Effect of Glutathione *S*-transferase M1 Polymorphisms on Biomarkers of Exposure and Effects

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Genotypes responsible for interindividual differences in ability to activate or detoxify genotoxic agents are recognized as biomarkers of susceptibility. Among the most studied genotypes are human glutathione transferases. The relationship of genetic susceptibility to biomarkers of exposure and effects was studied especially in relation to the genetic polymorphism of glutathione *S*-transferase M1 (*GSTM1*). For this review papers reporting the effect of *GSTM1* genotype on DNA adducts, protein adducts, urine mutagenicity, Comet assay parameters, chromosomal aberrations, sister chromatid exchanges (SCE), micronuclei, and hypoxanthine–guanine phosphoribosyl transferase mutations were assessed. Subjects in groups occupationally exposed to polycyclic aromatic hydrocarbons, benzidine, pesticides, and 1,3-butadiene were included. As environmentally exposed populations, autopsy donors, coal tar-treated patients, smokers, nonsmokers, mothers, postal workers, and firefighters were followed. From all biomarkers the effect of *GSTM1* and *N*-acetyl transferase 2 was seen in coke oven workers on mutagenicity of urine and of glutathione *S*-transferase T1 on the chromosomal aberrations in subjects from 1,3-butadiene monomer production units. Effects of genotypes on DNA adducts were found from lung tissue of autopsy donors and from placentas of mothers living in an air-polluted region. The *GSTM1* genotype affected mutagenicity of urine in smokers and subjects from polluted regions, protein adducts in smokers, SCE in smokers and nonsmokers, and Comet assay parameters in postal workers. A review of all studies on *GSTM1* polymorphisms suggests that research probably has not reached the stage where results can be interpreted to formulate preventive measures. The relationship between genotypes and biomarkers of exposure and effects may provide an important guide to the risk assessment of human exposure to mutagens and carcinogens. — *Environ Health Perspect* 106(Suppl 1):231–239 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-1/231-239sram/abstract.html*

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Introduction

Epidemiologic studies have indicated that most human cancers are originally caused by environmental exposure to genotoxic agents. According to Doll and Peto (*1*), up to 80 to 90% of all cancers are related to environmental factors, tobacco smoke, and diet. Individual susceptibility to cancer may result from several host factors including differences in metabolism, DNA repair, oncogene and tumor-suppressor gene activation, and nutritional status (*2*).

Groups of polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and nitroso compounds are regarded as the most important environmental carcinogens. It is increasingly clear that genetic differences among individuals in the ability to modulate metabolism of these carcinogens may play a primary role in susceptibility to environmentally induced diseases (*3–7*). Genetic polymorphism exists for a number of activating enzymes (phase I, represented by cytochrome P450 enzymes) and detoxifying enzymes (phase II). The relationship of genetic polymorphisms to carcinogenicity has been extensively studied for phase I enzymes on cytochrome P450 1A1 (*CYP1A1*) and cytochrome P450 2D6 (*CYP2D6*) genes or phase II enzymes on glutathione *S*-transferase M1 (*GSTM1*) and *N*-acetyl transferase 2 (*NAT2*) genes.

The knowledge of the genetic basis for individual metabolic variation has revealed new possibilities for studies focusing on increased susceptibility to environmental cancer (*8*). Individuals susceptible to various environmental carcinogens and mutagens could have greatly heightened genotoxic responses to exposures that induce little or no response in nonsusceptible individuals (*9*).

Recently new knowledge about susceptibility to environmental hazards was reviewed at the 12th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals: Susceptibility to Environmental Hazards (*10*). Several conclusions and recommendations were formulated, two of which are of particular interest. First, determination of susceptibility to chemicals in the workplace and general environment is becoming increasingly feasible through rapid advances in biologic sciences, particularly molecular biology. Parallel advances that have occurred in epidemiology, ecology, toxicology, and related sciences have greatly facilitated the understanding and measurement of susceptibility. Second, increased understanding of the pathways to chemical and physical agents leading to susceptibility of individuals, populations, and ecosystems in general is useful in protecting human health and the environment.

Olden (*11*) recently noted that "to address this problem the NIEHS [National Institute of Environmental Health Sciences] proposes to expand its molecular genetic research to identify susceptibility genes for environmentally induced diseases through a new environmental genome project."

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Abbreviations used: 4-ABP, 4-aminobiphenyl; B[*a*]P, benzo[*a*]pyrene; *CYP1A1*, cytochrome P450 1A1; *CYP2D6*, cytochrome P450 2D6; *CYP2E1*, cytochrome P450 2E1; ELISA, enzyme-linked immunosorbent assay; GSTs, glutathione *S*-transferases; *GSTM1*, glutathione *S*-transferase M1; *GSTP1*, glutathione *S*-transferase P1; *GSTT1*, glutathione *S*-transferase T1; *HPRT*, hypoxanthine–guanine phosphoribosyl transferase; *NAT2*, *N*-acetyl transferase 2; *NAT2*-ss, *N*-acetyl transferase 2 slow acetylator; *NQO1*, reduced nicotinamide adenine dinucleotide phosphate:guinone oxidoreductase; PAHs, polycyclic aromatic hydrocarbons; SCE, sister chromatid exchange.

Genotypes are responsible for interindividual differences in ability to activate or detoxify genotoxic agents as biomarkers of susceptibility. Genes of this type include those for cytochrome P450 and other enzymes that convert inactive carcinogens or mutagens to their genotoxic forms and those that conjugate and thereby detoxify these reactive forms. Many of these genes are polymorphic in human populations and potentially explain many of the interindividual differences observed both in genotoxic responses indicated by biomarkers and disease outcome.

Genetic Polymorphisms of *GSTM1*

Among the most studied genotypes are human glutathione *S*-transferases (GSTs). GSTs are multigene families of enzymes involved in the metabolism of a wide range of electrophilic compounds of both exogenous and endogenous origin. GSTs generally are recognized as detoxifying enzymes because of their ability to catalyze the conjugation of these compounds with glutathione. Their primary function is thought to be the detoxification of reactive electrophiles (*12*), but GSTs via their glutathione-dependent peroxidase activities also have an important role in free-radical scavenging, thus protecting the cell from deleterious effects of oxidative stress (*13*). GSTs may also be involved in the activation process of some carcinogens such as haloalkanes and haloalkenes (*14*).

Genetic susceptibility has been studied especially in relation to various biomarkers for genetic polymorphisms of *GSTM1*. The expression of *GSTM1* is inherited as autosomal dominant and between 40 and 60% of most populations express *GSTM1* (*15*). It is believed that genetic polymorphism exhibited by *GSTM1* may be a factor in determining an individual's susceptibility to the toxic effect of various xenobiotics. The high activity of *GSTM1* to convert PAHs to epoxide metabolites is believed to be particularly important (*16*). It was therefore suggested that these isoenzymes could serve as genetic markers for susceptibility to certain forms of cancer (*17–23*). Recently the glutathione *S*-transferase T1 (*GSTT1*) gene has been identified (*24–26*). This gene produces an enzyme, thus catalyzing detoxification of monohalomethanes. It is believed that homozygote individuals for the *GSTT1*-null allele will have altered cancer risk. Frequency of the null allele is expected to be between 10 to 30%.

The *GSTM1* gene is one of the most extensively studied genes related to metabolic polymorphisms and cancer risk. Seidegard et al. (*27*) first published the evidence that *GSTM1* deficiency may constitute a risk factor for development of carcinoma of the lungs, especially in smokers. McWilliams et al. (*28*) examined 12 case–control studies (*27,29–39*) on *GSTM1* status and lung cancer risk (a total of 1593 cases and 2135 controls). The results of this meta-analysis confirmed that *GSTM1* deficiency is a moderate risk factor for development of lung cancer, with an odds ratio of 1.41. *GSTM1* deficiency accounts for almost 17% of lung cancer cases. Ryberg et al. (*40*) suggested the simultaneous significance of *GSTM1* and glutathione *S*-transferase P1 (*GSTP1*) genotypes for lung cancer. Genetic factors may be more pronounced among patients who contract lung cancer at a younger age. The higher risk in *GSTM1*-null genotype smokers possibly is attributable to relevant carcinogens in cigarette smoke that may theoretically reach cellular DNA and form carcinogenic DNA adducts.

Kawajiri et al. (*41*) reported an association of smoking-induced lung cancer susceptibility with *CYP1A1* and *GSTM1* polymorphism with the *p53* gene. In non-small-cell lung cancer patients with a susceptible *CYP1A1* genotype they observed a remarkably high risk of mutation of the *p53* gene when combined with the *GSTM1*-null genotype.

Analysis of the contemporary literature on biomarkers of susceptibility, exposure, and effects shows that the relationship of the *GSTM1* genotype to other biomarkers was reported most frequently. Therefore for this review we considered those papers studying the effect of the *GSTM1* genotype. If other genotypes were studied simultaneously with the *GSTM1* genotype, their effects were also analyzed. Papers were divided according to the exposure dose into two groups: occupational and environmental exposure.

Occupational Exposure to Mutagens and Carcinogens

Table 1 summarizes the effect of genotypes on biomarkers of exposure and effects by occupationally exposed groups.

The effect of genetic polymorphisms for *GSTM1* and *CYP1A1* was first studied by Carstensen et al. (*42*) and Ichiba et al. (*43*) in chimney sweeps in Sweden. Occupational exposure to PAHs in this group was judged to be relatively low. The study of sweeps showed significantly increased aromatic DNA adduct levels in total white blood cells in workers with noninducible *CYP1A1* genotype m1/m1 and with combined *CYP1A1*-m1/m1 and *GSTM1*-null genotypes (*43*). The effect of these genotypes on micronuclei was not observed (*42,43*).

Another group exposed to PAHs was bus maintenance workers (garage workers exposed to diesel exhaust and mechanics exposed mainly to oils) (*44*). There was no significant difference in the DNA adduct levels or mutant frequency by hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) between *GSTM1*-null and *GSTM1*-positive individuals or between the slow and rapid acetylators. In slow acetylators lacking the *GSTM1* gene, significantly higher DNA adduct levels were observed. The exposure was classified according to type of job: workers highly exposed to diesel engine exhaust, mechanics exposed mainly to engine and lubricating oils, and others, which included electrical workers.

A similar scheme was used to determine the effect of *GSTM1* and *NAT2* genotypes on DNA adducts in Copenhagen bus drivers (*45*). The study group consisted of bus drivers driving in the center of Copenhagen, in suburban residential areas, and in rural and dormitory village settings. Exposure estimated as the air benzo[*a*]pyrene (B[*a*]P) concentrations in a busy street in Copenhagen was 3.9 ng B[*a*]P/m^3 compared with 0.09 to 0.5 ng B[*a*]P/m^3 in rural areas. No significant effects of *GSTM1* or *NAT2* on DNA adduct levels were observed either individually or combined. A nonsignificant trend was observed in individuals with the *GSTM1*-null genotype; they had higher DNA adduct levels in all exposure groups. Nielsen et al. (*45*) claimed that *GSTM1* function is less important at low level exposure.

One of the groups believed to be most heavily exposed to PAHs is foundry and coke oven workers. Hemminki et al. (*46*) analyzed aromatic DNA adducts in foundry workers in relation to exposure, lifestyle, and *CYP1A1* and *GSTM1* genotypes using groups in which no personal exposure monitoring was carried out. Occupational exposure to PAHs was extrapolated for the period 1991 to 1993 according to personal monitoring in 1990 and the decrease of production and reduced exposure to PAHs by workplace engineering improvements. Groups experiencing exposure to more than 5 ng/m^3 B[*a*]P were classified as high-exposure groups and those experiencing concentrations less than 5 ng/m^3 B[*a*]P were classified as low-exposure groups. [These

Table 1. Effect of genotypes on biomarkers of exposure and effects by occupationally exposed groups.

Group/ sample size	Exposure	Genotypes	Biomarkers: DNA adducts[a]	Urine[b]	CA	SCE	MN	*HPRT*	Reference
Chimney sweeps									
n=69	PAHs	*GSTM1*	NE	–	–	–	NE	–	
		CYP1A1	E	–	–	–	NE	–	(*42,43*)
Bus maintenance workers									
n=47	PAHs	*GSTM1*	NE	–	–	–	–	NE	(*44*)
		NAT2	NE	–	–	–	–	NE	
Bus drivers									
n=90	PAHs	*GSTM1*	NE	–	–	–	–	–	(*45*)
		NAT2	NE	–	–	–	–	–	
Foundry workers									
n=85	PAHs	*GSTM1*	NE	–	–	–	–	–	(*46*)
		CYP1A1	NE	–	–	–	–	–	
Coke oven workers									
n=46	PAHs	*GSTM1*	–	E	–	–	–	–	(*47*)
		NAT2	–	E	–	–	–	–	
n=23	PAHs	*GSTM1*	NE	–	NE	NE	–	–	(*48*)
		NAT2	NE	–	NE	NE	–	–	
n=69	PAHs	*GSTM1*	NE	–	–	–	–	–	(*49*)
		NAT2	NE	–	–	–	–	–	
Soldiers									
n=22	PAHs	*GSTM1*	NE	–	–	–	–	–	(*50*)
		CYP1A1	NE	–	–	–	–	–	
Dye production, benzidine workers									
n=30	Benzidine	*GSTM1*	NE	NE	–	–	–	–	(*51*)
Floriculturists									
n=23	Pesticides	*GSTM1*	–	–	NE	NE	NE	–	(*52*)
n=30		*GSTM1*	–	–	NE	NE	NE	–	(*53*)
		GSTT1	–	–	NE	NE	NE	–	
		NAT2	–	–	NE	NE	NE	–	
Monomer production workers									
n=40	1,3-Butadiene	*GSTT1*	–	–	–	E	–	–	(*56*)
n=53		*GSTM1*	–	–	NE	NE	NE	–	(*57*)
		GSTT1	–	–	E	NE	NE		

Abbreviations: CA, chromosome aberrations; E, significant effect of genotype on biomarker; NE, no effect of genotype on biomarker; MN, micronuclei in lymphocytes. [a]By ^{32}P-postlabeling or ELISA. [b]Urine mutagenicity.

PAH levels in Finland seem low; in fact they almost correspond to the environmental exposure in the busy streets of Copenhagen.] Neither *GSTM1* nor *CYP1A1* genotypes affected the level of DNA adducts determined by ^{32}P-postlabeling. *GSTM1* appeared to modify the DNA adduct levels compared to individuals lacking this gene. The authors suggested that the effect of genotypes in their study should not be used as negative evidence because exposure to PAHs was low.

Gabbani et al. (*47*) analyzed the effect of *GSTM1* and *NAT2* on urinary mutagens in coke oven workers. No data on exposure were presented. They observed the combined effect between genotypes and smoking. Smokers with the genotype combination *GSTM1* null/*NAT2*-ss (slow acetylator) showed the highest frequency of positive urine mutagenicity among all subjects. Smokers with the slow acetylator genotype showed a higher frequency of positive urine samples than smokers with fast acetylator genotypes. The results suggest that coke oven workers who are smokers and carry genotypes unfavorable for detoxification of aromatic amines (*NAT2*-ss) and PAHs (*GSTM1* null) may have an increased risk of developing bladder cancer.

Binková et al. (*48*) studied coke-oven workers by using personal monitors to evaluate PAH exposure and analyzing DNA adducts by ^{32}P-postlabeling, chromosomal aberrations, sister chromatid exchange (SCE), and *GSTM1* and *NAT2* polymorphism. Exposure to carcinogenic PAHs ranged between 0.6 and 632 μg/m^3. No effect of either genotype was observed on any biomarker. A similar scheme was used by Costa et al. (*49*) to examine the influence of *GSTM1* and *NAT2* genotypes on association between DNA adducts and personal exposure to PAHs (up to 200 μg/m^3). Again no effect of either genotype on DNA adduct level was observed. These results suggest that these detoxification enzymes have less effect on the complex dose–response relationship at high exposure to PAHs.

Another group exposed to PAHs is U.S. Army soldiers affected during the Gulf War (*50*). Analysis of DNA adducts by ^{32}P-postlabeling and dissociation-enhanced lanthanide fluoroimmunoassay revealed no observed association between any biomarker and *GSTM1* or *CYP1A1* genotypes.

The impact of occupational exposure to benzidine in relation to the *GSTM1* genotype was studied in workers currently exposed to benzidine (manufacturing benzidine dihydrochloride and benzidine-based dyes) by analyzing DNA adducts in the urothelial cells and urinary mutagenicity (*51*). The *GSTM1* genotype had no impact on DNA adducts and urinary mutagenicity levels in these exposed workers. The authors concluded that the *GSTM1*-null genotype does not have any impact on bladder cancer caused by benzidine exposure.

Scarpato et al. (*52*) studied the impact of *GSTM1*, *GSTT1*, and *NAT2* genotypes on chromosomal aberrations, SCEs, and micronuclei in floroculturists exposed to different types of pesticides. The level of cytogenetic damage was not significantly affected by the agrochemical exposure of the subjects regardless of the level of pesticide used. *GSTM1*, *GSTT1*, and *NAT2* genotypes did not influence the level of cytogenetic damage among floroculturists and control subjects. Surprisingly, *GSTM1*-null individuals who smoked had higher frequencies of chromosomal aberrations than *GSTM1*-positive smokers. *NAT2* polymorphism could not be related to any spontaneous or induced differences in the cytogenetic parameters studied. Later Scarpato et al. (*53*) increased the number of greenhouse workers in their study from 23 to 30 subjects. *GSTM1*-null smokers had significantly increased chromatid-type aberrations. The effect was more pronounced in both *GSTM1* and *GSTT1*-null genotype individuals, which suggests their possible interaction.

Wiencke et al. (*54*) worked out a technique to identify subjects sensitive to epoxide-induced damage. Peripheral lymphocytes of *GSTM1*-deficient and -nondeficient individuals were treated with trans-stilbene oxide. The *GSTM1*-null genotype individuals were associated with a significant increase of SCE. The results indicated that *GSTM1* is also a marker of susceptibility to the induction of cytogenetic damage by a certain class of mutagens.

Later Pemble et al. (*55*) identified a null allele at the *GSTT1* locus. The same approach was used by Kelsey et al. (*56*) to identify *in vitro* sensitivity of lymphocytes in *GSTT1*-null subjects to diepoxybutane.

Personal monitoring was also used in studies analyzing the effect of exposure to 1,3-butadiene. Kelsey et al. (*56*) observed in workers exposed to approximately 0.5 mg/m^3 1,3-butadiene that a proportional population with the *GSTT1*-null genotype had lymphocytes with increased sensitivity to diepoxybutane *in vitro*, determined as SCE. Because diepoxybutane is one of the 1,3-butadiene metabolites, lack of a *GSTT1* gene was postulated to increase the risk of 1,3-butadiene exposure. Later Sorsa et al. (*57*) confirmed this idea on a larger sample of workers exposed to a low level of 1,3-butadiene during monomer production. The *GSTM1* genotype had no effect on chromosomal aberrations, SCE, and micronuclei, but significantly increased levels of chromosomal aberrations were observed in workers lacking the *GSTT1* gene. The doubling of chromosomal aberration rate among the workers lacking the *GSTT1* gene suggests the importance of the *GSTT1* gene in the detoxification pathway of 1,3-butadiene *in vivo*. These results formed the basis of an idea to use *GSTT1* genotype determination for 1,3-butadiene-exposed workers as part of their preventive medical examinations before they began work in monomer production and polymerization units.

Table 2. Effect of genotypes on biomarkers of exposure and effects by environmentally exposed populations.

Group/ sample size	Genotypes	Biomarkers: DNA adducts[a]	Protein adducts[b]	Urine[c]	Comet	CA	SCE	MN	*HPRT*	Reference
Autopsy donors										
$n=38$	*GSTM1*	E	–	–	–	–	–	–	–	(*58*)
	CYP1A1	NE	–	–	–	–	–	–	–	
$n=90$	*GSTM1*	E	–	–	–	–	–	–	–	(*59*)
	CYP2D6	E	–	–	–	–	–	–	–	
	CYP2E1	E	–	–	–	–	–	–	–	
Patients, coal tar										
$n=57$	*GSTM1*	NE	NE	–	–	–	–	–	–	(*60*)
Smokers										
$n=17$	*GSTM1*	–	–	E	–	–	–	–	–	(*61*)
	NAT2	–	–	NE	–	–	–	–	–	
$n=63$	*GSTM1*	NE	–	–	–	–	–	–	–	(*62*)
$n=159$	*GSTM1*	NE	–	–	–	–	–	–	–	(*63*)
	CYP1A1	E	–	–	–	–	–	–	–	
$n=156$	*GSTM1*	–	–	–	–	–	E	NE	–	(*64*)
$n=21$	*GSTM1*	NE	–	–	–	–	–	–	–	(*65*)
	GSTP1	NE	–	–	–	–	–	–	–	
$n=151$	*GSTM1*	–	E	–	–	–	–	–	–	(*66*)
Nonsmokers										
$n=120$	*GSTM1*	NE	NE	–	–	–	–	–	–	(*67*)
$n=76$	*GSTM1*	–	–	–	–	–	–	–	NE	(*68*)
$n=22$	*GSTM1*	–	–	–	–	–	E	–	NE	(*69–71*)
$n=38$	*GSTM1*	–	–	–	–	–	–	NE	–	(*72*)
Mothers, blood										
$n=128$	*GSTM1*	–	NE	–	–	–	–	–	–	(*73*)
$n=74$	*GSTM1*	–	–	–	NE	–	–	–	–	(*74*)
Mothers, placenta										
$n=98$	*GSTM1*	E	–	–	–	–	–	–	–	(*75*)
Postal workers, gardeners										
$n=65$	*GSTM1*	NE	NE	E	E	NE	NE	–	–	(*76*)
Firefighters										
$n=47$	*GSTM1*	NE	–	–	–	–	–	–	–	(*77*)
	CYP1A1	NE	–	–	–	–	–	–	–	

Comet, Comet assay. [a]By ^{32}P-postlabeling or ELISA. [b]Protein adducts, hemoglobin, or albumin adducts. [c]Urine mutagenicity.

Environmental Exposure to Mutagens and Carcinogens

Table 2 summarizes the effect of genotypes on biomarkers of exposure and effects by environmentally exposed populations.

The first study attempting to establish a relationship between genotypes and DNA adducts in parenchymal lung tissue obtained from autopsy donors was that of Shields et al. (*58*). Higher DNA adduct levels were associated with the *GSTM1*-null genotype. No correlation was found between PAH–DNA adducts and *CYP1A1* exon 7 mutations. This study proved the effect of the *GSTM1*-null genotype on DNA adducts level detected by ^{32}P-postlabeling assay for bulky aromatic adducts.

Kato et al. (*59*) examined the effect of *GSTM1*, *CYP1A1*, *CYP2D6*, and cytochrome P450 2E1 (*CYP2E1*) genotypes on DNA adducts in lung tissues from autopsy donors. The *GSTM1*-null genotype was associated with higher levels of PAH-derived DNA adducts, *CYP2D6*, and *CYP2E1* genotypes with 7-methyl-2′-deoxyguanosine-3-monophosphate adduct levels in nonsmokers. These findings suggest that genetic polymorphisms may predict carcinogen–DNA adduct levels and thus might predict an individual's lifetime response to carcinogen exposure.

Santella et al. (*60*) tried to determine if the levels of DNA and protein adducts in coal tar-treated psoriasis patients might be affected by the *GSTM1* genotype. DNA adducts and PAH–albumin adducts were determined by enzyme-linked immunosorbent assay (ELISA). DNA adducts but not PAH–albumin adducts were elevated in patients. However no relationship was found between DNA or protein adduct levels and the *GSTM1* genotype.

Most environmental studies have been concerned with the effect of exposure to tobacco smoke. One of the first papers was by Hirvonen et al. (*61*), who examined the effect of *GSTM1* and *NAT2* genotypes on urinary mutagenicity using *Salmonella typhimurium* tester strains TA98 and YG1024. Smokers lacking the *GSTM1* gene had several times higher urine mutagenicity than smokers who had the gene. Such an effect was not observed among nonsmokers. No effect of *NAT2* genotypes was detected among smokers or nonsmokers.

Grinberg-Funes et al. (*62*) observed no increase of DNA adducts determined by competitive ELISA in *GSTM1*-null smokers. When DNA adducts were stratified according to *GSTM1* genotype and plasma levels of vitamins E and C in these individuals, a relationship between DNA adduct levels and the *GSTM1* genotype was observed. This finding is consistent with inverse associations between antioxidant micronutrient status and the *GSTM1*

genotype, which could modulate DNA adduct formation.

Mooney et al. (*63*) analyzed the effect of *GSTM1* and *CYP1A1* genotypes on DNA adducts by competitive ELISA in heavy smokers. No effect of *GSTM1*-null genotype was detected. DNA adducts were increased in smokers with the *CYP1A1* exon 7 valine polymorphism. There was no apparent interaction between *CYP1A1* and *GSTM1* genotypes with respect to DNA adducts in the smokers. A decreased level of β-carotene was detected in *GSTM1*-null genotype subjects. Association between DNA adducts and β-carotene levels in smokers lacking the *GSTM1* gene indicates that vitamin levels may be more critical in persons who do not have the ability to detoxify PAHs via the *GSTM1* pathway than in other individuals. The authors proposed that smokers with either *CYP1A1* exon 7 polymorphism or low levels of micronutrients alone or in combination with the *GSTM1*-null genotype sustain more genetic damage from cigarette smoking and other environmental exposures to PAHs than individuals without these factors.

SCE in peripheral lymphocytes and micronuclei in sputum cells were used as biomarkers for increased cytogenetic damage (*64*). SCEs were higher in smokers with the *GSTM1*-deficient phenotype. This effect of phenotype was not observed in micronuclei of sputum cells.

Savela et al. (*65*) investigated DNA adduct formation in bronchoalveolar macrophages of smokers and nonsmokers in relationship to *GSTM1* and *GSTP1* genotypes. The number of cigarettes smoked per day had a stronger influence on DNA adducts than polymorphic genotypes.

Yu et al. (*66*) studied the effect of the *GSTM1* genotype on 3- and 4-aminobiphenyl (ABP) hemoglobin adduct levels in white, black, and Asian smokers and nonsmokers. 4-ABP hemoglobin adducts were significantly higher in subjects possessing the *GSTM1*-null genotype.

Nielsen et al. (*67*) studied the impact of exposure to urban and rural air pollution in healthy male nonsmokers from Denmark and Greece. No effect of *GSTM1* on DNA and protein adduct level was observed, probably because of the low level of pollution.

Another group of healthy male nonsmokers was followed in Stockholm using *HPRT* T-cell cloning assay (*68*). The difference in *HPRT* mutant frequency was not significant between *GSTM1*-negative and *GSTM1*-positive individuals. The study showed that age contributes more than *GSTM1* polymorphism to the large interindividual variation in *HPRT* mutant frequency of nonsmokers.

Cheng et al. (*69,70*) observed the effect of the *GSTM1* genotype on SCE frequency in nonsmokers; SCE frequency was higher in subjects of both sexes lacking this gene. A similar effect was not found in former or current smokers. In the same group the mutant frequency at the *HPRT* locus (*71*) and micronuclei in human lymphocytes (*72*) were studied. Mutant frequency at *HPRT* and micronuclei frequency were not associated with *GSTM1* polymorphism.

Autrup et al. (*73*) determined the transplacental transfer of genotoxic material using a competitive ELISA assay to measure PAH–albumin adduct level in serum isolated from the mother and the umbilical cord. Air pollution measured by suspended particulate matter in the city of Aarhus, Denmark, between 1988 and 1990 was estimated on average to be 62 $\mu g/m^3$. Protein adducts were related in decreasing order to rural areas, Aarhus, and suburban areas. The *GSTM1* genotype did not significantly alter the serum albumin adduct level.

The Comet assay, which determines DNA single-strand breaks, was used to evaluate the impact of air pollution in a polluted region. The assay used whole blood from mothers and umbilical cords (*58*). No effect of the *GSTM1* genotype on comet parameters (as a percentage of DNA in tail or tail length) was observed (*74*).

Topinka et al. (*75*) used human placentas to study DNA adduct levels in relation to the *GSTM1* genotype in mothers living in two regions with different average air pollution levels. Total DNA adduct levels were significantly higher in the polluted region and more pronounced in winter. Higher DNA adduct levels were detected in the group of mothers with the *GSTM1*-null genotype. This finding seems more relevant to subjects living in polluted industrial areas.

An environmental study that used personal monitoring of carcinogenic PAHs in a group of healthy women who worked outdoors was reported by Binková et al. (*76*). The series of biomarkers included PAH metabolites in urine, urine mutagenicity, PAH–DNA adducts in white blood cells determined by ^{32}P-postlabeling, PAH–albumin adducts determined by ELISA, DNA damage in lymphocytes by Comet assay, chromosomal aberrations, SCE, and the *GSTM1* genotype. There were no observed effects of the *GSTM1* genotype on DNA and protein adduct levels, chromosomal aberrations, or SCE. Urinary PAH metabolites were significantly increased in *GSTM1*-null genotype subjects in the polluted district. In the Comet assay there was a significant increase of DNA percentage in tail and an increase of urinary mutagenicity associated with the *GSTM1*-null genotype, but the effect of personal exposures to PAHs on the variability of biomarkers was more pronounced than the effect of the *GSTM1* genotype. DNA adducts tended to show an increase in the *GSTM1*-null genotype.

In a group of firefighters (*77*) the level of PAH–DNA adducts measured by ELISA in white blood cells was not significantly related to *GSTM1* or *CYP1A1* genotypes. However there was a positive association between consuming charbroiled food and PAH–DNA adduct formation. These results suggest that the *GSTM1*-null genotype and *CYP1A1* exon 7 polymorphisms are not associated with increased susceptibility to PAH–DNA adduct formation in peripheral white blood cells measured by ELISA in nonsmoking populations.

Discussion

Literature on the effect of genotypes and different sites of cancer is extensive. However, only 34 recent papers were found that study the effect of *GSTM1* together with other genotypes on biomarkers of exposure and of effects related to occupational or environmental exposure to mutagens and carcinogens. Using a well-known paradigm of environmental cancer (*78*), biomarkers for the entire spectrum of human genotoxicant interactions begin with exposure. Many papers lack data on air pollution measured during the same period that the blood samples were collected. With regard to occupational studies, only four papers (*48,49,56,57*) have been found that compared biomarkers to personal exposures. This may be one of the reasons epidemiologists usually acknowledge the limitations of biomarkers in cancer epidemiology (*79*).

Validated biologic markers of susceptibility can be used as effect modifiers in epidemiologic studies. Interpretation of effect modification depends on the statistical method (e.g., multiplicative or additive) used to model interaction. From the biologic perspective effect modification can explain why two similarly exposed individuals may not both develop a disease. In part the answer is individual variability in metabolic, detoxification, and repair capabilities (*80*).

Possession of a susceptible gene should decrease the biologically effective dose or

elevate the risk of disease. Khoury et al. (*81*) proposed six possible patterns of gene–environment interaction to evaluate genetic marker-disease associations and their interactions with specific environmental risk factors. According to Rothman et al. (*82*), susceptibility genes are common in the population, are generally considered polymorphisms, and may interact with a particular exposure.

Our review suggests that exposure to carcinogens is usually affected by several genotypes. It is possible that if individuals lack a certain genotype, detoxification may proceed through another pathway. There are only a few studies that analyze the significance of several genotypes simultaneously. New input is expected from the environmental genome project (*11*).

It is difficult to decide how genetic polymorphisms should be interpreted in risk assessment. Until now there have been only two studies on the effect of genotype on human health. Rothman et al. (*83*) evaluated the impact of genetic predispositions that activate (e.g., *CYP2E1*) and detoxify reduced nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase (*NQO1*) benzene and its metabolites. Occupational benzene poisoning is associated with subsequent development of acute nonlymphocytic leukemia and the related myelodysplastic syndromes. The authors showed that subjects homozygous for the *NQO1* ^{609}C–T mutation had a 7.6-fold increased risk of benzene poisoning. This is the first report providing evidence that interindividual variation in metabolic process among humans is associated with the risk of benzene-associated disease. The significance of benzene poisoning for hematologic malignancy may be used to screen new workers with benzene exposure for *NQO1* polymorphisms. The Rothman et al. study (*83*) suggests that in the future other genotypes may also be found to be related to specific types of carcinogen exposure.

Another example is *GSTT1* polymorphisms for 1,3-butadiene occupational exposure (*57*). The fact that subjects with *GSTT1*-null genotype have frequencies of chromosomal aberrations twice as high as *GSTT1* carriers means that *GSTT1*-null genotype subjects will have higher risk of malignancies (*84,85*). This agrees with reports (*84,85*) showing that chromosomal aberrations are increased before clinical manifestation of cancer.

At present the most sensitive biomarker of exposure is thought to be identification of DNA adducts by ^{32}P-postlabeling (*86,87*). However, no studies involving high exposure to PAHs found any effects of genotype on DNA adducts (Table 1). Effects of *GSTM1* and *NAT2* were observed in coke oven workers on mutagenicity of urine (*47*) regardless of biomarker used; these effects, however, were restricted to the subgroup of smokers. Another effect was observed with *GSTT1* genotype affecting the frequency of chromosomal aberrations among workers from the 1,3-butadiene monomer production unit.

In an analysis of environmentally exposed populations, effects of genotypes on DNA adducts from lung tissue of autopsy donors (*58,59*) and from placentas of mothers living in an air-polluted region were found (*75*). *GSTM1*-null genotype increased the affected mutagenicity of urine in smokers (*61*) and in subjects from polluted regions (*76*). A *GSTM1* effect was also observed from other biomarkers in smokers on protein adducts (*66*) and SCE (*64*), in nonsmokers on SCE (*69*), and in postal workers on Comet assay parameters (*76*). These results, however, appear too limited to allow any significant conclusion. DNA adducts were affected by *GSTM1* in lung tissue of autopsy donors (*58,59*) and in placentas of mothers (*75*); tissues in both cases correspond to the target tissue.

Vineis and Marton (*88*) speculated that the effect of genotype is more pronounced at low doses and that individual susceptibility is irrelevant under exceptionally high exposure conditions. If one compares the effects of genotypes on occupationally and environmentally exposed populations in Tables 1 and 2, their idea appears consistent with the results of the reviewed studies.

Another important feature in epidemiologic studies may be an adaptive response, as originally demonstrated for ionizing radiation (*89*). Coke oven workers are exposed to high concentrations of PAHs but their health risks do not correspond to the levels of carcinogen exposure. Experience with exposures in Eastern Europe indicates that the impact of pollution on human health is less dramatic than the sum of pollutants in these regions (*90,91*). Similar unexpected results were observed by Natarajan et al. (*92*) among Indians in Argentina who were exposed to high concentrations of arsenic but did not show any sign of chronic arsenic poisoning. These examples suggest an adaptive response in human populations—a response that surely is determined by genotype.

Our review covered several studies and analyzed the effect of genotypes, especially *GSTM1*, on biomarkers of exposure and effects for occupationally and environmentally exposed populations. Considering all studies with *GSTM1*, we probably have not reached the the stage where results could be interpreted for preventive measures, e.g., to predict risk because of occupational exposure to mutagens and carcinogens, or to identify hypersusceptible workers and exclude them from working in jobs in which they may be exposed to high levels of carcinogens. The effect of genotypes was sometimes based on small sample size. For this reason further studies should consider the most suitable epidemiologic design for each biomarker (*82*).

Conclusion

The impact of genetic polymorphisms as biomarkers of susceptibility is of key significance to the understanding of the process of genetic damage involved in mutagenesis and carcinogenesis. Studies published so far are promising. The relationship between genotypes, biomarkers of exposure, and biomarkers of effects could be important in risk assessment of human exposure to mutagens and carcinogens.

Probably, at least for proved human carcinogens, should be accepted: If the genetic polymorphism of genes responsible for detoxification pathways is known it is reasonable to suggest that subjects lacking these genes not be employed in occupations in which certain types of exposure are likely to occur.

Certain ethical questions must be addressed if knowledge about individual genotypes is to be used to prescribe preventive measures among specific groups. For example, should the genotypes of workers be identified as prerequisites to their working in environments with high exposures to carcinogens such as PAHs in coke ovens? A consensus must be reached about when or where the use of new knowledge is ethically acceptable.

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