauterine growth was considered as the outcome variable. Ethanol use in pregnancy was associated with poorer growth, including a dose–response effect for offspring birth weight, birth length, and head circumference. In an analysis of variance model, controlling for other confounders affecting offspring growth, gestational age and the two-way interaction between ethanol intake in pregnancy and the absence of an \(ADH2^*3\) allele were the strongest predictors of poorer offspring birth weight. With that interaction in the model, none of the other variables related to ethanol or substance use were significantly associated with differences in offspring growth.

Thus, in this African American sample, ethanol use in pregnancy was associated with poorer offspring growth and mental development similar to that reported by others. The presence of the \(ADH2^*3\) allele was associated with protection from adverse outcome, measured as poorer mental development at 1 year of age, as well as lower birth weight. Importantly, the interaction between ethanol and \(ADH2\) genotype was the most significant predictor of poorer mental development. The significance of this interaction suggests that the mechanism of the \(ADH2\) genotype effect on offspring outcome is ethanol-related. We believe that the mechanism for the genotype's effect is that it encodes for a high capacity enzyme. Damage from intrauterine ethanol exposure has been linked to binge drinking. During such occasions, ethanol concentrations of 20–40 mM would be expected. At these blood alcohol concentrations, the enzyme encoded by the \(ADH2^*1\) allele would be saturated, whereas that encoded by \(ADH2^*3\) would not be (Table 1). In addition, the maximal velocity of the enzyme encoded by \(ADH2^*3\) is much greater. Thus, at high blood alcohol concentrations, the presence of the \(ADH2^*3\) allele would enhance ethanol elimination. These observations are the first documentation of a genetic risk for alcohol related birth defects. Because the \(ADH2\) allele that is unique to African Americans appears protective, the \(ADH2\) polymorphism does not appear to explain the increased risk in the African American population, but does provide meaningful insight about susceptibility within that population.

GENETIC RISK AND CANCER: ROLE OF N-ACETYLTRANSFERASE POLYMORPHISMS (NAT1 AND NAT2) IN COLORECTAL CANCERS (D. A. Bell)

Exposures to carcinogenic aromatic amines that are present in the diet, cigarette smoke, or in the environment have been associated with increased risk of cancer. These compounds can be \(N\)- or \(O\)-acetylated by the NAT1 and NAT2 enzymes which may result in activation to a DNA reactive metabolite or, in some cases, detoxification. Recent studies have focused on genetic variation in NAT2 and its potential as a risk factor in colorectal cancer (Lang et al., 1994). However, the NAT1 gene is highly expressed in colonic mucosa and other tissue and also exhibits phenotypic variation among human tissue samples. We hypothesized that specific genetic variants in the polyadenylation signal of the
MATERNAL GENOTYPE

Offspring Genotype

FIG. 1. Impact of maternal (left) and offspring (right) ADH2 genotype upon the outcome of offspring of women abstaining (open bars) or drinking during pregnancy (dark bars). Offspring neurobehavioral outcome was measured as the Mental Developmental Index (MDI) score of the Bayley Scales of Infant Development at 1 year of age (N = 243 infants). Bayley scores were significantly lower among offspring whose mothers lacked an ADH2*3 allele and consumed ethanol during pregnancy (left, **p < 0.01, ANOVA, Duncan's post hoc). Similarly, Bayley MDI scores were significantly lower among offspring without an ADH2*3 allele whose mothers consumed ethanol during pregnancy (right, *p < 0.05, ANOVA, Duncan's post hoc). Data shown as mean ± standard deviation.

NAT1 gene would alter tissue levels of NAT1 enzyme. After developing a PCR-based method for distinguishing the polymorphic alleles, NAT1 genotype was compared against NAT1 phenotype in colon and bladder tissue (Bell et al., 1995a). We observe twofold higher NAT1 enzyme activity in tissue among individuals with a variant polyadenylation signal (p = 0.006). This was the first observation relating a NAT1 genotype to NAT1 phenotype in tissue samples.

We tested to see if the NAT1*10 allele could affect risk of colorectal cancer. Using a gene–environment interaction hypothesis, we would predict that individuals inheriting the NAT1*10 allele would generate more DNA reactive carcinogens and have higher risk for cancer relative to other NAT1 genotypes. We analyzed for genetic polymorphism in both NAT1 and NAT2 in a group of 202 colorectal cancer patients and 112 control subjects from Staffordshire, England (Bell et al., 1995b). We found significantly increased risk (OR = 1.9, 95% CI 1.2–3.2; p = 0.009) associated with the NAT1*10 allele, an allele that contains a variant polyadenylation signal. Individuals with higher stage tumors (Dukes C) were more likely to inherit this variant allele (OR = 2.5, p = 0.005). In contrast, rapid acetylation genotypes of NAT2 were not a significant risk factor in this English population. However, we found that the risk associated with the NAT1 variant allele (NAT1*10) was most apparent among NAT2 rapid acetylators (OR = 2.8, 95% CI 1.4–5.7, p = 0.003) suggesting a possible gene–gene interaction between NAT1 and NAT2. These positive findings suggest that: (1) the NAT1 enzyme may have a central role in carcinogen metabolism in the colon and (2) human variability in the NAT1 gene may be an important determinant of colorectal cancer risk.

We have preliminary data that suggests that NAT1*10 genotype is associated with higher levels of DNA adducts in bladder tissue and that NAT1 genotype interacts with smoking exposure in bladder cancer (Badawi et al., 1995; Taylor et al., 1995). Significant risks for bladder cancer ranging from 2 to 24 were associated with various combinations of NAT1 genotype and lifetime smoking exposures (Taylor et al., 1995). Other new alleles of NAT1 have recently been cloned and it will be important to determine what impact these low activity alleles have on risk of cancer (Hughes et al., 1997).

POLYMORPHISMS IN THE HUMAN GLUTATHIONE S-TRANSFERASES AS GENETIC DETERMINANTS OF SUSCEPTIBILITY TO ENVIRONMENTAL CARCINOGENS (D. L. Eaton, T. K. Bammler, and J. van Loo)

The cytosolic GSTs are a multigene family of dimeric enzymes implicated in detoxification, and in a few instances activation, of a variety of xenobiotics. The GSTs consist of four major classes of enzymes, referred to as alpha, mu, pi,
and theta. The nomenclature for these enzymes has been confusing, but a standard nomenclature is now accepted that identifies the class by a capital Arabic letter (A, M, P or T, for alpha, mu, pi and theta, respectively), followed by a subfamily number (Mannervik et al., 1992; Hayes and Pulford, 1995). Four members of this family, glutathione S-transferase M1 (GSTM1), glutathione S-transferase M3 (GSTM3), glutathione S-transferase T1 (GSTT1), and glutathione S-transferase P1 (GSTP1) are polymorphic in the human population.

GST M1 Polymorphism

In 1985 Seidegard and coworkers first described a human genetic polymorphism in lymphocytic glutathione S-transferase activity when measured toward the substrate, trans-stilbene oxide (TSO; Seidegard et al., 1985; Seidegard and Pero, 1985). Individuals with little or no activity toward TSO were later shown to be homozygous for a deletion of a major portion of the GSTM1 gene (Seidegard et al., 1988, 1989). It is now recognized that approximately 45–55% of the Caucasian population is homozygous for the GSTM1 gene deletion, but ethnic differences in gene frequency are evident (Table 2).

There are actually three allelic variants of the GSTM1 gene, referred to as GSTM1*A, GSTM1*B, and GSTM1*, with the latter representing the deleted, or null, allele. GSTM1*A and GSTM1*B differ only in the amino acid present at position 173 and have essentially identical catalytic activities toward substrates examined thus far. Most epidemiological studies have utilized phenotyping (activity toward TSO) or genotyping (PCR-based) assays that do not distinguish between the M1*A and the M1*B allelic variants or distinguish heterozygotes (GSTM1/GSTM1*) from homozygotes for the null allele (GSTM1*0/GSTM1*0).

**Table 2**

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>GSTM1<em>0/GSTM1</em>0 genotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronesian/Polynesian</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Chinese</td>
<td>58</td>
</tr>
<tr>
<td>Caucasian</td>
<td>45–52</td>
</tr>
<tr>
<td>Japanese</td>
<td>48</td>
</tr>
<tr>
<td>French National</td>
<td>43</td>
</tr>
<tr>
<td>African American</td>
<td>28–35</td>
</tr>
<tr>
<td>Indian</td>
<td>33</td>
</tr>
<tr>
<td>Nigerians</td>
<td>22</td>
</tr>
</tbody>
</table>

Note. Data from Hayes and Pulford (1995), Chen et al. (1996c), and Zhao et al. (1995).

Functional Significance of the GSTM1 Polymorphism

GSTM1 is active in the detoxification of a variety of epoxides, including oxidative products of some known carcinogens such as benzo[a]pyrene (Robertson et al., 1986), a polyaromatic hydrocarbon (PAH) present as a combustion by-product in cigarette smoke and in some occupational environments, and aflatoxin B1, (Raney et al., 1992), a fungal toxin contaminant of peanuts and corn.

Some, but not all, in vitro studies with human cells and/or body fluids have suggested that GSTM1 deficient individuals may be more susceptible to the genotoxic actions of epoxide carcinogens. For example, it was found that urine from smokers with the GSTM1*0/GSTM1*0 genotype was about three times more mutagenic in the Ames test than urine from smokers with one or two active GSTM1 alleles (Hirvonen et al., 1994). Other studies have found that lymphocyte sister chromatid exchange rate, but not micronucleus formation, is increased in GSTM1 deficient smokers compared with GSTM1 positive smokers (Cheng et al., 1995; van Poppel et al., 1993). However, DNA adduct levels in nontarget tissue such as lymphocytes may not be reflective of adduct levels in target tissue such as lung. For example, when PAH–DNA adducts were examined directly in lymphocytes from smokers, no association between adduct levels and GSTM1 alleles was found (Rothman et al., 1995), whereas a significant increase (OR = 8.6, 95% CI 1.03–100) in PAH–DNA adducts was found in lung tissue in GSTM1 deficient samples compared to GSTM1-positive controls (Kato et al., 1995). Such differences could result from differences in the type and/or level of expression of individual GST isoforms, and/or tissue-specific differences in activity of oxidative enzymes necessary to form the epoxide that serves as the GST substrate. Obviously, the lack of a functional GST allele will have no consequence in tissues which are incapable of forming an epoxide substrate because of lack of expression of the particular cytochrome P450 necessary to form the epoxide.

Cancer Susceptibility for the GSTM1 Polymorphism

Numerous molecular epidemiology studies have examined the potential significance of the GSTM1 polymorphism as a susceptibility factor for environmentally and/or occupationally related cancers. Positive associations (absence of both alleles conferring increased risk) have been convincingly found for lung and bladder cancers, with limited evidence for an association with several other types of cancer.

(a) Lung cancer. Over a dozen molecular epidemiology studies have examined whether the absence of the GSTM1 gene places individuals, especially smokers, at increased risk for lung cancer (Alexandrie et al., 1994; Anttila et al., 1995; Bell et al., 1992; Brockmoller et al., 1993; Hayashi et al., 1992; Heckbert et al., 1992; Hirvonen et al., 1993; Kawajiri
et al., 1995, 1996; Kihara et al., 1995a,b; Liu and Wang, 1988; London et al., 1995; McWilliams et al., 1995; Nazar et al., 1993; Seidegard et al., 1986; ToFigueras et al., 1996; Zhong et al., 1991). Although not all studies have found a statistically significant association between the GSTM1 null genotype and increased risk for lung cancer (Brockmoller et al., 1993; Heckbert et al., 1996; London et al., 1995; Zhong et al., 1991), most have at least suggested a trend for such an association. A meta-analysis of 11 studies completed prior to 1995 found a composite odds ratio for an association between the GSTM1 null genotype and lung cancer risk of 1.6 (95% CI 1.26–2.04) (dErrico et al., 1996). When these studies were controlled for smoking using only incident cases and healthy controls, the composite odds ratio increased slightly to 1.76 (95% CI 1.43–2.17) (dErrico et al., 1996). Several studies have suggested that Japanese populations with the GSTM1 null genotype might be at especially high risk for lung cancer when combined with other susceptibility genotypes such as the CYP1A1 MspI variant allele.

Thus, there is convincing evidence that the GSTM1 null genotype is a significant determinant of lung cancer in smokers, especially when combined with other susceptibility genotypes. As both the gene frequency for this deletion and the prevalence of lung cancer are quite common, Caporaso (1996) estimated that an increased risk of 60–70% associated with the GST null genotype in smokers would result in an attributable risk due to this one gene that would exceed the cancer risks of the breast cancer (BRCA1) gene and the human nonadenoma polyposis carcinoma (HNPCC) gene combined.

(b) Bladder cancer. Over a dozen studies have examined the relationship between the GSTM1 null genotype and bladder cancer, and most have demonstrated a significant association (Anwar et al., 1996; Bell et al., 1993; Brockmoller et al., 1994, 1996; Daly et al., 1993; Katoh et al., 1995; Lafuente et al., 1990, 1993, 1996; Lin et al., 1993, 1994; Okkels et al., 1996; Rothman et al., 1996; Zhong et al., 1993). A meta-analysis of six studies in Caucasians completed prior to 1995 found an aggregate odds ratio of 1.54 (95% CI 1.28–1.85) (dErrico et al., 1996). Based on only two studies with a small sample size, a similar association was found for African Americans (OR = 1.41, 95% CI 0.48–4.10) (Bell et al., 1993; Lin et al., 1994) and Asians (OR = 2.40, 95% CI 1.3–4.45) (Katoh et al., 1995; Lin et al., 1994). The attributable risk for total bladder cancers for the GSTM1 polymorphism has been estimated to range from 17 to 25% (Bell et al., 1993; Brockmoller et al., 1994).

Because smoking is associated with bladder cancer, it is not surprising that the strongest association has been identified in smokers, although several studies have also found increased risk among nonsmokers. Other studies have demonstrated that urine from smokers with the GSTM1 null genotype is more mutagenic than urine from GSTM1-positive smokers (Hirvonen et al., 1994). In addition, individuals (both smokers and non-smokers) with the null GSTM1 genotype have a higher level of 3- and 4-amino-biphenyl hemoglobin adducts (Yu et al., 1995a), lending biological plausibility to such an association. In one relatively large case–control study no statistically significant overall association between the GSTM1 null genotype and bladder cancer was found (OR = 1.33, 95% CI 0.91–1.94), but a significant relationship was found when surviving cases were examined (Okkels et al., 1996).

(c) Stomach and colorectal cancers. Several studies have suggested that the GSTM1 null genotype is associated with increased risk for stomach cancer (Harada et al., 1992; Kato et al., 1996; Strange et al., 1991), although other studies have failed to find an association (Deakin et al., 1996; Kato et al., 1996). All of the studies to date have been relatively small, and further research is necessary to determine whether the GSTM1 null polymorphism is a significant risk factor for stomach cancer.

Several case–control studies on GSTM1 genotype and colon cancer have been completed, again with mixed findings. Two initial studies both suggested an approximate increase in risk of 60–70% for the null genotype (Strange et al., 1991; Zhong et al., 1993), but more recent studies (Chen et al., 1995a,b; Liu and Wang, 1995, 1996; Kato et al., 1996) have not found a significant overall association. No association was found between anal cancer and the GST null genotype in a population based case–control study (Chen et al., 1996a).

(d) Other cancers. Case–control studies on the association between GSTM1 null genotype and breast (Ambrosone et al., 1995; Harada et al., 1992; Zhong et al., 1993), brain (Elexpuru et al., 1995; Hand et al., 1996), skin (Heagerty et al., 1994, 1996; Lear et al., 1996; Yengi et al., 1996), ovarian (Sarhanis et al., 1996), cervical (Warwick et al., 1994), and oral (Deakin et al., 1996; Kato, 1995) cancers, and myelodysplastic syndromes (Chen et al., 1996d), have been negative, although in a few instances a trend was noticed or subclassification resulted in marginally significant associations. One small study on head and neck cancers (Trzina et al., 1995) found an odds ratio of 3.1 (95% CI 1.24–7.75) when compared with matched controls for the GSTM1 null genotype. No association between the GSTM1 null genotype and liver cancer was found in two studies (Hsieh et al., 1996; Yu et al., 1995b), but GSTM1 null individuals exposed to aflatoxin B1 in the diet may be at slightly increased risk relative to GSTM1-positive individuals (Chen et al., 1996b; McGlynn et al., 1995).

GSTM3 Polymorphism

A more recently described GSTM3 polymorphism has been shown to have a frequency of 16% in the Caucasian
TABLE 3

Ethnic Variability in the Frequency of the GSTT1*0 Genotype (Homozygous Null)

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Frequency of GSTT1*0 genotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>12–25</td>
</tr>
<tr>
<td>English</td>
<td>16</td>
</tr>
<tr>
<td>Germans</td>
<td>12</td>
</tr>
<tr>
<td>US-&quot;nationwide&quot;</td>
<td>24</td>
</tr>
<tr>
<td>US-New England</td>
<td>16</td>
</tr>
<tr>
<td>US-general</td>
<td>15</td>
</tr>
<tr>
<td>Asian</td>
<td></td>
</tr>
<tr>
<td>Japanese</td>
<td>45</td>
</tr>
<tr>
<td>Chinese</td>
<td>58–62</td>
</tr>
<tr>
<td>Blacks</td>
<td>24</td>
</tr>
<tr>
<td>African American</td>
<td></td>
</tr>
</tbody>
</table>

Note. Data from Nelson et al. (1995) and Lee et al. (1995).

A polymorphism for the theta class GSTT1 enzyme in humans was first described in 1994 (Pemble et al., 1994). Like the GSTM1 polymorphism, the GSTT1 polymorphism also occurs because of a deletion of a substantial part of the gene, and thus individuals who are homozygous for the deleted form lack GSTT1 activity in all tissues. The frequency of the homozygous GSTT1*0 genotype is quite variable among different ethnic groups, ranging from 12 to 62% (Table 3).

This enzyme is of particular functional significance because it is involved in both the activation and the detoxification of a variety of oxidative metabolites of important industrial chemicals, including methylene chloride, ethylene dichloride, methyl bromide, ethylene oxide, and 1,3-butadiene (Guengerich 1995; Hayes and Pulford, 1995). Because of the dichotomous role in activation and detoxification, the gene deletion could conceivably decrease risk to some exposures (e.g., ethylene dichloride, where the GSTT1 enzyme activates ethylene dichloride to a genotoxic metabolite), while increasing risk to other chemicals (e.g., butadiene, where GSTT1 detoxifies butadiene epoxides). The significance of the deletion toward a particular substrate will also depend on the level of expression of other GSTs that may act upon the same substrate. Thus, interpretation of epidemiological studies on the relationship between disease endpoints and the GSTT1 null genotype is greatly complicated under circumstances where mixed exposures to potential GSTT1 substrates occurs.

The potential significance of GSTT1 as a detoxification enzyme is suggested by several studies on butadiene exposed workers. GSTT1-null individuals occupationally exposed to butadiene demonstrated a 16-fold increase in sister chromatid exchange compared to GSTT1-positive individuals with similar exposures (Wiencke et al., 1995). Chromosomal aberrations in butadiene workers were also significantly higher in GSTT1-null individuals compared to GSTT1-positives (Sorsa et al., 1996). In contrast to these studies with exposure to butadiene, a cytogenetic study of floriculturists exposed to a variety of pesticides found a significant (albeit marginal) increase in lymphocyte sister chromatid exchanges in GSTT1-positive individuals, compared to the GSTT1 nulls, but no effect on micronuclei or chromosomal aberrations (Scarpato et al., 1996).

Relatively few molecular epidemiology studies have been completed on the relationship between the GSTT1 deletion and cancer. The GSTT1 deletion polymorphism has been associated with increased risk for brain astrocytoma [OR = 2.7, p < 0.001 (Elexpuru et al., 1995)] and meningioma [OR = 4.5, p < 0.001 (Elexpuru et al., 1995)], although the sample size was small. A recent study (Chen et al., 1996d) found a significant, 4.3-fold increase in risk for myelodysplastic diseases among individuals homozygous for the theta deletion. An association between colon cancer and the GSTT1 null allele was found in one study [OR = 1.88, p = 0.001 (Deakin et al., 1996)], but not another (Katoh et al., 1996). A third study of colon cancer patients found that, although the frequency was not significantly different between cases and controls, GSTT1 null homozygotes were more common in patients who were diagnosed before the age of 70 (Chenevix et al., 1995). The authors suggested from this that GSTT1 genotype might influence the age of onset of colorectal cancer (Chenevix et al., 1995).

No association between GSTT1 null genotype and skin cancer was found in one study (Heagerty et al., 1996), but a longitudinal study suggested that accrual of basal cell carcinomas occurred at a significantly greater rate in GSTT1 null individuals [rate ratio = 2.15, p < 0.001 (Lear et al., 1996)]. GSTT1 deletion was associated with increased risk for bladder cancer in nonsmokers (OR = 2.6, 95% CI 1.1–6.0), but not in intermediate or heavy smokers (Brockmoller et al., 1996). Other studies on lung (Deakin et al., 1996), oral (Deakin et al., 1996), or gastric (Deakin et al., 1996; Katoh et al., 1996) cancers have failed to find any statistically significant association with the GSTT1 null genotype. However, very few epidemiology studies of adequate power have...
been completed for the GSTT1 polymorphism, and the significance of this polymorphism will require further study.

**GSTP1 Polymorphism**

The gene encoding the isoenzyme known as GSTP1 is located on chromosome 11 (Suzuki et al., 1987; Hayes and Pulford, 1995) and the enzyme appears to be the most widely abundant form found in many tissues (Suzuki et al., 1987). While members of all GST classes are normally expressed in the human lung (Awasthi et al., 1987), GSTP1 is the predominant form, accounting for most of the immunohistochemical GST staining (Awasthi et al., 1987) and 90 to 97% of GST activity (Koskela et al., 1981; Partridge et al., 1984; Di Ilio et al., 1988). GSTP1 also has the highest specific activity toward the active benzo[a]pyrene metabolite of tobacco smoke, benzo[a]pyrene-7,8-dihydriodiol-9,10-epoxide (BPDE), and is almost exclusively active toward the (+)-enantiomer of anti-BPDE (Jernstrom et al., 1989; Robertson et al., 1986), which is believed to be the ultimate mutagenic form of benzo[a]pyrene. Thus, GSTP1 may be even more important than GSTM1 in preventing tobacco-induced lung cancer. However, this relationship has not been studied in humans.

Recently two genetic polymorphisms in the human GSTP1 gene have been identified (Harries et al., 1997; Ali-Osman et al., 1997). Two variant alleles have been described. GSTP1*B results from a transition mutation in codon 104 (nucleotide +313) that changes Ile to Val, and GSTP1*C has the same codon 104 as GSTP1*B, but also has a second transition mutation in codon 113 that changes Ala to Val (Ali-Osman et al., 1997). Interestingly, the codon 113 change has not been identified as an allele by itself, although only a relatively small number of samples (75) have been examined (Ali-Osman et al., 1997). Molecular modeling studies have shown that the amino acids at both codon 104 and 113 lie in the hydrophobic binding site for electrophilic substrates and would be expected to affect substrate binding (Zimniak et al., 1994; Ali-Osman et al., 1997). In fact, cDNA expressed GSTP1b and GSTP1c proteins have altered enzymatic activity when compared to the wild type GSTP1a protein (Ali-Osman et al., 1997). The catalytic efficiency ($K_{cat}/K_{m}$) of both variant alleles is about three- or fourfold lower than the wild type allele (Ali-Osman et al., 1997). The allele frequency for the combined GSTP1*B + GSTP1*C variant alleles is about 30% (Harries et al., 1997; Ali-Osman et al., 1997), and homozygotes for the variant B + C alleles occurred in 6.5% (Harries et al., 1997) and 12% of samples examined (Ali-Osman et al., 1997). Homozygotes for the altered GSTP1 alleles may exhibit increased susceptibility to smoking-induced cancers if the variant proteins exhibit a similar loss in activity toward relevant substrates such as benzo[a]pyrene diol epoxide as has been shown for the surrogate substrate, CDNB (Harries et al., 1997; Robertson et al., 1986; Jernstrom et al., 1989). Ali-Osman et al. (1997) found that the GSTP1*C allele was fourfold higher in cells obtained from malignant gliomas than from normal tissue, although it was not possible to tell whether this was due to differences in the subjects' genotypes, to loss of heterozygosity, or to other genetic changes that may have occurred in the tumor tissue. Harries et al. (1997) examined the association between the GSTP1*B gene and a variety of cancers. The authors did not distinguish between GSTP1*B and GST1*C alleles, but the method they used would have measured both variant alleles (*B + *C) if Ali-Osman et al. are correct in noting that all GSTP1*C alleles also carry the GSTP1*B allele change. Harries et al. (1997) found statistically significant associations between homozygotes for the variant allele(s) and testicular (OR = 3.3, 95% CI 1.5–7.7) and bladder (OR = 3.6, 95% CI 1.4–9.2) cancers, and teratomas (OR = 3.4, 95% CI 1.4–8.4), but not for breast or colon cancer. A nonsignificant increase in lung cancer (OR 1.9, 95% CI 0.7–4.8) was suggested. Interestingly, although the sample size was small and the results were not statistically significant, a protective effect for prostate cancer was suggested (OR = 0.4, 95% CI 0.02–3.3). Clearly, additional studies are needed to assess the significance of the polymorphism identified in human GSTP1.

**THE GENETIC BASIS OF THE CYP2C9 AND CYP2C19 POLYMORPHISMS (J. A. Goldstein)**

There are four members of the human CYP2C subfamily (CYP2C8, CYP2C9, CYP2C18, and CYP2C19). Two principal polymorphisms occur in this subfamily in humans. A genetic polymorphism in the metabolism of the anticonvulsant drug mephenytoin has been known since the 1980s (Wilkinson et al., 1989). Population studies show a bimodal distribution of metabolism of the drug in humans. Individuals can be characterized phenotypically as extensive metabolizers (EMs) or poor metabolizers (PMs). The recessive inheritance of the PM trait has been shown in family studies. Mephenytoin exists as a racemic mixture, and it is the 4'-hydroxylation of the S-enantiomer which is specifically impaired in man. This polymorphism also affects the metabolism of a number of other clinically used drugs, such as the anti-ulcer drug omeprazole, certain barbiturates and antidepressants, the antimalarial proguanil, the β-blocker propranolol, and the anxiolytic diazepam (reviewed in Goldstein, 1994). There is a marked ethnic difference in the distribution of PMs with the PM trait representing 2–5% of Caucasians, but as much as 13–23% of Oriental populations. CYP2C19 has been shown to be the enzyme responsible for this polymorphism (Wrighton et al., 1993; Goldstein et al., 1994). Recombinant CYP2C19 metabolizes mephenytoin in vitro, and mephenytoin 4'-hydroxylase activity of human microsomes correlates with CYP2C19 content.
Studies in our laboratory have identified four defective CYP2C19 alleles. The two principal genetic defects (termed CYP2C19*1 and CYP2C19*2) account for >99% of PM alleles in Orientals but only ~87% of Caucasian PM alleles (de Morais et al., 1994a,b; Sarich et al., 1997; Brøsen et al., 1995; Goldstein et al., 1997). Our laboratory has developed genetic tests for these alleles (Goldstein and Blaisdell, 1996). CYP2C19*1 consists of a single G → A base pair mutation in exon 5 which creates a new aberrant splice site. This splice site is apparently used almost exclusively over the normal splice site in PMs who are homozygous for this defect. mRNA reverse transcribed from these individuals lacks the first 40 bp of exon 5. The abnormal splicing produces a premature stop codon resulting in a truncated 234-amino-acid protein which would be inactive. CYP2C19*2, is the most common defective allele, representing ~75–87% of PM alleles in Caucasians and Orientals. A second defect, CYP2C19*3, is found primarily in Orientals (~20–25% of PM alleles), but is extremely rare in Caucasians (~1% of PM alleles) (deMorais et al., 1994a; Brøsen et al., 1995). This defect consists of a single base pair change in exon 4 which produces a premature stop codon and would also produce a truncated inactive protein.

Subsequent studies from our laboratory have revealed additional defective CYP2C19 alleles. CYP2C19*4 consists of a G → A mutation in the initiation codon which appears to interfere with translation of the protein (Ferguson et al., in press). This mutation accounts for approximately 3% of Caucasian PM alleles. A fourth rare (CYP2C19*5) mutation consists of a single base change resulting in an Arg → Trp substitution in the heme binding region (Xiao et al., 1997). This mutation accounts for ~0.8–1.5% of PM alleles in Orientals (Xiao et al., 1997) and Caucasians (Goldstein, unpublished data). Genetic tests for CYP2C19 mutant alleles can now account for ~100% of Oriental PM alleles and ~91% of Caucasian PM alleles (de Morais et al., 1994a,b; Sarich et al., 1997; Brøsen et al., 1995; Goldstein et al., 1997; Xiao et al., 1997; Ferguson et al., in press).

A second rare polymorphism has been reported in the metabolism of the antidiabetic drug tolbutamide and the anticonvulsant phenytoin (reviewed in Goldstein, 1994). The frequency of this polymorphism has been estimated to be ~0.2% in Caucasians. The primary enzyme responsible for the metabolism of these substrates is CYP2C9. This enzyme also metabolizes many clinically important drugs such as the anticoagulant warfarin as well as a number of antiinflammatory drugs. Several alleles of CYP2C9 have been reported. The principal allele (CYP2C9*1), containing Arg144 and Ile359, represents ~86% of the alleles in Caucasians. A second allele, CYP2C9*2, containing Cys144 has been reported to have lower activity toward warfarin in some cDNA expression systems (Rettie et al., 1994). In two separate studies, sequencing of CYP2C9 in two poor metabolizers of tolbutamide and the angiotensin II receptor antagonist, losartin, indicated that both individuals were homozygous for CYP2C9*3, which contains Leu359 (Sullivan-Klose et al., 1996; Spielberg et al., 1996). The use of cDNA expression studies has indicated that CYP2C9*3 has a lower affinity for drugs such as tolbutamide and warfarin (Sullivan-Klose et al., 1996). Genetic tests indicated that the incidence of the CYP2C9*3 allele was ~0.06 in Caucasians and 0.03 in Chinese. CYP2C9*2 was very rare in Orientals, and the frequencies of both CYP2C9*2 and CYP2C9*3 was low in African-Americans. According to the Hardy–Weinberg law, the incidence of homozygous CYP2C9*3 individuals is estimated to be (0.06)², or 0.04%, in Caucasians, which is consistent estimates of the incidence of PMs of tolbutamide and phenytoin in Caucasians. Based on frequencies of known CYP2C9 mutant alleles, this polymorphism appears to be rare in Oriental and African-American populations.

In summary, a polymorphism in CYP2C19 affects the metabolism of the anticonvulsant drug phenytoin, omeprazole, proguanil, certain antidepressants and barbiturates, and diazepam. This polymorphism shows marked racial heterogeneity, with a higher frequency in Oriental populations than in Caucasians. Four mutant alleles of CYP2C19 have been reported which account for ~100% of Oriental and ~91% of Caucasian PM alleles. A second rare polymorphism in the CYP2C subfamily which affects the metabolism of drugs such as tolbutamide, phenytoin, and warfarin is largely attributed to a single Ile → Leu359 substitution in CYP2C9 which alters the affinity of the enzyme for many drugs and may also reflect a somewhat lower activity of the Arg → Cys144 variant for some substrates.

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