Glutathione S-Transferase M1 Genotype Affects Aminobiphenyl-hemoglobin Adduct Levels in White, Black, and Asian Smokers and Nonsmokers

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Abstract
Cigarette smoking is the major cause of bladder cancer in men in the United States, and the arylamines contained in cigarette smoke, including 4-amino-biphenyl (4-ABP), are believed to play an important role in the induction of bladder cancer among smokers. As a result, we have demonstrated that the prevalence of slow acetylators, which is catalyzed by the genetically controlled hepatic N-acetyltransferase enzyme displaying two phenotypes (slow versus rapid), is a detoxification pathway for arylamines with regard to bladder carcinogenesis. In Los Angeles, CA, non-Hispanic white (54%), black (34%), and Asian males have comparable smoking habits and yet dramatically different risks of bladder cancer (31 of 100,000 in whites, 16 of 100,000 in blacks, and 13 of 100,000 in Chinese and Japanese). Previously, we have demonstrated that the prevalence of slow acetylators (the high-risk phenotype) was highest in whites (54%), intermediate in blacks (34%), and lowest in Asians (14%). We also showed that mean 3- and 4-ABP hemoglobin adduct levels were significantly higher in cigarette smokers relative to non-smokers, and that the level increased with increasing number of cigarettes smoked/day. Most importantly, slow acetylators consistently exhibited higher mean levels of ABP hemoglobin adducts relative to rapid acetylators, regardless of race and level of cigarette smoking.

We assessed 151 residents of Los Angeles County (CA) who were either white, black, or Asian (Chinese or Japanese) and over the age of 30 years for their glutathione S-transferase M1 (GSTM1) genotype (null versus non-null), acetylator phenotype (slow versus rapid), levels of 3- and 4-ABP hemoglobin adducts, and current use of tobacco products. Whites (27%) had the highest prevalence of the highest risk profile (slow acetylator, GSTM1 null), followed by blacks (15%) and Asians (2.7%), and the difference was statistically significant (P = 0.006). Whites also had less than one-half the prevalence of the “protective” profile (rapid acetylator, GSTM1 non-null) relative to blacks and Asians (23 versus 57%; P = 0.0001). Regardless of race and level of cigarette smoking, mean levels of 3- and 4-ABP hemoglobin adducts were higher in subjects possessing the higher risk GSTM1/acetylator profile. Mean level of 4-ABP hemoglobin adduct (adjusting for race, cigarette smoking, and acetylator phenotype) was significantly higher in subjects possessing the GSTM1-null versus GSTM1-non-null genotype (46.5 versus 36.0 pg/g Hb; P = 0.037). The comparable difference in mean levels of 3-ABP hemoglobin adduct was borderline significant (1.6 versus 1.1 pg/g Hb; P = 0.07). Thus, our results suggest that GSTM1 is involved in the detoxification of 3- and 4-ABP and may contribute to the racial variation in bladder cancer incidence among white, black, and Asian males in Los Angeles, CA.

Introduction
Epidemiological studies of workers exposed to industrial arylamines, such as 2-naphthylamine and 4-ABP, coupled with laboratory investigations have established these compounds as human bladder carcinogens (1). These chemicals require metabolic activation in order to become carcinogenic, and N-hydroxyl, which is catalyzed by the hepatic cytochrome P450 1A2 isoenzyme (CYP1A2), is considered to be the first critical step (2). N-acetylation, which is regulated by N-acetyltransferase activity in the liver, represents a competing detoxification pathway for many of these arylamines (3). The enzyme has been shown to be genetically controlled, coded by a single gene with several so-called “mutant” alleles (4). Individuals homozygous for any of the mutant alleles display the slow acetylator phenotype, whereas individuals heterozygous or homozygous for the wild-type allele display the rapid acetylator phenotype (4). After the discovery that the polymorphic N-acetyltransferase enzyme is involved in caffeine metabolism (5), a urinary assay for phenotypic determination was developed, that uses this substance (contained in such commonly consumed beverages as coffee, tea, and cola) as the test compound (6).

Aside from occupational exposure to industrial arylamines, cigarette smoking is the only other established cause of bladder cancer in the United States, and it is the major cause of

1 The abbreviations used are: ABP, aminobiphenyl; CYP1A2, cytochrome P450 1A2 isoenzyme; GSTM1, glutathione S-transferase M1; Hb, hemoglobin.
bladder cancer in men in the United States (1). Cigarette smoke also contains a number of carcinogenic arylamines, including 2-naphthylamine and 3- and 4-ABP, which are thought to be part of the constitutes responsible for inducing bladder cancer among smokers (1, 7).

In Los Angeles, non-Hispanic white (white), black, and Asian males have comparable smoking habits and yet dramatically different risks of bladder cancer (8). Annual incidence is highest in whites (31 of 100,000), intermediate in blacks (16 of 100,000), and lowest in Chinese and Japanese (13 of 100,000). Previously, we conducted a cross-sectional survey among male adults from these three racial-ethnic groups in Los Angeles in an attempt to identify the determinants that could explain the disparate observations described above. The survey results suggest that acetylation phenotype (i.e., individual’s genetically determined ability to detoxify arylamines) could be an important determinant of bladder cancer risk, and that this genetic determinant might be a major factor in the varying bladder cancer risk among whites, blacks, and Asians. We observed that the prevalence of slow acetylators (the high-risk phenotype) was highest (54%) among whites at high risk for bladder cancer and lowest (14%) among Asians with the lowest risk for bladder cancer. Black males whose bladder cancer risk is intermediate between those of the other two groups, showed an intermediate rate of slow acetylators (34%). We also showed that mean 3- and 4-ABP hemoglobin adduct levels were significantly higher in cigarette smokers relative to nonsmokers, and that the level increased with increasing number of cigarettes smoked/day. Most importantly, slow acetylators consistently exhibited higher mean levels of ABP hemoglobin adducts relative to rapid acetylators, regardless of race and level of cigarette smoking (8). It has been shown that in humans, ABP hemoglobin adduct levels closely correlate with DNA adduct levels in urothelial cells (9).

GSTM1 is part of a family of enzymes that detoxify reactive chemical entities by promoting their conjugation to glutathione. Metabolites of many constituents of cigarette smoke, including polycyclic aromatic hydrocarbons, arylamines, and nitrosamines (10, 11), are potential substrates for GSTM1. GSTM1 is polymorphic in humans, with inherited homozygous deficiency (−/−) being associated with no GSTM1 enzymatic activity in affected individuals. It is known that about 50% of whites in the United States possess the homozygous null genotype. Recently, several case-control studies of bladder cancer conducted in the United States and Western Europe have reported an excess of GSTM1 homozygous-null genotype among cases relative to controls (12–16), although at least one other case-control study failed to confirm the association (17).

We explored the possibility that the hydroxylamine metabolites derived from certain arylamines that are present in cigarette smoke, including 3- and 4-ABP, are substrates for GSTM1. Thus, at least part of the excess risk for bladder cancer in individuals possessing the GSTM1 homozygous-null genotype could be due to their reduced capacity in arylamine detoxification. We tested this hypothesis on 151 black, Asian, and white residents of Los Angeles County, who were either subjects of the cross-sectional survey described above (8) or controls for a subsequent case-control study of bladder cancer that used the same protocol for urine and blood collection as the multiethnic survey.

Materials and Methods
Subjects. Our study subjects were derived from two separate sources: (a) those who participated in the multiethnic survey described previously (8); and (b) control subjects for an ongoing case-control study of bladder cancer that utilized an identical protocol for urine and blood collection and included identical questions on current tobacco use as the multiethnic survey. In brief, the survey participants were male residents of Los Angeles County who were over the age of 35 years and were either white (n = 32), black (n = 39), or Asian (n = 37). Chinese and 21 Japanese. By design, 58 (54%) subjects were lifelong nonsmokers; the remaining 50 subjects were current cigarette smokers of varying intensity.

The study data also included the first 43 control subjects recruited for an ongoing case-control study of bladder cancer among non-Asian residents of Los Angeles ages 25–64 years. The case-control study was initiated immediately after the termination of the multiethnic survey. Ten of the control subjects were women. We did not exclude the female subjects from the study for the following reasons: (a) there is no a priori reason to believe that genetic determinants such as acetylation phenotype and GSTM1 genotype are gender related; and (b) a comparison of the distributions of acetylation phenotype, GSTM1 genotype, and 3- and 4-ABP hemoglobin adduct levels between the male and female subjects indicated strong comparability. The ages of the 43 controls ranged from 32 to 68 years; the mean age was 56 years. Ten subjects were current smokers, and four smoked two or more packs of cigarettes/day.

A blood specimen was obtained from each subject after an in-person interview during which the subject was asked about cigarette and other tobacco use during the past 60 days. Red cells extracted from whole blood were processed on the same day of collection and then stored at −20°C until analysis for 3- and 4-ABP hemoglobin adduct levels (8). DNA samples extracted from whole blood were stored at −70°C until GSTM1 genotyping, which utilized the method as described in Bell et al. (13).

In addition, each subject was asked to collect an overnight urine sample (ending with the first morning void) after drinking coffee prepared from two packets of Nescafe instant coffee (about 70 mg of caffeine) between 3 and 6 p.m. the previous day. All urine specimens were acidified (20 mg of ascorbic acid/ml of urine) within 24 h of collection and subsequently stored at −20°C until acetylator phenotype determination (8). Statistical Analysis. Initially, data from the multiethnic survey and the case-control study of bladder cancer were analyzed separately. Results were found to be similar; consequently, the two data sets were pooled. The prevalence of the highest (slow acetylator phenotype, GSTM1-null genotype) and the lowest risk profile (rapid acetylator phenotype, GSTM1-non-null genotype) was compared across the three races studied by means of the multinomial test. The distributions of 3- and 4-ABP hemoglobin adduct levels in our study populations were markedly skewed; therefore, formal statistical testing was performed on logarithmically transformed values of adduct levels and geometric (as opposed to arithmetic) mean values are presented. The analysis of covariance method (with number of cigarettes smoked/day as the covariate and race, GSTM1 genotype, and acetylator phenotype as main effects) was used to test the simultaneous effects of GSTM1 genotype and acetylator phenotype on 3- and 4-ABP hemoglobin adduct levels, while adjusting for the effects of race and cigarette smoking on ABP hemoglobin adduct levels (18). All P values quoted are two sided.
Results

Table 1 presents the frequencies of acetylator phenotype by GSTM1 genotype in the study subjects. Whites (27%) had the highest prevalence of the highest risk profile (slow acetylator, GSTM1 null), followed by blacks (15%), with Asians showing the lowest prevalence (2.7%). The difference in prevalence of the highest risk profile across the three races was statistically significant (P = 0.006), and the three rates were as predicted by the varying bladder cancer incidence across the three races. Conversely, the prevalence of the lowest risk profile (rapid acetylator, GSTM1 null) among whites (23%) was less than one-half those in blacks and Asians (both were 57%), and this difference in the three rates also was statistically significant (P = 0.0001). We examined the frequency distributions of GSTM1/acetylation phenotype according to cigarette smoking status and found no association between the two factors (P = 0.42; data not shown).

Table 2 shows the geometric mean levels of 4-ABP hemoglobin adducts, with adjustment for race and level of cigarette smoking. Mean level was highest among subjects possessing the slow acetylator phenotype and having at least one functional GSTM1 allele (33.1 pg/g Hb). The difference in mean levels between subjects with the highest versus the lowest risk profile was 1.6-fold. Overall, the mean level was 21% higher in slow versus rapid acetylators (P = 0.13) and 29% higher in GSTM1-null versus GSTM1-non-null subjects (P = 0.037). The residual difference in mean levels across the three races after adjustment for acetylator phenotype, GSTM1 genotype, and level of cigarette smoking still approached statistical significance (P = 0.08).

Results for 3-ABP hemoglobin adducts were similar to those for 4-ABP hemoglobin adducts (Table 3). Again, subjects who were slow acetylators and displayed the GSTM1-null genotype had the highest mean level (2.4 pg/g Hb), whereas rapid acetylators with at least one functional GSTM1 allele had the lowest mean level (0.8 pg/g Hb); the difference was 3.0-fold. Overall, the mean level was 211% higher in slow versus rapid acetylators (P = 0.0004) and 45% higher in GSTM1-null versus GSTM1-non-null subjects (P = 0.07). There was a significant difference in mean levels across the three racial groups even after adjustment for acetylator phenotype, GSTM1 genotype, and level of cigarette smoking (P = 0.017).

Table 4 presents the geometric mean levels of 3- and 4-ABP hemoglobin adducts according to GSTM1/acetylation profile for cigarette smokers and nonsmokers separately. The mean levels of 4-ABP hemoglobin adducts were 3-4-fold higher in slow versus rapid acetylators (P = 0.0004), and 45% higher in GSTM1-null versus GSTM1-non-null subjects (P = 0.07). There was a significant difference in mean levels across the three racial groups even after adjustment for acetylator phenotype, GSTM1 genotype, and level of cigarette smoking (P = 0.017).
higher in smokers than in nonsmokers with comparable GSTM1 acetylation profile. These differences were much more pronounced for 3-ABP hemoglobin adducts; mean levels between smokers and nonsmokers were 18–29-fold apart. For both smokers and nonsmokers, individuals possessing the GSTM1-null genotype and slow acetylator phenotype had the highest mean levels of 3- and 4-ABP hemoglobin adducts, and those having at least one functional allele of the GSTM1 gene and possessing the rapid acetylator phenotype had the lowest mean levels. Subjects with an intermediate risk profile (slow acetylator/GSTM1 non-null or rapid acetylator/GSTM1 null) exhibited intermediate mean levels of 3- and 4-ABP hemoglobin adducts.

Discussion
Among our study subjects, we determined that 51% of whites, 23% of blacks, and 32% of Asians (Chinese and Japanese) possessed the GSTM1 −/− genotype. A number of studies have reported the prevalence of GSTM1 homozygous-null genotype in European- or North American-white populations; most fall within the range of 50–60% (13, 15, 16). Two studies have examined the prevalence of the GSTM1-null genotype in blacks in the United States and reported rates of 35 (13) and 31% (15), respectively. Several studies conducted among Chinese and Japanese have observed GSTM1-null rates ranging from 35 to 50% (15). Thus, our results of GSTM1 genotyping among blacks, whites, and Asians in the United States are generally consistent with previous findings.

Our data suggest that GSTM1 is involved in the detoxification of 3- and 4-ABP and may contribute to the racial variation in bladder cancer incidence. We observed a 10-fold increase in the prevalence of the highest risk profile (i.e., slow acetylator, GSTM1 null) between Asians (2.7%) who are at low risk and whites (27%) who are at high risk for bladder cancer. Intermediate-risk blacks have an intermediate rate (15%) of the highest risk profile. High-risk whites also have less than half the rate of the “protective” profile (rapid acetylator, GSTM1 non-null) relative to blacks and Asians (23 versus 57%). We further noted that 3- and 4-ABP hemoglobin adduct levels were consistently higher in subjects possessing the higher risk GSTM1/acetylator profile and that GSTM1 genotype and acetylator phenotype independently contribute to the differential in ABP hemoglobin adduct levels.

It is interesting that our data suggest that whites, blacks, and Asians who possessed identical acetylator phenotype and GSTM1 genotype and who had comparable smoking habits might be exposed to varying levels of activated 3- and 4-ABP in vivo. This could indicate the presence of one or more additional arylamine metabolizing enzyme(s) that show variation in activities by race. One such candidate enzyme may be CYP1A2. Kalow and Tang (19) have proposed a caffeine-based urinary assay for possible CYPIA2 phenotypic determination. Unfortunately, our survey participants were not assessed for this “CYP1A2 index.” Although our data are internally consistent with the notion that GSTM1 is involved in arylamine metabolism, we caution that the hypothesis is a novel one, and that the sample size of the present study is relatively modest. Furthermore, metabolites of ABP have not been shown to be substrates for GSTM1.

Therefore, there is a particular need to replicate these findings and to explore the GSTM1/acetylation/arylamine/bladder cancer relationships in greater detail.

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