Review

Molecular Epidemiology of the Human Glutathione S-Transferase Genotypes GSTM1 and GSTT1 in Cancer Susceptibility

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Abstract

The \( \mu \) (GSTM1) and \( \theta \) (GSTT1) members of the glutathione S-transferase multigene family are candidate cancer susceptibility genes because of their ability to regulate the conjugation of carcinogenic compounds to excretable hydrophilic metabolites. Deletion variants that are associated with a lack of enzyme function exist at both of these loci. Individuals who are carriers of homozygous deletions in the GSTM1 or GSTT1 genes may have an impaired ability to metabolically eliminate carcinogenic compounds and may therefore be at increased cancer risk. Molecular epidemiological studies have provided three pieces of information about the relationship of GSTM1 and GSTT1 with cancer susceptibility. First, the frequencies of homozygous GSTM1 and GSTT1 deletion carriers is very high (i.e., \( 20\% - 50\% \)) in most populations studied to date. Second, GSTM1 and, possibly, GSTT1 may be involved in the etiology of cancer at more than one site. Third, the risk conferred to individuals who carry homozygous deletions in GSTM1 or GSTT1 appears to be small in magnitude (e.g., odds ratio of \(< 2 \)). However, the magnitude of risk is larger (e.g., odds ratio of 3–5) when interactions of GSTM1 or GSTT1 with other factors (e.g., cigarette smoking) are considered. These findings have implications for studies of GSTM1 and GSTT1 in cancer susceptibility and for future applications of these biomarkers in cancer prevention or control strategies. First, molecular epidemiological studies should consider both the common frequency of deletion genotypes and the relatively low cancer risk these deletion genotypes may impart. For example, the common frequency of deletion variants may improve statistical power in some molecular epidemiological studies, but large samples may still be required to detect relatively small effect sizes or important interaction effects. Second, the fact that deletion genotypes are common implies that the proportion of cancer attributable to these variants may be large in the general population. However, these genotypes may be less suited for individual cancer risk assessment because of their relatively small contribution to the absolute risk of cancer.

Rationale for GSTM1 and GSTT1 in Cancer Susceptibility

The etiology of most commonly occurring cancers cannot be explained by allelic variability at a single locus. Instead, the major burden of cancer in the general population probably results from the complex interactions of multiple genetic and environmental factors over time. An understanding of the interplay of xenobiotic exposures, endogenous physiology, and genetic variability at multiple loci will facilitate knowledge about cancer etiology and the identification of individuals who are at increased risk for developing cancer.

Members of the GST\(^\theta\) family (E.C. 2.5.1.18) are important candidates for involvement in susceptibility to commonly occurring forms of cancer because they may regulate an individual's ability to metabolize environmental carcinogens (1). Two genes encode the cytosolic enzymes GST-\(\mu\) (GSTM1: chromosome 1p13.3) and GST-\(\theta\) (GSTT1: chromosome 22q11.2). These enzymes catalyze the addition (conjugation) of aliphatic aromatic heterocyclic radicals, epoxides, or arene oxides to glutathione. Conjugation reaction at the electrophilic center of these compounds occurs at the sulfur atom of the glutathione molecule. These molecules act as glutathione peroxidases but do not require a selenium cofactor to accomplish conjugation reactions. A number of recent reviews (2–12) have described the protein structure, enzymology, inducibility, and expression (including tissue- and gender-specific expression) of GSTM1 and GSTT1. These reviews summarize the role of GSTM1 or GSTT1 in the metabolism of and induction by numerous known or suspected carcinogenic compounds. These include benzo(a)pyrene, styrene-7,8-oxide, and trans-stilbene oxide by GSTM1 and epoxynbutanes, ethylene oxide, halomethanes, and methyl bromide by GSTT1. As this list implies, there does not appear to be a single class of chemical compounds that is associated with GSTM1 or GSTT1 metabolism or induction (12). Instead, GSTM1 and GSTT1 are involved in the metabolism of a wide range of xenobiotics, including environmental carcinogens, chemotherapeutic agents, and reactive oxygen species. Furthermore, GSTM1 appears to make a distinct contribution to cancer susceptibility because its substrate specificities may be different from that of the other classes of GSTs (13).

Knowledge of GST biochemistry has led to hypotheses about the role of the GSTM1 and GSTT1 genes in cancer etiology. Normal or increased GST enzyme activity or levels

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1 The abbreviations used are: GST, glutathione S-transferase; PAH, polycyclic aromatic hydrocarbon; SCE, sister chromatid exchange; LOH, loss of constitutional heterozygosity; OR, odds ratio; CI, confidence interval.
may protect susceptible tissues from somatic mutations in DNA by facilitating the conjugation and subsequent elimination of electrophilic carcinogens. Absent or deficient GST enzyme activity may result in poorer elimination of electrophilic carcinogens, particularly in the presence of very active electrophilic activation by phase I enzymes (e.g., the cytochromes P-450). GST deficiencies may therefore result in increased risk of somatic mutation, leading to tumor formation (14, 15). If an individual’s inherited genotype at a GST locus does not permit the efficient metabolism of compounds involved in carcinogenesis, then that individual may be at increased cancer risk. This review presents a synopsis of the molecular epidemiological evidence that evaluates this hypothesis.

Polymorphism in GSTM1 and GSTT1

Carriers of homozygous deletions in the GSTM1 and GSTT1 genes have an absence of GST-μ and GST-θ enzyme activity, respectively (1, 16, 17). These deletion variants have been useful for molecular epidemiological studies of cancer because they divide study subjects into two well-defined susceptibility classes: those who are and those who are not able to detoxify potential carcinogens by the metabolic pathways regulated by GSTM1 or GSTT1.

Phenotypic assays have been used to identify individuals who lack GST-μ or GST-θ activity (e.g., by induction of protein expression after exposure to trans-stilbene-oxide or methyl chloride, respectively). However, these assays are more time-consuming and susceptible to interassay variability relative to genotyping assays and are therefore not ideally suited for application to molecular epidemiological studies. Fortunately, there is a strong correlation between the phenotypic induction assays and genotype assays in identifying individuals who lack GST-μ (18–23) or GST-θ (17, 24, 25). These studies indicate that the rate of misclassification in GST-μ and GST-θ phenotype by GSTM1 and GSTT1 genotype assays is extremely low (i.e., <1%). Therefore, genotype assays are appropriate for use in molecular epidemiological studies that require both reliability and inexpensiveness, high-throughput capability.

Three alleles at the GSTM1 locus have been described: GSTM1α (also denoted ψ), GSTM1β (also denoted μ), and GSTM1-0 (deletion) alleles. The GSTM1α and GSTM1β alleles differ by a C→G substitution at base position 534. This DNA variant results in a Lys→Asn substitution at amino acid 172 (1). However, there is no evidence to date that the GSTM1α and GSTM1β alleles are functionally different from one another (26). As noted above, comparisons of the homozygous deletion genotype (for simplicity, denoted below as GSTM1-0) with genotypes containing at least one GSTM1α or GSTM1β allele (collectively denoted here as GSTM1-1) are of primary interest to molecular epidemiological studies. GSTM1-0 genotypes can be reliably determined by Southern blotting and PCR-based assays (18, 19, 22, 27–29), the latter being preferable in epidemiological studies. Because of the high frequency of deletion homozygotes (e.g., 50%), most individuals who are phenotypically GSTM1-1 are expected to be deletion heterozygotes rather than nondeletion homozygotes (42 versus 9%, respectively; Ref. 1). The ability to accurately distinguish deletion heterozygotes from deletion homozygotes by PCR-based methods may be complicated by limitations of consistent PCR product quality and quantity across samples. Scanning densitometry has been used to aid in distinguishing deletion heterozygotes from nondeletion homozygotes (30), but most studies to date have compared GSTM1-θ with GSTM1-1 genotype carriers. Similarly, two functionally different genotypes in GSTT1 have been identified (17) that are denoted here as GSTT1-0 (homozygous deletion genotype) and GSTT1-1 (genotypes with one or two undeleted alleles).

Because the PCR-based assays involve the detection of a gene deletion in a member of a multigene family with many homologues, PCR detection approaches must be carefully undertaken with adequate internal (e.g., simultaneous amplification of genes at other loci) and external (e.g., known genotype) controls. Artifacts in PCR amplification of the GSTM1 gene and detection of the deletion polymorphism have been reported (30), which may in part be remedied by the choice of exon for amplification. Because the variant of interest is a gene deletion, PCR-based assays of the GSTM1 or GSTT1 gene often include a positive internal control PCR amplification product in the β-globin gene or in another member of the GST family. Assays that involve the simultaneous amplification of PCR products that “sandwich” the GSTM1 product between two positive control (e.g., β-globin) bands are optimal because they confirm that the absence of a GSTM1 band is a true deletion, rather than the result of DNA degradation or PCR amplification failure (30). Adaptations of the original PCR assay for the GSTT1 gene (17) have also included coamplification of a region of the β-globin gene along with the GSTT1 PCR product (31). Single PCR protocols have also been proposed to simultaneously characterize GSTM1 and GSTT1 genotypes with (32) and without (33) positive internal controls.

Table 1 summarizes the high frequency of homozygous deletion polymorphisms in both GSTM1 and GSTT1. For example, the homozygous deletion genotype frequency in GSTM1 ranges from 0.38 to 0.67 in Caucasians, from 0.33 to 0.63 in East Asians, and from 0.22 to 0.35 in Africans and African Americans. Pacific Islanders have the highest reported frequency of homozygous deletion genotypes of any group studied to date. For example, natives of Kiribati are apparently completely monomorphic for the homozygous deletion form of GSTM1 (33). The GSTT1 deletion polymorphism has been studied in fewer populations than GSTM1. Table 1 presents GSTT1 null genotype frequencies, many of which are derived from control populations of case-control studies. These esti-
mates suggest that the GSTTI null frequency in Caucasians is 20% or less but that the null genotype frequency in African and Asian populations may be similar to that of GSTMI.

As stated previously, the homozygous deletion variants in GSTMI and GSTTI facilitate molecular epidemiological studies because they are both highly polymorphic and allow studies to contrast two biologically meaningful genotypic classes. There are two additional issues to consider when dealing with very common variant genotype frequencies at these loci in molecular epidemiological studies. First, the reported size of effects (e.g., relative risk and relative odds) that are associated with these deletion polymorphisms is likely to be small (see review of case-control studies, below). Thus, the benefit (with respect to statistical power) of having common variant genotypes may be offset by the relatively low magnitude of effects in association studies of GSTMI or GSTTI. Second, the fact that deletion polymorphisms in GSTMI or GSTTI are common implies that the population attributable risk associated with these genotypes may be quite high. A large proportion of cancer in the general population may be explained by genotypes at GSTMI or GSTTI because carriers of homozygous deletion genotypes at these loci are so common. As an illustration of the potential population impact of these genes, it has been estimated that 17% of lung cancers (34) and 17% of bladder cancers (35) may be attributable to GSTMI genotypes. These estimates are based on case-control data from multiple studies using two cancer sites for which consistent associations with GSTMI have been reported. Although these values provide only a crude measure of the potential population impact of these genes, the estimate of 17% suggests that GSTMI could explain a substantial fraction of cancer in the population. In contrast, the absolute risk to the individual associated with these deletion polymorphisms may be quite small, as suggested by the low relative risk reported for these genotypes. Therefore, genotypes at GSTMI or GSTTI may not have the same role in cancer prevention and control strategies as would germ-line mutations in genes such as APC, p53, or BRCA1, which have a low population frequency and a low population attributable risk but confer a high individual cancer risk.

### Molecular Epidemiological Evidence for GSTMI and GSTTI as Cancer Susceptibility Genes

Multiple lines of evidence from molecular epidemiological studies suggest that GSTMI and GSTTI are involved in cancer susceptibility. This evidence derives primarily from three types of studies: “transitional” studies relating GSTMI or GSTTI genotypes with intermediate biomarkers of exposure or effect; case-series studies that explore the relationship between inheritance of a particular genotype and the manifestation of a tumor,

#### Table 2: Studies of GSTMI genotype and biomarkers of exposure and effect

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sample description</th>
<th>Relationship with GSTMI*</th>
<th>Refs. (population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAH DNA adducts</td>
<td>90 autopsy lung specimens</td>
<td>Adducts more common in 0 vs. 1</td>
<td>66 (United States)</td>
</tr>
<tr>
<td></td>
<td>63 male smokers</td>
<td>Adducts inversely associated with vitamins E and C in 0 vs. controls in 0, no association in 1</td>
<td>37 (United States)</td>
</tr>
<tr>
<td></td>
<td>69 chimney sweeps, 35 controls</td>
<td>Increased adduct levels in cases vs. controls in 0, no association in 1</td>
<td>67 (Sweden)</td>
</tr>
<tr>
<td></td>
<td>63 lung cancer patients</td>
<td>No association</td>
<td>68 (Norway)</td>
</tr>
<tr>
<td></td>
<td>130 pregnant women</td>
<td>No association</td>
<td>69 (Denmark)</td>
</tr>
<tr>
<td></td>
<td>47 occupationally exposed and 22 unexposed nonsmokers</td>
<td>No association</td>
<td>70 (Sweden)</td>
</tr>
<tr>
<td></td>
<td>47 nonsmoking firefighters</td>
<td>No association</td>
<td>71 (United States)</td>
</tr>
<tr>
<td></td>
<td>60 women</td>
<td>Increased adduct levels in 0 vs. 1</td>
<td>72 (Bohemia)</td>
</tr>
<tr>
<td></td>
<td>90 nonsmoking bus drivers</td>
<td>No association (trend in 0)</td>
<td>73 (Denmark)</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>Healthy Chinese volunteers</td>
<td>Adducts increased in 0 vs. 1</td>
<td>74 (United States)</td>
</tr>
<tr>
<td>DNA adducts</td>
<td>49 male gold miners</td>
<td>Adducts increased in 0 vs. 1</td>
<td>75 (Ghana)</td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>151 control subjects</td>
<td>Adducts increased in 0 vs. 1</td>
<td>76 (United States)</td>
</tr>
<tr>
<td>hemoglobin adducts</td>
<td>154 smokers, 38 nonsmokers</td>
<td>SCE increased in 0 vs. 1</td>
<td>77 (The Netherlands)</td>
</tr>
<tr>
<td></td>
<td>20 volunteers</td>
<td>No association</td>
<td>78 (Finland)</td>
</tr>
<tr>
<td></td>
<td>12 healthy volunteers</td>
<td>SCE increased in 0 vs. 1</td>
<td>79 (Finland)</td>
</tr>
<tr>
<td></td>
<td>81 control subjects</td>
<td>SCE increased in 0 vs. 1</td>
<td>80 (United States)</td>
</tr>
<tr>
<td>Lymphocyte micronuclei</td>
<td>23 volunteers</td>
<td>No association</td>
<td>81 (Italy)</td>
</tr>
<tr>
<td></td>
<td>154 sputum samples in smokers</td>
<td>No association</td>
<td>77 (Finland)</td>
</tr>
<tr>
<td></td>
<td>71 chimney sweeps, 59 controls</td>
<td>No association</td>
<td>82 (Sweden)</td>
</tr>
<tr>
<td></td>
<td>69 chimney sweeps, 35 controls</td>
<td>No association</td>
<td>67 (Sweden)</td>
</tr>
<tr>
<td>Specific somatic genetic mutations</td>
<td>97 pituitary tumors</td>
<td>No p53 expression changes or ras/gap mutation differences by genotype</td>
<td>83 (United Kingdom)</td>
</tr>
<tr>
<td></td>
<td>148 non-small cell lung cancers</td>
<td>Increased p53 and Ki-ras mutation in GSTMI × CTP/1A</td>
<td>84 (Japan)</td>
</tr>
<tr>
<td></td>
<td>76 nonsmoking men</td>
<td>No association with HPRT mutation</td>
<td>85 (Sweden)</td>
</tr>
<tr>
<td>Urine mutagenicity and metabolites</td>
<td>16 occupationally exposed men</td>
<td>Increased HPRT mutation in DNA/25 slow acetylators</td>
<td>86 (Sweden)</td>
</tr>
<tr>
<td></td>
<td>29 nonsmokers</td>
<td>Increased mutagenicity in 0 vs. 1</td>
<td>87 (Finland)</td>
</tr>
<tr>
<td></td>
<td>60 women</td>
<td>Increased mutagenicity in 0 vs. 1</td>
<td>72 (Bohemia)</td>
</tr>
<tr>
<td></td>
<td>351 men</td>
<td>No effect on hippuric acid, phenol, or o- and p-cresol levels</td>
<td>88 (Japan)</td>
</tr>
<tr>
<td>LOH</td>
<td>28 ductal breast tumor/normal pairs</td>
<td>Increased LOH in 0 vs. 1</td>
<td>89 (United States)</td>
</tr>
</tbody>
</table>

*0, homozygous deletion carriers; 1, nondeleted genotype carriers.
usually with respect to age of cancer onset or tissue type; and case-control studies that are used to identify associations of genotypes at GSTMI or GSTT1 in cancer etiology. These studies are summarized in the subsequent sections.

**Relationship of GSTMI and GSTT1 and Intermediate Biomarkers of Exposure and Effect**

The hypothesized role of GSTMI and GSTT1 in carcinogenesis suggests that (deleterious) somatic changes may occur at a higher frequency in homozygous GSTMI-0 or GSTT1-0 genotype carriers than in individuals with GSTMI-1 or GSTT1-1 genotypes. These somatic changes may hallmark susceptibility to the carcinogenic effects of xenobiotic compounds in individuals who cannot efficiently detoxify potentially carcinogenic exposures. Measures of somatic genetic damage may reflect exposure to carcinogenic compounds (i.e., biomarkers of exposure; Ref. 36) or the early effects of these compounds in the carcinogenesis pathway (i.e., biomarkers of effect; Ref. 36).

A number of studies consistent with this hypothesis have been undertaken that stratify levels of DNA damage by inherited GSTMI (Table 2) or GSTT1 (Table 3) genotype. These studies report that levels of PAH DNA adducts, aflatoxin DNA adducts, SCE, specific somatic genetic mutations, or LOH may be increased in carriers of GSTMI-0 or GSTT1-0 genotypes. Some of these studies support the hypothesis that individuals who lack the ability to eliminate potentially harmful active carcinogenic compounds by GSTMI or GSTT1 activity have elevated levels of DNA damage, as measured by biomarkers of exposure or effect. However, few of these associations have been consistently reported (a notable exception being increases in SCE in GSTT1-0 compared with GSTT1-1 genotypes). One report (37) suggests an inverse relationship of GSTMI and serum micronutrients with PAH DNA adducts. This type of information may direct cancer chemoprevention strategies that include information about GSTMI genotype. As described above, these inconsistencies may be traced, in part, to issues of study design.

In general, the studies described in the previous paragraph have not been conceived as formal epidemiological (e.g., case-control) studies, but rather as transitional studies (36). Although a carefully designed transitional study can provide important insights into the relationship between inherited variability and somatic genetic changes in carcinogenesis, inferences from these studies are often limited. For example, subject ascertainment and inclusion/exclusion criteria are often not carefully defined. An assessment of the effects of various sources of bias (particularly selection bias) on point estimates or confidence intervals is generally not presented. Few studies of this type correct for the effect of confounder variables. As a result, it is often difficult to assess potential biases in point estimates or to determine the population of inference for whom these results are valid. Many studies appear to have insufficient statistical power to detect potential genotypic differences, despite the presentation of point estimates that are suggestive of important effects. Although many of these studies suggest important relationships of GSTMI with intermediate traits, additional carefully designed investigations will be required to confirm many of the results reported to date.

**Case-Series Studies: Definition of Etiological Heterogeneity**

Etiological heterogeneity implies that two or more groups of cases in the population may have been caused by different sets of events. The ability to define etiologically heterogeneous subgroups in the population may facilitate a number of research and clinical issues. Studies of etiologically homogeneous subgroups in the general population may improve the ability to identify etiological agents with small magnitudes of effect that may not be detectable in samples with an etiologically heterogeneous case mix. Appropriately designed studies of case-series samples can be used to identify genotype-environment or genotype-genotype interactions (38, 39) that may guide more formal epidemiological investigations. Identification of homogeneous groups of cancer cases may also have implications for optimizing cancer diagnosis or treatment and may allow a more effectively application of cancer detection and prevention strategies. A small number of studies have been undertaken in case-series samples that identify differences in age at cancer manifestation (e.g., age at diagnosis or detection) by GST genotypes (Table 4). These studies have considered genotype effects of both continuous age distributions (e.g., Ref. 31) and categorical dichotomizations of age (e.g., Refs. 40 and 41). The addition of control populations that simultaneously consider the distribution of GST genotypes with age to more formally estimate disease penetration associated with GSTMI or GSTT1 have not been reported. In addition, there have been reports of an increased frequency of GSTMI-0 in hospital controls as compared with older postmortem (42) or geriatric (40) subjects, suggesting that some selection against GSTMI-0 individuals may occur with age. This may in part be explained by susceptibility to cancers at multiple sites conferred by GSTMI or GSTT1 (40). However, not all reports indicate an association of GSTMI-0 frequency with age (43). Although additional studies are required to resolve this issue, these data suggest that the selection of appropriate controls in molecular epidemiological studies involving GSTMI or GSTT1 may require consideration of matching or adjustment for age (or other age-related characteristics) before this class of study is undertaken (12). Additional characterization of GSTMI and GSTT1 using age-specific data may help to resolve whether genotype distributions at these loci are influenced by removal of GSTMI or GSTT1 genotypes from the aging population because of competing causes that are directly or indirectly related to GST-mediated cancer susceptibility.

**Case-Control Studies of Cancer Etiology: Evidence for Causality**

Using results of case-control studies, a set of criteria proposed by Hill (44) can be applied to evaluate whether GSTMI or

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Table 3: Studies of GSTT1 genotype and biomarkers of exposure and effect

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Study design</th>
<th>Relationship with GSTT1</th>
<th>Refs. (population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCE</td>
<td>20 volunteers</td>
<td>Increase in SCE in 0 vs. 1</td>
<td>78 (Finland)</td>
</tr>
<tr>
<td></td>
<td>78 volunteers</td>
<td>Increase after dipeoxynitroxy exposure in 0 vs. 1</td>
<td>90 (United States)</td>
</tr>
<tr>
<td></td>
<td>30 volunteers</td>
<td>Increase in SCE in 0 vs. 1, affected by smoking</td>
<td>91 (Germany)</td>
</tr>
<tr>
<td></td>
<td>23 volunteers</td>
<td>Increase in SCE in 0 vs. 1, affected by smoking</td>
<td>81 (Italy)</td>
</tr>
</tbody>
</table>

*0, homozygous deletion carriers; 1, nondeleted genotype carriers.*

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GSTTI are causally involved in cancer susceptibility. Those to be considered here are the criteria of biological plausibility, temporality in the exposure-disease relationship, dose-response relationships, consistency across studies, and strength of association. The following discussion includes only published articles and selected reviews. No results published in abstract form only were included because of the limited information relating to study design and statistical power in abstracts. Note that whenever possible, crude OR estimates were taken directly from the published work or recomputed using data presented in the original article to facilitate comparability across studies. When multiple ORs were presented in the original paper, the ORs presented in the original paper, the ORs presented in Tables 5 and 6 represent the maximum OR attained in the study.

Biological Plausibility. As stated previously, there is substantial information from the fields of molecular biology, biochemistry, and physiology that support a role of GSTM1 or GSTTI in carcinogenesis. For epidemiological studies to provide meaningful information about the role of GSTs in cancer etiology, these studies must also be consistent with knowledge about carcinogen metabolism and its relationship with carcinogenesis. One piece of support for the biological plausibility of GSTs in cancer etiology is the following: when an association has been reported between GSTM1-0 or GSTTI-1 and cancer, the risk is always elevated among homozygous deletion carriers. This finding is consistent with the hypothesis that the excretion of carcinogenic compounds is hindered by the absence of the detoxifying enzyme. However, there is also evidence that some metabolites in GSTTI-1 individuals may produce tissue-specific mutagenic metabolites (12, 45). Thus knowledge of specific environmental compounds in the carcinogenesis process may be necessary before relationships between GST genotype, xenobiotic, and cancer susceptibility may fully be evaluated. The second major piece of evidence in support of GSTs in carcinogen metabolism is the finding that compounds known to be metabolized by GSTM1 or GSTTI are involved in the carcinogenic process. As described below, epidemiological studies of interactions between GSTs and these compounds (e.g., smoking) support the biological plausibility of GSTs in cancer susceptibility.

Temporal Relationship. The temporal relationship of an exposure and disease is a key component in determining causality. Because GSTM1 and GSTTI genotypes are inherited in the germ line, this causality condition is fulfilled. Studies to date of interactions between GSTs and xenobiotics also support the fact that exposures prior to cancer development (e.g., cigarette smoking or germ-line variants at other loci such as CYP1A1) are associated with cancer susceptibility in the presence of a GSTM1-0 genotype.

Dose-Response. Dose-response criteria can be thought of in terms of genotype-environment interaction or genotype-genotype interaction. First, the relationship of GST-mediated effects of xenobiotics (e.g., smoking) should be consistent with an increased (relative) risk with increasing levels of exposure (i.e., genotype-environment interaction). This appears to be the case for most studies reporting significant effects that have considered a smoking-GSTM1 interaction to date. For example, there is evidence that dose-dependent interactions with smoking may contribute to GSTM1-mediated susceptibility to lung, bladder, and colon cancers (Table 5). Second, the “dose” of a susceptibility locus may be defined as the effect of genotypes of a multilocus system, such as CYP1A1 (i.e., genotype-genotype interaction). For example, a DNA variant in the CYP1A1 gene results in an Ile substitution for the 462 Val amino acid in exon 7. The Val allele is associated with higher aryl hydrocarbon hydroxylase enzyme activity and mutagenicity (46). OR estimates that increase in those groups with an increasing number of “high-risk” alleles or genotypes (e.g., GSTM1-0 or CYP1A1-Val) would support a genotypic dose-response relationship. In support of this hypothesis, the ORs associated with GSTM1 and CYP1A1 genotypes in lung cancer as reported by Kawajiri et al. (46) are 5.4 for GSTM1-0:CYP1A1-Val/Val, 1.47 for GSTM1-0:CYP1A1-Ile/Val, 1.68 for GSTM1-0:CYP1A1-Ile/Ile, 1.55 for GSTM1-1:CYP1A1-Val/Val, and 1.59 for GSTM1-1:CYP1A1-Ile/Ile as the reference group. In other words, the OR is substantially higher in the GSTM1-0:CYP1A1-Val/Val group (i.e., individuals with two high-risk genotypes) compared with the ORs in the groups with only one high-risk genotype. This result supports the notion of a genotypic dose-response relationship for interactions between GSTM1 and candidate genes at other loci. However, this relationship is more consistent in some populations (e.g., Japanese) than others (e.g., Caucasians).

Consistency across Studies. In general, there is equivocal evidence for consistency of associations involving GSTM1 or GSTTI. The criterion of consistency across studies may only be evaluated for GSTM1 in lung and bladder cancers, for which multiple studies of good quality have been conducted (Table 5). There is support for a role of GSTM1 at both of these sites, although the results are not entirely consistent in all studies. This consistency is greater when interactions (e.g., among smokers, an increased risk of lung cancer in GSTM1-0 but not in GSTM1-1) are considered. Consistency among the results for

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of cases</th>
<th>Association with GSTM1 or GSTTI*</th>
<th>Refs. (population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>185</td>
<td>Accelerated age of first breast cancer diagnosis among GSTTI-0 vs. GSTTI-1; no association with GSTM1</td>
<td>31 (United States)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>175</td>
<td>Increased frequency of GSTM1-0 in proximal but not distal tumors</td>
<td>92 (United Kingdom)</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>Decreased frequency of GSTM1-0 in cases diagnosed after age 70</td>
<td>40 (Australia)</td>
</tr>
<tr>
<td>Lung</td>
<td>228</td>
<td>Association with GSTM1 in squamous and small cell carcinoma but not adenocarcinoma</td>
<td>21 (United Kingdom)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>282</td>
<td>Association with GSTM1 in squamous and small cell carcinoma but not adenocarcinoma</td>
<td>46 (Japan)</td>
</tr>
<tr>
<td>Nevoid basal cell carcinoma</td>
<td>124</td>
<td>No GSTM1 genotype frequency difference in proportion of cases diagnosed before and after age 49</td>
<td>41 (Australia)</td>
</tr>
<tr>
<td>Nevoid basal cell</td>
<td>62</td>
<td>No GSTM1 genotype frequency difference in proportion of cases diagnosed before and after age 20</td>
<td>41 (Australia)</td>
</tr>
</tbody>
</table>

* 0, homozygous deletion carriers; 1, nondeleleted genotype carriers.

Table 4  Case-series studies involving GSTM1 or GSTTI genotype
cancers at other sites or for a role of GSTT1 is largely un-evaluable at this time due to the small number of such reports. The studies that provide the most consistent evidence for a genotype-disease relationship have been those of GSTM1 and lung cancer. This is particularly true in those studies in which genotype-environment or genotype-genotype interaction effects have been evaluated (Table 5). For example, all of the studies in Japanese populations that consider interactions of GSTM1 with smoking and/or CYP1A1 genotypes report a significantly elevated OR for lung cancer. This relationship is not as consistent among similar studies conducted outside of Japan. Even less consistent are the univariate (i.e., single genotype-disease)
studies to date. For example, these studies have, at best, reported small elevations in lung cancer risk associated with homozygous deletions in GSTM1. McWilliams et al. (34) have conducted a meta-analysis of 12 case-control studies with a total of 1593 cases and 2135 controls to evaluate the relationship between GSTM1 and lung cancer. They report a small increased risk of 1.4 (95% CI, 1.2–1.6) associated with the GSTM1-0 genotype. This modest increase in risk was found for all histological subtypes and for both genotyping and pheno- 
typing studies. However, when studies were stratified by race, an elevated OR was detected in Japanese populations (OR = 1.6; 95% CI, 1.3–2.1) but not in Caucasians (OR = 1.2; 95% CI, 0.98–1.4). Thus, the most convincing argument that supports GSTM1 as a cancer susceptibility gene comes from studies of lung cancer in Japanese populations. However, the associated magnitude of risk is small unless smoking and/or CYP1A1 genotypes are also considered.

Multiple associations of GSTM1 with bladder, colorectal, and stomach cancers have also been presented (Table 5). These studies do not provide consistent inferences, but six of nine published studies suggest bladder cancers may be associated with GSTM1. Multiple associations of GSTM1 in colorectal and stomach cancers have also been reported. However, these reports are conflicting in their inferences and do not provide consistent evidence for an association of GSTM1 with cancer at these sites. The remaining studies of GSTM1 and cancer at numerous sites have reported inconsistent relationships with GSTM1 and have generally not been confirmed in an independent sample. No elevated risk has been reported with GSTM1 and astrocytoma, basal cell carcinoma, breast cancer, cervical (squamous cell) cancer, meningioma, or myelodysplasia. Fewer studies of GSTT1 have been undertaken (Table 6), but the study design issues and results of these analyses are similar to those of GSTM1. Relatively large, significant univariate OR effects of GSTT1 on astrocytoma (2.7), meningioma (4.5), and myelodysplasia (4.3) have been reported, but these studies have yet to be confirmed in independent samples.

The inconsistent inferences across studies may be explained in part by study design characteristics. First, selection of cancer cases has often been accomplished by using clinic- or hospital-based subjects for which no inclusion/exclusion criteria are presented and for which little information about the source of tumor pathology or patient characteristics is presented. Control selection in many of these studies appears to be even less well defined. Often, “healthy” clinic patients or volunteers are used as control subjects, again without explanation of inclusion/exclusion criteria. Similarly, generalizability of these results to a specific reference population is difficult. OR estimates are often not corrected for important study subject attributes (i.e., confounder variables), such as age, race, or sex. The biases that may result from these analytical deficiencies may be important, given the relatively small magnitude of effects that are likely to be detected in an association study involving GSTM1 or GSTT1. Finally, a number of studies that suggest no association may have had insufficient statistical power to detect the effect of interest. Reports of elevated ORs of 1.5–2.0 in a number of small studies (Tables 5 and 6) that were not significantly different from a value of 1 suggest this possibility. Similarly, the power to detect important statistical interactions between loci or with other risk factors is often inadequate.

As an alternative to the possibility that inadequate study design may cause true associations to be missed, some reports of associations may in fact be spurious due to study design issues. GSTM1 or GSTT1 genotype association studies have the advantage of comparing two genotype levels and therefore suffer less from the multiple comparisons problems inherent to studies of other genotype-disease associations in which multiple alleles may exist. Thus, the need for adjusting significance levels to account for effects from multiple alleles at a locus may not be as important a consideration in studies of GSTM1 or GSTT1. However, studies using small sample sizes that may have been collected in an ill-defined manner (e.g., by using a poorly chosen control population) may be prone to produce a spurious association. This limitation, which is applicable to numerous GST association studies published to date, suggests that replication of findings in a well-designed study using an independent sample is essential before any association of GST with cancer can be accepted.

Many studies of GSTM1 or GSTT1 have considered only single-locus associations with disease. Given the role of GSTs in the metabolism of specific carcinogens and the obvious complexity in cancer etiology, it is unlikely that a single gene such as GSTM1 or GSTT1 will be sufficient to explain most cancer susceptibility in the absence of knowledge about other susceptibility genotypes or specific exposures. Studies of interactions with xenobiotics (e.g., smoking) or other susceptibility genotypes (e.g., cytochromes P-450) may provide the most convincing evidence for a role of GSTs in cancer susceptibility. Not coincidentally, the strongest and most consistent associations to date come from studies that consider genotype-genotype or genotype-environment interaction effects (Table 5). However, a number of issues have yet to be addressed that are likely to affect the relationship of GST genotypes and cancer risk. For example, age- and gender-specific effects of the relationships between genotype and xenobiotics have yet to be studied. These effects are likely to be important in understanding the role of GST genotypes in cancer etiology, as well as the

<table>
<thead>
<tr>
<th>Site</th>
<th>Cases/controls</th>
<th>Interaction with</th>
<th>OR estimates*</th>
<th>Refs. (population)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Main effect</td>
<td>Interaction(s)</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>112/577</td>
<td>CYP2D6, GSTM1</td>
<td>2.7</td>
<td>NS, NS</td>
</tr>
<tr>
<td>Basal cell</td>
<td>737/563</td>
<td>CYP2D6, GSTM1</td>
<td>NS</td>
<td>NS, NS</td>
</tr>
<tr>
<td>Bladder</td>
<td>374/373</td>
<td>Smoking</td>
<td>NS</td>
<td>2.6</td>
</tr>
<tr>
<td>Colorectal</td>
<td>125/148</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Head/neck</td>
<td>218/448</td>
<td>GSTM1</td>
<td>1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Larynx</td>
<td>169/145</td>
<td></td>
<td>1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Meningioma</td>
<td>50/577</td>
<td>CYP2D6, GSTM1</td>
<td>4.5</td>
<td>NS, NS</td>
</tr>
<tr>
<td>Myelodysplasia</td>
<td>96/190</td>
<td></td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

* NS indicates the OR effects were not significantly different from a value of 1.
utility of these biomarkers in cancer prevention and control strategies. Because potential associations of GSTM1 or GSTT1 genotypes with cancer susceptibility appear to be quite broad, the question may be raised whether GSTM1 or GSTT1 are markers of susceptibility to cancer in general or whether the effects of these genotypes are site-specific. One hypothesis of GST-mediated carcinogenesis is that carcinogenic compounds may be generally elevated in GSTM1-0 or GSTT1-0 individuals compared with those carrying undeleted alleles. This hypothesis suggests that interindividual variability in GST genotypes could modify cancer risk by systemically regulating levels of active carcinogens and may not be site- or substrate-specific mediators of carcinogenesis. In contrast, biochemical and physiological evidence suggests that GSTs are differentially expressed in different tissues (6). These enzymes may therefore exert tissue-specific effects or may predispose to specific cancer subtypes (e.g., postmenopausal but not premenopausal breast cancer; squamous cell carcinoma but not adenocarcinoma of the lung). To date, the sites at which a consistent effect of GST1 has been reported coincide roughly with levels of GST-µ tissue expression. For example, GST-µ is not expressed in breast tissue at high levels (6), and studies to date have not demonstrated an effect of GSTM1 on breast cancer susceptibility (Tables 2 and 5). In contrast, GSTM1 is expressed at relatively high levels in both bladder and lung tissues (6). As previously mentioned, positive associations of GSTM1 and cancers have been most consistently reported at these sites. Although not completely evaluable at this point, additional molecular epidemiological studies should be undertaken to evaluate whether GSTM1 and GSTT1 confer susceptibility to cancer in general or whether the effect of these genes is site specific. Aside from studies of the relationship between smoking and GST genotypes in cancer susceptibility, evaluation of substrate specificity in molecular epidemiological studies of GSTM1 or GSTT1 has been largely ignored. Although accurate measurement of specific exposures is difficult to accomplish in the context of a powerful, well-designed epidemiological study, this type of study will be extremely powerful in elucidating the mechanisms by which GST genotypes may act in cancer susceptibility.

Magnitude of Effects. The magnitude of effects of a genotype-disease relationship may also provide evidence in support of a causal relationship. For example, the inference that a very large OR estimate is greater than a value of 1 is less likely to be due to chance or bias than an OR value near 1. In the case of GSTM1 or GSTT1, the magnitude of effect is not expected to be large. As shown in Tables 5 and 6, the magnitude of OR effects reported to date for most univariate studies have been small (i.e., less than 2). In contrast, the magnitudes of effects of interaction ORs are substantially larger, and provide even greater support for the notion that the effects of GSTM1 are causative of cancer under the influence of xenobiotic compounds or in conjunction with genotypes at other loci. For example, the OR estimates for interactions of smoking and/or CYP1A1 associated with lung cancer risk are substantially higher than the univariate OR estimates of GSTM1 alone (Table 5).

Summary

There is evidence that GSTM1 is involved in the etiology of cancers at a few sites (particularly lung and bladder), and there is suggestive evidence that GSTM1 and GSTT1 are involved at numerous other sites. However, the complexity with which these and other metabolic enzymes are likely to act in chemical carcinogenesis tempers inferences about the role of these loci in cancer susceptibility. The major effects of these loci may be exerted (and most readily identified in epidemiological studies) as effect modifiers of xenobiotics and in the context of other genetic or biochemical traits. Although univariate relationships between GSTM1 or GSTT1 and cancer may confer a small magnitude of risk that is difficult to translate into clinical (e.g., cancer risk assessment) applications, the consideration of GST genotypes in conjunction with multiple susceptibility loci and avoidance of deleterious xenobiotics (e.g., smoking) may be valuable. Despite numerous studies published to date, the role of GSTM1 and GSTT1 in cancer susceptibility remains unclear. The resolution of this ambiguity will require carefully designed studies with sufficient sample sizes to detect small effects. The potentially high attributable risk associated with GSTM1 or GSTT1 suggest that these genes are important candidates for studies that attempt to understand the complex and multifactorial etiology of cancer in the general population. However, studies that specifically evaluate the utility of these genotypes in cancer risk prediction have yet to be conducted. These studies will be crucial to establish the value of GSTM1 and GSTT1 in cancer prevention or control strategies.

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