Gene Deletion of Glutathione S-transferase θ: Correlation with Induced Genetic Damage and Potential Role in Endogenous Mutagenesis

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Abstract
Genetic traits that confer increased susceptibility to DNA and chromosomal damage from reactive epoxide and peroxides could be important individual risk factors in the development of human cancers. To provide an index of individual sensitivity to epoxides, we previously studied sister chromatid exchange (SCE) induction in peripheral blood lymphocytes and identified a trait involving sensitivity to chromosomal damage by monoepoxybutene and diepoxbutane (DEB), both potential carcinogenic metabolites of 1,3-butadiene. Individuals sensitive to DEB induction of SCEs also had an increased number of background or "spontaneous" SCEs. The present investigation was conducted to test whether a newly described deletion polymorphism in the glutathione S-transferase class θ (GSTT1) was significantly associated with the previously described inherited chromosomal sensitivity to DEB. The background and DEB-induced SCE frequencies in peripheral blood lymphocytes from 78 healthy volunteers were determined with the use of fluorescence plus Giemsa staining. The presence or absence of the homozygous deletion of the GSTT1 gene was determined for each participant using PCR methods. In the present study, we report a close correlation of the DEB sensitivity trait with the novel polymorphism in GSTT1. The GSTT1 polymorphism was also highly associated with the background frequencies of SCE. These studies raise the possibility that DEB is a substrate for GST-θ. Individuals who carry a homozygous deletion of the GSTT1 gene may be at increased risk for genotoxic damage from environmental or occupational 1,3-butadiene exposures. The association of the GSTT1 deletion polymorphism with increases in background SCEs indicates that substrates for this isozyme are encountered commonly in the environment or are endogenous in nature. Possible substrates known to be either widely distributed in the diet or endogenous include halomethanes, ethylene oxide, and peroxides of fatty acids such as 13-hydroperoxy-9,11-octadecadienoic acid. The data indicate that a large component of individual variation in SCEs is not spontaneous but are genetically determined. Furthermore, because the GSTT1 gene codes for an enzyme involved in the metabolism of exogenous and endogenous compounds, the increase in SCEs associated with the GSTT1 polymorphism can be viewed as resulting from gene-environment interactions. Study of SCEs related to the GSTT1 gene may provide insights into the nature of common environmental or dietary exposures that produce chromosomal damage.

Introduction
Human cancer risk associated with exposures to exogenous or endogenous carcinogens may be modified by genetic variations in metabolic detoxification mechanisms (1). One important pathway for the metabolism of organic epoxides and peroxides involves the soluble GST1 (2.5.1.18) supergene family which consists of four unlinked gene loci designated α, π, μ, and θ (2–4). The μ class GSTs are highly efficient in conjugating arylepoxides [e.g., benzo(a)pyrene-7,8-diol-9,10-epoxide (5, 6)] and are polymorphic in humans; approximately 50% of Caucasian individuals carry a homozygous deletion of the GSTM1 locus (7). The GSTM1 homozygous deletion has been associated with lung (8–10), bladder (11, 12), and cutaneous cancers (13), as well as nonmalignant but cancer-associated pulmonary asbestosis (14). We previously investigated interindividual variation in susceptibility to epoxides at the cellular level by testing the effects of GSTM1 deficiency on the induction of SCEs by mutagenic epoxides in cultured peripheral blood lymphocytes. Our investigations demonstrated that the GSTM1 gene deletion leads to increased SCE formation by trans-stilbene oxide but has no effect on cis-stilbene oxide-induced SCEs (15). The results indicated that SCE could be a marker of the stereospecific detoxification of epoxide mutagens by GST-μ in human blood, since trans-stilbene oxide is an excellent substrate for GST-μ, whereas cis-

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The abbreviations used are: GST, glutathione S-transferase; SCE, sister chromatid exchange; DEB, 1,3-butadiene diepoxide; MEB, monoepoxybutane; ETO, ethylene oxide.
stilbene oxide is a very poor substrate. Other researchers have shown that smokers at risk for lung cancer who are also GSTM1 deficient have increased SCE levels in vivo in their blood lymphocytes compared with GSTM1-positive smokers (16). In the absence of cigarette smoking both this study and ours reported that the GSTM1 deletion does not affect the SCE frequency of peripheral blood lymphocytes of an individual.

As a part of these investigations we also studied inter-individual variation in SCE and chromosomal aberration induction by epoxide metabolites of 1,3-butadiene (MEB and DEB), which are not thought to be substrates for GST-μ but are potential environmental and occupational mutagens. We observed a bimodal distribution of SCE induction by DEB in healthy individuals; approximately 20% of subjects were hypersensitive to SCE induction (17). In addition, those lymphocytes from individuals sensitive to SCE induction contained increased numbers of chromosomal aberrations, including a 16-fold excess of chromatid exchanges compared with resistant individuals. We found no association of DEB sensitivity with the GSTM1 deletion, but studies carried out in identical twins revealed a complete concordance of the DEB-sensitivity trait among genetically identical twins (18) and suggested that the bimodal response to DEB could be due to the action of a highly penetrant single gene. Surprisingly, the background or spontaneous frequencies of SCEs were found to be strongly associated with DEB sensitivity; DEB-sensitive individuals had higher background SCEs, and this effect could not be attributed to demographic factors or cigarette smoking (19, 20). We hypothesized that the association of DEB-induced SCE with background SCEs could be explained by one of two general mechanisms: (a) an intrinsic sensitivity to SCE formation in DEB-sensitive persons; or (b) a heretofore unrecognized variation in a metabolic pathway that is responsible for the detoxification of a ubiquitous exogenous mutagen or an endogenously derived inducer of SCEs (21).

Recently, a second deletion polymorphism unrelated to the GSTM1 locus has been described within the GST supergene family that involves GST-θ; a prevalence of 38 or 22% was reported in European subjects for the GST-θ-deficient genotype (22) or phenotype (23), respectively. In humans, GST-θ was first recognized as being involved in the glutathione-dependent detoxification of naturally occurring monoclonal cell lines (e.g., methyl bromide and methyl chloride) by erythrocytes (24, 25). Subsequent studies using SCE induction as a marker of enzyme detoxification in whole blood indicated that alkyl halides (e.g., dichloromethane) and ETO were also potential substrates for the human θ class GST of RBC (23). Studies of the rat GST 5–5, which is likely an orthologous enzyme to the human GST-θ, have also implicated dihaloethanes (e.g., ethylene dibromide) and dihalomethanes (e.g., dichloromethane) as potential substrates for the human GST-θ (26).

Because of the structural similarity of DEB or MEB and known substrates for the polymorphic GST-θ (e.g., ETO), and the close agreement of its prevalence with that of the DEB sensitivity trait, we have carried out studies comparing the prevalence of the GST-θ deletion among DEB-sensitive individuals. Further, we have tested the hypothesis that the recently discovered GST-θ polymorphism is associated with variations in background SCE frequencies in healthy individuals.

Materials and Methods

Subjects. Studies were carried out in two populations of healthy individuals; these included 38 employees of the University of California Medical Center in San Francisco (20) and 40 employees of a major Houston-based oil-refining company. All participants completed a questionnaire and provided a blood sample. The questionnaire elicited demographic data, age, occupation, medical status (including history of cancer), diet, smoking history, and prior or current exposure to medication or environmental agents that could affect the SCE assay (e.g., X-rays, estrogens, thyroid hormones, anticancer chemotherapy, etc.). Work histories were obtained and used to ensure that potential exposure to occupational genotoxins did not contribute to the induction of SCE in lymphocytes.

Cell Culture and Cytogenetics. Venous blood was drawn from donors into sodium-heparinized vacutainers. For cell cultures, 0.5 ml of whole blood was added to a final volume of 5 ml of RPMI 1640 tissue culture containing 10% FCS-0.1 ml of phytohemagglutinin (DIFCO Laboratories, Detroit, MI), penicillin (100 units/ml), and streptomycin (100 μg/ml in 1-ounce glass prescription bottles). Lymphocytes were treated with DEB (Aldrich Chemical Co., Milwaukee, WI) at 21 h of culture. DEB was diluted in sterile water, and a fresh stock solution was prepared for each experiment. At 24 h of culture, 50 μm 5-bromo-2-deoxyuridine was added to each culture. Cells were cultured for 72 h at 37.5°C in 5% CO2 with 98% relative humidity. Two h before fixation, Colcemid (final concentration, 2 × 10–7 M; CIBA Pharmaceuticals, Summit, NJ) was added. Cells collected by centrifugation were exposed for 8 min to 0.075 μKCl at 37°C to spread the chromosomes and fixed three times in methanol-acetic acid (3:1). The resulting suspension was dropped onto microscope slides and differentially stained by a modification of the fluorescence plus Giemsa technique (27). The slides were immersed for 15 min in a solution of 5 μg Hoechst 33258 (Riedel-De Haen AG, Hannover, Germany)/ml in Sorensen’s buffer (pH 6.8) and then washed, dried, mounted with buffer under the coverslip, and exposed for 8 min to black light 2 cm from 2 block-light bulb GF tubes at 55°C. The slides were then stained for 4 min in a 3% Giemsa solution made in the same Sorensen’s buffer. To estimate background SCE frequencies, 50 second-division metaphases were scored per point; for DEB-treated cultures, 30 second-division metaphases were scored.

PCR Analysis of GSTT1 Deletions. PCR reactions were carried out in 50-μl volume containing 10 mM Tris-HCl (pH 8.3) containing 50 mM KCl-1 mM 2-mercaptopethanol-1% glycerol-1 mM MgCl2-0.2 mM deoxyribonucleotide triphosphates-2.5 units of AmpliTaq. Target DNA was obtained from 3 μl of heparinized whole blood by Chelex extraction (Sigma Chemical Co., St. Louis, MO). PCR conditions were 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were analyzed on a 1.5% agarose gel. As a control, coamplification of two genes was used in each reaction tube. Primers were used corresponding to the 3'-coding region of the human GSTT1: 5'-TTCTTACTAGGTGCCTCACATCTC and 5'-TACCCGGATCATG-GCCAGCA (22). The amplified product was 480 base pairs in length, and absence of this PCR product was indicative of the null genotype. In addition, a fragment of the CYP1A1
gene was coamplified with \textit{GSTT1} in each reaction. This fragment of \textit{CYP1A1} corresponds to exon 7 of \textit{CYP1A1} and yields a 312-base pair product. Primers for the \textit{CYP1A1} fragment were 5'-GAACTGCCACTTCAGCTGTCT and 5'-TCACCGGATCATGGCCAGCA.

\textbf{Statistical Analysis.} Mean SCEs per cell for individuals who were compared with Student's \textit{t} test after stratifying on \textit{GSTT1} status or DEB sensitivity status. DEB sensitivity status was defined as described previously (19, 20); persons were DEB sensitive if their lymphocytes contained an SCE frequency of >90 SCEs/cell and DEB resistant if <90 SCEs/cell were induced following treatment with 6 \mu M DEB. Fisher's exact test was used to test for associations of \textit{GSTT1} status with DEB sensitivity. ANOVA was used to analyze the effect of variables, as indicated, on baseline SCE frequencies.

\textbf{Results} 

Table 1 summarizes the demographic characteristics of the study population. The group consisted of approximately equal numbers of healthy men and women who were predominantly nonsmokers. Fig. 1 is a photograph of an agarose gel showing the 480-base pair band in \textit{GSTT1}-positive individuals and the deletion of this PCR product in deficient individuals. Concomitant amplification of the \textit{CYP1A1} gene fragment was used as a positive control. Because even 1 copy of the \textit{GSTT1} gene will be amplified and will yield a detectable PCR product, we did not attempt to distinguish homozygote wild type and heterozygous deleted individuals.

![Agarose gel of products of multiplex PCR reactions for detection of \textit{GSTT1} deletion polymorphism (see "Materials and Methods" for details). Lane 1, molecular weight ladder; Lanes 2 and 3, PCR products from 2 \textit{GSTT1}-deleted individuals showing absence of 418-base pair \textit{bp} \textit{GSTT1} fragment and positive amplification of the 312-base pair internal control \textit{CYP1A1} fragment. Lanes 4 and 5, PCR products from 2 \textit{GSTT1}-positive individuals showing presence of both \textit{GSTT1} and \textit{CYP1A1} fragments; Lane 6, negative control (amplification carried out without addition of target DNA).](image)

![Agarose gel showing the 480-base pair band in \textit{GSTT1}-positive individuals and the deletion of this PCR product in deficient individuals. Concomitant amplification of the \textit{CYP1A1} gene fragment was used as a positive control. Because even 1 copy of the \textit{GSTT1} gene will be amplified and will yield a detectable PCR product, we did not attempt to distinguish homozygote wild type and heterozygous deleted individuals.](image)

Table 1. Demographic characteristics of the study population according to DEB sensitivity and \textit{GSTT1} status.

<table>
<thead>
<tr>
<th>Group</th>
<th>Men</th>
<th>Women</th>
<th>Smoking status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>Current No. (%)</td>
</tr>
<tr>
<td>All subjects</td>
<td>78 (47)</td>
<td>41 (53)</td>
<td>8 (10.3)</td>
</tr>
<tr>
<td>DEB resistant</td>
<td>60 (53)</td>
<td>28 (47)</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td>DEB sensitive</td>
<td>18 (28)</td>
<td>13 (72)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>\textit{GSTT1} deleted</td>
<td>12 (25)</td>
<td>9 (75)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>\textit{GSTT1} positive</td>
<td>66 (52)</td>
<td>32 (48)</td>
<td>8 (12.1)</td>
</tr>
<tr>
<td>\textit{GSTT1} positive and DEB sensitive</td>
<td>6 (33)</td>
<td>2 (67)</td>
<td>3 (50.0)</td>
</tr>
</tbody>
</table>

\textbf{DEB Sensitivity} 

\textbf{\textit{GSTT1} status*} 

<table>
<thead>
<tr>
<th></th>
<th>Deleted</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>12 (60)</td>
<td>6 (60)</td>
<td>18</td>
</tr>
<tr>
<td>Resistant</td>
<td>0 (0)</td>
<td>60 (60)</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>12 (66)</td>
<td>66 (66)</td>
<td>78</td>
</tr>
</tbody>
</table>

* \textit{GSTT1} status is significantly associated with DEB sensitivity (Fisher exact test, \( P < 0.001 \)). \textit{GSTT1} status determined by PCR analysis and DEB sensitivity by SCE induction; see "Materials and Methods" for details.

Table 2 shows the association of DEB sensitivity with the presence of the \textit{GSTT1} gene deletion. A highly significant association of the DEB sensitivity trait with the homozygous deletion of \textit{GSTT1} was found to carry the \textit{GSTT1} deletion. Twelve of the 18 DEB-sensitive individuals carried the homozygous deletion of the \textit{GSTT1} gene, compared with 0 of 60 DEB-resistant subjects. The concordance between the gene deletion and the phenotype of high SCE induction, however, was not complete because 6 DEB-sensitive individuals had detectable \textit{GSTT1} products by PCR analysis. Repeated analysis of discordant samples failed to implicate technical error as a factor in the results (data not shown). Fig. 2 illustrates the distribution of SCE induction values for the entire group and shows that the outlying high SCE induction scores segregate with the \textit{GSTT1} gene deletion.

Because of the high concordance between the phenotype of DEB sensitivity and the \textit{GSTT1} polymorphism, the yields of DEB-induced SCE frequencies were very similar in DEB-sensitive and \textit{GSTT1}-deleted subgroups (Table 3); DEB-sensitive lymphocytes contained an increase of 44.3 SCEs/cell, and \textit{GSTT1}-deleted cells contained an increase of 46.5 SCEs/cell compared with DEB-resistant and \textit{GSTT1}-positive subgroups, respectively. Of interest was the significantly lower SCE induction scores among persons with detectable \textit{GSTT1} who were also DEB sensitive. Lymphocytes from these subjects contained 16 fewer SCEs/cell compared with \textit{GSTT1}-deficient/DEB-sensitive individuals (100.5 versus 116.3 SCEs/cell; \( P = 0.026 \)).

\textit{GSTT1} was also associated with higher background numbers of SCEs. Lymphocytes from DEB-sensitive individuals or \textit{GSTT1}-deleted individuals contained increases of 1.9 and 1.6 background SCEs/cell, respectively (\( P < 0.001 \)). Interestingly, the 6 persons who were DEB sensitive and \textit{GSTT1} positive (i.e., nondeleted) also showed increased background SCEs compared with DEB-resistant individuals (\( P < 0.001 \)). Consequently, in persons discordant for the two
traits, the DEB-sensitive phenotype appears to be the most important predictor of increased background SCE frequencies.

To test the relative contribution of DEB sensitivity, GSTT1 deletion, and cigarette smoking to variations in background SCE frequencies, we carried out an ANOVA. Variables other than cigarette smoking, DEB sensitivity, and GSTT1 genotype that were included in the original model included age, gender, geographic location (i.e., California or Texas), caffeine, and alcohol consumption. Table 4 shows the results of the ANOVA when the significant predictor variables (i.e., DEB sensitivity, GSTT1 genotype, and smoking) are treated as dichotomous variables; all other variables tested were found to have no significant association with the SCE outcome. Depending on the model, approximately 6–16% of the variance in SCEs could be attributed to smoking, whereas 27–37% was accounted for by GSTT1 status or DEB sensitivity, respectively.

**Discussion**

The current investigation has established that individual variation in the induction of chromosomal damage in lymphocytes following *in vitro* treatments with DEB in healthy individuals is highly correlated with the deletion polymorphism at the GSTT1 locus. A previously considered explanation for DEB sensitivity was the possibility of a variation in the intrinsic mechanism of SCE formation, (e.g., DNA-cross-link repair). Given the known involvement of GST-θ in the metabolism of epoxides (e.g., ETO) and other reactive compounds, this explanation now seems highly unlikely. Our results can be explained by proposing that DEB is a heretofore unrecognized substrate for the GST-θ enzyme of human erythrocytes. More definitive *in vitro* biochemical studies will be needed to test this proposal; however, this interpretation is consistent with our observation that DEB-sensitive individuals are not hypersensitive to nitrogen mustard (21), which, like DEB, produces interstrand DNA cross-links. In addition, DEB-sensitive individuals also have increased yields of SCEs induced by the monofunctional MEB, which is incapable of forming DNA cross-links (21). The mechanisms responsible for the intermediate yields of SCEs induced by DEB in some GSTT1-positive individuals are unknown. Lack of concordance of sensitivity and GSTT1 may represent a gene dosage effect; het-
erozygotes for GSTT1 may have less GST-θ protein and hence show less detoxifying activity toward DEB. Alternatively, individual variations in other activation and detoxification pathways (e.g., epoxide hydrolase) may also be involved. We observed indications of nongenetic modification of DEB sensitivity in earlier studies of cigarette smokers who had lower DEB-induced SCE frequencies (17) compared with nonsmokers. Since smoking is associated with increased red cell mass (higher hematocrit) it may also lead to greater amounts of red cell-associated GSTT1 activity. In the present studies, however, intermediate SCE induction could not be explained by cigarette smoking.

Given these results, we postulate that risks associated with toxicologically harmful agents may be modified by the presence or absence of the GSTT1 gene; furthermore, these risks may arise from occupational as well as low level environmental exposures. MEB and DEB are products of the P-450-catalyzed oxidation of 1,3-butadiene (28) and are considered to be the active metabolites responsible for the genotoxic and carcinogenic effects of this compound (29). From the current findings it should be considered that GSTT1 individuals exposed to 1,3-butadiene represent a sensitive subgroup within the population that may be at increased risk for adverse health outcomes linked to internal exposure to MEB and DEB. Low level environmental exposure to 1,3-butadiene occurs during exposure to motor vehicle exhaust and cigarette smoke; commercially this compound is used in the manufacture of synthetic rubber (30). Epidemiological studies have found excesses of lymphohematopoietic malignancies among occupational groups exposed to 1,3-butadiene (31). GST-θ could play a role as a protective mechanism in the bone marrow and at sites of hematopoiesis; GSTT1 deficiency should be investigated as a susceptibility factor in the occupational carcinogenicity of 1,3-butadiene. Assessing the risks associated with GSTT1 deficiency in 1,3-butadiene exposure, however, will have to rely on human studies, since GST-θ activity has not been observed in erythrocytes of animals including mice, rats, cattle, sheep, pigs, and rhesus monkeys (24). Increased risks from occupational exposures to other toxic substrates (e.g., methyl bromide) have been discussed previously (23). The possibility of complex patterns of risk associated with the GSTT1 deletion due to the involvement of GST-θ in both metabolic activation as well as detoxification has also been demonstrated (26). Thus, GST-θ deficiency would appear to be a highly significant genetic variation in humans with great potential for modifying the toxic and carcinogenic effects of a significant number of industrial chemicals.

The striking association of the GSTT1 polymorphism to individual variations in background SCE was independent of cigarette smoking, explaining nearly twice as much variation as smoking in our ANOVA. A variety of other demographic factors (e.g., age, sex, alcohol consumption) proved to have no significant effect on SCE levels. While the underlying mechanism for this association is unknown, it seems reasonable to propose that the higher SCE frequencies in GSTT1-null individuals may reflect exposure to an unrecognized mutagen substrate which, like DEB, is capable of differentially affecting deficient cells. The association of background SCEs with GSTT1 deletion has been observed in independent samples of individuals residing in California and Texas and, furthermore, among persons with widely different occupations. For these reasons we propose that the source of the putative SCE-inducing agent is either a ubiquitous environmental agent(s) or an endogenously derived substrate for GSTT1. It seems unlikely that DEB or MEB exposures themselves could be responsible for the association of GSTT1 with background SCE values, since nonoccupational exposure to 1,3-butadiene is extremely low.

Dietary factors and endogenous mutagens have been implicated in human cancer and aging (32). It is of interest to note that some of these factors are substrates for GSTT1-θ. Although exposure levels can be anticipated to be very low, halogenated alkane substrates of GST-θ are present in diet and drinking water (e.g., methyl bromide; Refs. 33 and 34). Diet is indirectly implicated in the endogenous production of ETO and other alkene epoxides. Hydrocarbon gases (methane, ethane, butane, and pentane) have long been known to be present in expired air as a result of the oxidation of edible fats (35). Ethylene is also produced endogenously, and although the exact sources are not known, it is proposed to be derived from unsaturated lipid degradation by intestinal bacteria (36). Oxidation of ethylene leading to ETO has been demonstrated by the formation of N-(2-hydroxyethyl)valine in hemoglobin; approximately 20 pmol/g of this protein adduct are produced in the blood of nonsmokers (37). ETO is a well documented inducer of SCE in peripheral blood lymphocytes and is a probable human leukemogen.

Support for the role of diet in both cancer risk and background SCE production has been proposed previously by Wulf et al. (38) in their studies of Seventh-Day Adventists who have low risk for diet-related gastrointestinal cancers (39). Seventh-Day Adventists practicing vegetarianism were observed to have markedly lower frequencies of SCE in their peripheral blood lymphocytes compared to persons eating meat and dairy products. Similarly, longitudinal studies in rodents reported correlations of increases in animal fat intake with increased SCE frequencies in hepatocytes (40); increased SCE frequencies of liver cells were hypothesized to be related to lipid peroxidation. Products of lipid peroxidation are known to be genotoxic in mammalian cells, 4-Hydroxynonenal induced SCEs in Chinese hamster ovary cells (41). In V9 cells, ferric nitritoacetate was a potent inducer of SCEs and lipid peroxidation; in contrast, ferric citrate produced neither SCEs nor peroxides (42). Since current work also indicates a significant contribution of diets rich in animal fat and dairy products to the intake of conjugated diene fatty acids (43, 44), the possible involvement of lipid hydroperoxides such as 13-hydroperoxy-9,11-octadecadienoic acid should also be considered; 13-hydroperoxy-9,11-octadecadienoic acid is a potential substrate for GSTT1-θ (45) and has recently been shown to form covalent DNA adducts in mammals in vivo (46). In fact, early work on the θ isoform proposed that this class of GSTs represents the most ancient evolutionary group within the GST superfamily that may have evolved in aerobic organisms as a defense against oxygen toxicity (47). Thus, the SCE assay combined with the identification of GSTT1 individuals may provide a novel approach to search for dietary factors leading to DNA damage.

Finally, our earlier observations on GST-µ and the current observations on GST-θ provide strong evidence that deletion polymorphisms within the GST superfamily are common variants within human populations that both play important roles in determining heterogeneity in individual sensitivity to the production of chromosomal damage. Because some exposures are known to involve multiple chem-
ical substrates for both GST-μ and GST-θ (e.g., cigarette smoke), the possibility should be considered that combined polymorphic deletion of GST-μ and GST-θ may interact to produce marked cancer risk for those exposures involving mutagen substrates of both isozymes.

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References
Gene deletion of glutathione S-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis.

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