BIOMARKERS, VOL. 8, NO. 2 (MARCH–APRIL 2003), 142–155

Taylor & Francis
healthsciences

# A cross-sectional study of polycyclic aromatic hydrocarbon–DNA adducts and polymorphism of glutathione *S*-transferases among heavy smokers by race/ethnicity

KERA F. WEISERBS[1], JUDITH S. JACOBSON[1], MELISSA D. BEGG[2], LIAN WEN WANG[3], QIAO WANG[3], MEENAKSH AGRAWAL[4], EDWARD P. NORKUS[4], TIE-LAN YOUNG[3] and REGINA M. SANTELLA[3]*

[1] Department of Epidemiology, Mailman School of Public Health of Columbia University, New York, USA
[2] Department of Biostatistics, Mailman School of Public Health of Columbia University, New York, USA
[3] Department of Environmental Health Sciences, Mailman School of Public Health of Columbia University, New York, USA
[4] Our Lady of Mercy Medical Center, Bronx, New York, USA

*Received 5 August 2002, revised form accepted 10 January 2003*

Differences in lung cancer risk by race/ethnicity have been observed among smokers. To determine whether these observations might reflect differences in the formation of carcinogen–DNA adducts, we analysed blood specimens ($n = 151$) collected from smokers who were recruited for possible participation in an antioxidant vitamin intervention study. Mononuclear cells were analysed for polycyclic aromatic hydrocarbon (PAH)–DNA adducts by competitive enzyme-linked immunosorbent assay. Genotypes of glutathione *S*-transferase M1 and P1 (*GSTM1* and *GSTP1*), enzymes involved in the detoxification of PAH metabolites, were determined by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism, respectively. *GSTM1* was present in 65 out of 88 (73.4%), 16 out of 32 (50.0%) and 16 out of 29 (54.8%) of African-Americans, Caucasians and Latinos, respectively ($p = 0.022$). Homozygosity for the *GSTP1* codon 105 variant was found in 25.6%, 6.3% and 10.0% of African-Americans, Caucasians and Latinos, respectively ($p = 0.023$). Regression analysis of the log-transformed adduct levels confirmed that Caucasian and Latino subjects had lower PAH–DNA adduct levels than African–American subjects, after adjustment for gender, education, α-tocopherol and β-carotene levels, and *GSTM1* status. Further adjustment for age and current smoking habits had no impact on these findings. Although crude analysis suggested that the *GSTM1*-positive genotype may be associated with lower PAH–DNA levels in Caucasians (but not in African-Americans or Latinos), a formal test for interaction between *GSTM1* and ethnicity was not significant. We found no association between adduct levels and *GSTP1* genotype. Although the mechanism is unclear, ethnic differences in DNA damage levels may in part explain why African-Americans have higher lung cancer incidence rates than other ethnic groups.

*Keywords:* polycyclic aromatic hydrocarbons, DNA adducts, glutathione S-transferases.

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

## Introduction

In the USA, the incidence and mortality rates of lung cancer are higher among African-Americans than among those of other races. Socioeconomic factors such as

* Corresponding author: Regina M. Santella, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 701 West 168th Street, New York, NY 10032, USA. Tel: (+1) 212 305 1996; fax: (+1) 212 305 5328; e-mail: rps1@columbia.edu

*Biomarkers* ISSN 1354-750X print/ISSN 1366-5804 online © 2003 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/1354750031000086269

RIGHTSLINK

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

diet, health care utilization, perceived disease risk, occupational exposures and ambient air pollution may account for some of the excess cancer risk among African-Americans (Williams 1996, Shakoor-Abdullah *et al*. 1997, Perez-Stable *et al*. 1998). Smoking behaviour may also play a role. African-Americans smoke mentholated cigarettes more frequently than other groups. Mentholated cigarettes, which have a relatively high tar and nicotine content, provide cooler smoke, encouraging greater inhalation and longer puffs (Shields 2000). Other smoking patterns, such as age at smoking initiation, daily cigarette use and quitting rates, may also differ by race/ethnicity. Some data have suggested that the genes controlling the metabolism of carcinogens may be associated with race/ethnicity. For example, the higher serum cotinine levels found among African-American smokers compared with white smokers may reflect genetic differences in nicotine metabolism (Wagenknecht *et al*. 1990). A recent study using urinary biomarkers of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) found that white smokers had a higher glucuronide to parent NNK ratio than African-American smokers (Wynder and Muscat 1995). This ratio is believed to be a measure of detoxification capacity.

In addition to NNK, cigarette smoke is known to contain more than 55 carcinogenic agents, including polycyclic aromatic hydrocarbons (PAHs) and aromatic amines (Hecht 1999). PAHs are metabolically activated by phase I enzymes such as the cytochrome P450 enzymes to reactive electrophiles, which produce PAH–DNA adducts (Vineis *et al*. 1999). Among smokers, PAH–DNA adducts in mononuclear cells have been associated with lung (Tang *et al*. 1998), bladder (Bonassi *et al*. 1989) and laryngeal (Degawa *et al*. 1994, Szyfter *et al*. 1994) cancers. However, phase II enzymes, such as glutathione *S*-transferase (GST) M1 and P1, conjugate and detoxify reactive intermediates of PAH (Vineis *et al*. 1999). We therefore assessed the relationship between *GSTM1* and *GSTP1* alleles and PAH–DNA adduct levels in African-American, Caucasian and Latino participants in the screening phase of a chemoprevention trial among heavy smokers.

## Methods

The study design was submitted to and approved by the Institutional Review Board of the Columbia Presbyterian Medical Center. This cross-sectional study evaluated heavy smokers initially recruited for an intervention trial of the effects of antioxidant vitamins on DNA damage levels (Jacobson *et al*. 2000). Signs, posted at the Columbia Presbyterian Medical Center and in the surrounding community, invited adults 18 years of age and older who were heavy smokers and who were not current vitamin users to participate in a randomized, placebo-controlled, double-blind trial. The data reported here pertain only to the screening evaluation, which occurred prior to the start of the intervention trial.

A total of 172 individuals received an eligibility screening at which informed consent, a baseline questionnaire regarding demographic factors, diet, personal health and smoking habits, and biological specimens including 45 ml of blood, $\sim$100 ml of urine and oral cell specimens were collected. The biospecimen collection and laboratory analyses have been described previously (Bell *et al*. 1993, Harries *et al*. 1997). PAH–DNA adducts, measured by competitive enzyme-linked immunosorbent assay (ELISA) were expressed as the number of adducts per $10^8$ nucleotides, with a limit of sensitivity of approximately $2/10^8$ nucleotides. Samples with $<20\%$ inhibition were assigned a value of $1/10^8$ nucleotides, a value midway between the lowest detectable value and zero. Plasma vitamins and β-carotene were determined by high performance liquid chromatography using an internal standard for analyte recovery. The *GST* genotype was determined using a polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism (RFLP) method. As plasma lipoproteins are non-specific carriers for carotenoids and vitamin E in plasma, their ratio to the total cholesterol has proven to be the best way to control for confounding effects due to differences in lipoprotein levels between subjects

RIGHTSLINK

(Thurnham *et al*. 1986). Criteria for inclusion in the current analysis consisted of participation in the eligibility screening, *GSTM1* genotype and PAH–DNA adduct data, and self-reported African-American, Caucasian or Latino descent. *GSTM1* genotype and PAH–DNA adduct levels were available from 155 participants. Four individuals who identified themselves as being of 'other' ethnicity were excluded from this analysis. Thus the final analytic sample comprised 151 individuals.

*Statistical analysis*

The major objective of the study was to compare PAH–DNA adducts per $10^8$ nucleotides among heavy smokers by race/ethnicity and *GST* genotype. Preliminary analyses revealed that the distribution of PAH–DNA adducts was highly skewed, as has been noted in other studies. Consequently, we applied a natural log transformation to the PAH–DNA adduct measurements in order to facilitate standard analyses (for which outcome measures are assumed to be approximately normally distributed).

In addition to cross-classifying race/ethnicity by *GSTM1* and *GSTP1* categories, we tabulated race/ethnicity against a number of potential correlates, including gender, age, education ($>12$ years versus $\leq 12$ years), age when began smoking, pack-years, current smoking habits, cotinine levels, and plasma levels of three antioxidant micronutrients (β-carotene, ascorbic acid and α-tocopherol). For these analyses, continuous variables were categorized for simplicity in presentation. To assure interpretability, age was dichotomized at 40 years, and pack-years was dichotomized at 20. The three vitamin variables were dichotomized at their respective median values in order to yield groups of comparable sizes. Median PAH–DNA levels and mean log PAH–DNA levels were computed against these same covariates and formally compared using the Kruskal–Wallis test.

These unadjusted analyses were followed by a linear regression analysis of log adduct levels, permitting us to make comparisons by race/ethnicity and genetic characteristics while controlling for potential confounders. Regression coefficients from these models represent average changes in adduct levels on the log scale. To aid in interpretation, we also present the exponentiated coefficients from these models; these represent ratios of the geometric means of the PAH–DNA levels according to levels of each covariate. For example, race/ethnicity was coded using indicator variables, with the largest subgroup in our sample, African-American, serving as the referent category. The exponentiated coefficient of the Caucasian indicator variable, then, represents the ratio of the (geometric) mean adduct level in Caucasians to that in African-Americans. Every regression estimate is accompanied by 95% confidence limits and a $p$ value (corresponding to the test of each covariate's contribution to the regression model, controlling for the other covariates).

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

## Results

Table 1 presents demographic, genetic, smoking, and antioxidant measurements for the screening study participants by race/ethnicity. Caucasian study participants were slightly, but not significantly, older than African-American and Latino study participants ($p = 0.125$). A larger proportion of Caucasians than of African-Americans and Latinos had graduated from high school ($p = 0.016$). A smaller proportion of African-American (26.1%) than of Caucasian (50.0%) or Latino (45.2%) study participants had the *GSTM1* null genotype ($p = 0.022$), and a larger proportion had the *GSTP1* homozygous variant *GG* genotype ($p = 0.023$) compared with the other two categories (*AA* and *AG*) combined. Race/ethnicity was not associated with gender, micronutrient levels or smoking-related variables in this sample of volunteers.

Table 2 presents median PAH–DNA and mean log PAH–DNA adducts/$10^8$ nucleotides by categories of demographic, genetic, smoking and antioxidant micronutrient variables. PAH–DNA adduct levels differed by race/ethnicity; African-Americans had the highest median PAH–DNA level (20.1 adducts/$10^8$ nucleotides) compared with Caucasians (8.6 adducts/$10^8$ nucleotides) and Latinos (14.1 adducts/$10^8$ nucleotides) ($p = 0.059$). The difference in PAH–DNA levels by ethnicity was driven in part by differences among the ethnic groups with regard to the proportion of subjects with adduct levels too low to detect: a non-detectable measurement of PAH–DNA was coded as 1, and hence the log PAH–DNA among

RIGHTSLINK

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.



Table 1. Demographic, genetic, smoking and plasma nutrient characteristics of participants at initial screening ($n = 151$).

| | | African-American | | Caucasian | | Latino | | |
|---|---|---|---|---|---|---|---|---|
| Characteristic | Total no. | No. | % | No. | % | No. | % | $p$ value[a] |
| Gender | | | | | | | | |
| Male | 86 | 55 | 62.5 | 14 | 43.8 | 17 | 54.8 | 0.18 |
| Female | 65 | 33 | 37.5 | 18 | 56.3 | 14 | 45.2 | |
| Age group | | | | | | | | |
| $<40$ years | 68 | 40 | 45.5 | 10 | 32.3 | 18 | 58.1 | 0.13 |
| $\geq 40$ years | 82 | 48 | 54.5 | 21 | 67.7 | 13 | 41.9 | |
| Education | | | | | | | | |
| $\leq 12$ years | 76 | 52 | 59.1 | 9 | 29.0 | 15 | 50.0 | 0.016 |
| $>12$ years | 73 | 36 | 40.9 | 22 | 71.0 | 15 | 50.0 | |
| Age began smoking | | | | | | | | |
| 15 years or younger | 63 | 41 | 47.7 | 12 | 38.7 | 10 | 32.3 | 0.29 |
| 16 years or older | 85 | 45 | 52.3 | 19 | 61.3 | 21 | 67.7 | |
| Pack-years | | | | | | | | |
| 1–20 | 40 | 21 | 25.3 | 8 | 26.7 | 11 | 39.3 | 0.36 |
| $>20$ | 101 | 62 | 74.7 | 22 | 73.3 | 17 | 60.7 | |
| Current smoking | | | | | | | | |
| $\leq 1$ pack per day | 62 | 40 | 47.6 | 9 | 30.0 | 13 | 46.4 | 0.24 |
| $>1$ pack per day | 80 | 44 | 52.4 | 21 | 70.0 | 15 | 53.6 | |
| Plasma cotinine (ng ml$^{-1}$) | | | | | | | | |
| 0 | 19 | 10 | 11.4 | 5 | 15.6 | 4 | 12.9 | 0.64 |
| 1–150 | 51 | 32 | 36.4 | 7 | 21.9 | 12 | 38.7 | |
| 151–250 | 64 | 38 | 43.2 | 14 | 43.8 | 12 | 38.7 | |
| $\geq 251$ | 17 | 8 | 9.1 | 6 | 18.8 | 3 | 9.7 | |
| Plasma β-carotene (nmol mmol$^{-1}$ cholesterol)[b] | | | | | | | | |
| Low ($<$median) | 75 | 43 | 50.0 | 15 | 46.9 | 17 | 56.7 | 0.73 |
| High ($\geq$median) | 73 | 43 | 50.0 | 17 | 53.1 | 13 | 43.3 | |

RIGHTSLINK

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

Table 1 (*Continued*)

| Characteristic | Total no. | African-American | | Caucasian | | Latino | | $p$ value[a] |
|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | |
| Plasma ascorbic acid (μM)[c] | | | | | | | | |
| Low (< median) | 61 | 37 | 52.1 | 14 | 51.9 | 10 | 35.7 | 0.31 |
| High (≥ median) | 65 | 34 | 47.9 | 13 | 48.2 | 18 | 64.3 | |
| Plasma α-tocopherol (μmol mmol$^{-1}$ cholesterol)[d] | | | | | | | | |
| Low (< median) | 74 | 45 | 53.6 | 16 | 57.1 | 13 | 44.8 | 0.62 |
| High (≥ median) | 67 | 39 | 46.4 | 12 | 42.9 | 16 | 55.2 | |
| *GSTM1* | | | | | | | | |
| Deletion | 53 | 23 | 26.1 | 16 | 50.0 | 14 | 45.2 | 0.022 |
| Wild-type | 98 | 65 | 73.4 | 16 | 50.0 | 17 | 54.8 | |
| *GSTP1* | | | | | | | | |
| *AA* | 53 | 26 | 30.2 | 14 | 43.8 | 13 | 43.3 | 0.09 |
| *AG* | 68 | 38 | 44.2 | 16 | 50.0 | 14 | 46.7 | |
| *GG* | 27 | 22 | 25.6 | 2 | 6.3 | 3 | 10.0 | (0.023[e]) |

[a]$p$ value from the Pearson $\chi^2$ comparing proportions across race/ethnicity categories.
[b]Median ≅ 25 nmol mmol$^{-1}$ cholesterol.
[c]Median ≅ 63 μM.
[d]Median ≅ 4.3 μmol mmol$^{-1}$ cholesterol.
[e]Homozygous variant compared with other groups.

RIGHTSLINK

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.



Table 2. Mean log PAH–DNA adducts/$10^8$ nucleotides by *GST* genotype, demographic characteristics, plasma nutrient levels and smoking history at initial screening ($n = 151$).

| | Total | | log PAH–DNA | | PAH–DNA | | |
|---|---|---|---|---|---|---|---|
| Characteristic | No. | % | Mean | SD | Median | IQR | $p$ value[a] |
| Ethnicity | | | | | | | |
| African-American | 88 | 58.3 | 2.26 | 1.38 | 11.4 | 20.1 | 0.059 |
| Caucasian | 32 | 21.2 | 1.83 | 1.12 | 8.0 | 8.6 | |
| Latino | 31 | 20.5 | 1.59 | 1.40 | 6.9 | 14.1 | |
| Gender | | | | | | | |
| Male | 86 | 57.0 | 2.11 | 1.37 | 9.7 | 16.6 | 0.31 |
| Female | 65 | 43.1 | 1.92 | 1.34 | 8.0 | 13.5 | |
| Age group | | | | | | | |
| $<40$ years | 68 | 45.3 | 2.02 | 1.41 | 9.7 | 17.6 | 0.91 |
| $\geq 40$ years | 82 | 54.7 | 2.03 | 1.32 | 8.6 | 14.9 | |
| Education | | | | | | | |
| $\leq 12$ years | 76 | 51.0 | 1.85 | 1.32 | 8.4 | 13.4 | 0.09 |
| $>12$ years | 73 | 49.0 | 2.23 | 1.37 | 10.6 | 15.7 | |
| Age began smoking | | | | | | | |
| 15 years or younger | 63 | 42.6 | 2.10 | 1.44 | 9.1 | 15.7 | 0.92 |
| 16 years or older | 85 | 57.4 | 2.00 | 1.30 | 10.2 | 14.7 | |
| Pack-years | | | | | | | |
| 1–20 | 40 | 28.4 | 2.22 | 1.55 | 10.4 | 23.6 | 0.54 |
| $\geq 20$ | 101 | 71.6 | 2.00 | 1.27 | 8.8 | 12.9 | |
| Current smoking | | | | | | | |
| $\leq 1$ pack per day | 62 | 43.7 | 2.04 | 1.55 | 9.4 | 19.0 | 0.92 |
| $>1$pack per day | 80 | 56.3 | 2.07 | 1.22 | 8.8 | 14.2 | |
| Plasma cotinine (ng $ml^{-1}$) | | | | | | | |
| 0 | 19 | 12.6 | 2.00 | 1.23 | 9.1 | 15.7 | 0.69 |
| 1–150 | 51 | 32.8 | 2.18 | 1.23 | 11.5 | 15.1 | |

RIGHTSLINK

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

Table 2 (*Continued*)

| Characteristic | Total | | log PAH–DNA | | PAH–DNA | | |
|---|---|---|---|---|---|---|---|
| | No. | % | Mean | SD | Median | IQR | *p* value[a] |
| 151–250 | 64 | 42.4 | 1.92 | 1.48 | 7.5 | 15.8 | |
| ≥ 251 | 17 | 11.3 | 2.02 | 1.45 | 12.7 | 24.1 | |
| Plasma β-carotene (nmol mmol$^{-1}$ cholesterol)[b] | | | | | | | |
| Low (< median) | 75 | 50.7 | 1.84 | 1.37 | 8.3 | 14.1 | 0.09 |
| High (≥ median) | 73 | 49.3 | 2.25 | 1.33 | 11.2 | 16.2 | |
| Plasma ascorbic acid (μM)[c] | | | | | | | |
| Low (< median) | 61 | 48.4 | 1.80 | 1.23 | 8.2 | 13.3 | 0.99 |
| High (≥ median) | 65 | 51.6 | 1.84 | 1.33 | 7.5 | 15.2 | |
| Plasma α-tocopherol (μmol mmol$^{-1}$ cholesterol)[d] | | | | | | | |
| Low (< median) | 74 | 52.5 | 1.83 | 1.38 | 8.3 | 14.1 | 0.024 |
| High (≥ median) | 67 | 47.5 | 2.36 | 1.30 | 12.3 | 15.7 | |
| *GSTM1* | | | | | | | |
| Deletion | 53 | 35.1 | 2.19 | 1.42 | 11.6 | 15.2 | 0.34 |
| Wild-type | 98 | 64.9 | 1.94 | 1.32 | 8.6 | 14.3 | |
| *GSTP1* | | | | | | | |
| *AA* | 53 | 35.8 | 1.98 | 1.38 | 8.2 | 18.1 | 0.94 |
| *AG* | 68 | 46.0 | 2.01 | 1.21 | 9.0 | 11.6 | |
| *GG* | 27 | 18.2 | 2.14 | 1.63 | 10.2 | 24.1 | |

IQR, interquartile range.
[a]*p* value from Kruskal–Wallis test comparing median values across categories of the exposure variable.
[b]Median ≅ 25 nmol mmol$^{-1}$ cholesterol.
[c]Median ≅ 63 μM.
[d]Median ≅ 4.3 μmol mmol$^{-1}$ cholesterol.

RIGHTSLINK

non-detectables was coded as 0. Among African-Americans, 18% of the subjects had non-detectable adduct levels; the corresponding figures in Caucasian and Latino subjects were 22% and 39%, respectively. Adduct levels were not associated with gender, age, genotype or smoking indices. They were somewhat higher in high school graduates, although this difference did not reach statistical significance ($p = 0.09$). Contrary to the findings of other studies, PAH–DNA adduct levels were higher in subjects with higher β-carotene and α-tocopherol levels ($p = 0.09$ and 0.024, respectively). Figure 1 illustrates the distribution of adducts by *GSTM1* genotype in subjects categorized by race/ethnic group. Only in Caucasians was the *GSTM1* genotype associated with PAH–DNA adduct levels ($p < 0.01$).

Regression analysis confirmed the finding that African-American subjects had higher adduct levels than Caucasian or Latino subjects. This finding remained strong after adjustment for gender, education, plasma antioxidant levels and *GSTM1* status (see Table 3). On the ratio scale, adduct levels among Caucasian and Latino subjects were only half as high, on average, as levels among African-Americans ($p = 0.015$). Adjustment for age, current smoking and lifetime smoking habits had no impact on these results (data not shown). *GSTM1* classification was not significantly associated with adduct levels after adjustment for the aforementioned factors. Surprisingly, we found marginally significant relationships between increased adduct levels and longer education and higher levels of α-tocopherol. Effect modification of the relationship between ethnicity and adduct levels by *GSTM1* status was not statistically significant. Because the *GSTP1* variant was unrelated to adduct levels in both the crude and adjusted analyses, it was omitted from the final reported regression model.

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

## Discussion

To explore the relationship between PAH–DNA adduct formation and *GSTM1* and *GSTP1* polymorphisms among heavy smokers of African-American, Caucasian and Latino descent, we used regression analysis to assess the effects of selected genetic markers on adduct levels, after controlling for confounders. Although PAH–DNA adduct levels and the distribution of *GSTM1* polymorphisms differed by race/ethnic group, no significant interaction between race/ethnic group and *GSTM1* deletion was detected in this sample. African-Americans had the highest levels of PAH–DNA adducts, but they did not have the highest rates of current

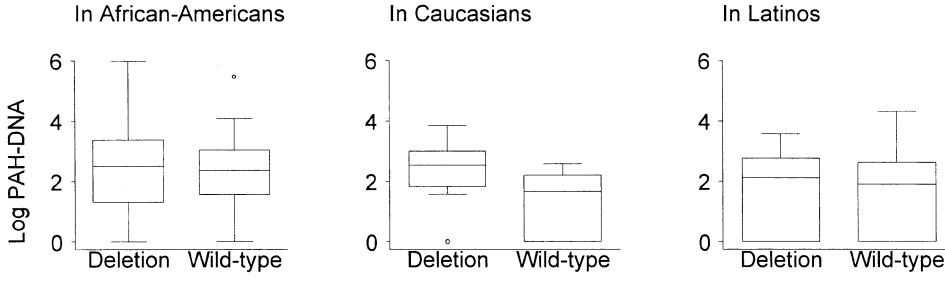


Figure 1. Distribution of PAH–DNA adducts by *GSTM1* genotype.

RIGHTSLINK

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.



Table 3. Multiple linear regression analysis of simultaneously adjusted predictors of natural log-transformed PAH–DNA adduct levels.

| | Estimated regression coefficient | | Estimated effect on ratio scale | | |
|---|---|---|---|---|---|
| Characteristic | | 95% CI | | 95% CI | $p$ value |
| Ethnicity | | | | | |
| Caucasian versus African-American | −0.66 | −1.26 to −0.07 | 0.52 | 0.28–0.94 | 0.015 |
| Latino versus African-American | −0.73 | −1.31 to −0.15 | 0.48 | 0.27–0.86 | |
| *GSTM1* (deletion versus wild-type) | −0.32 | −0.79 to 0.15 | 0.73 | 0.45–1.17 | 0.19 |
| Gender (male versus female) | 0.17 | −0.28 to 0.63 | 1.19 | 0.75–1.87 | 0.46 |
| Education ($>12$ years versus $\leq 12$ years) | 0.40 | −0.05 to 0.85 | 1.49 | 0.95–2.34 | 0.081 |
| β-Carotene (high versus low) | 0.30 | −0.18 to 0.78 | 1.35 | 0.84–2.17 | 0.22 |
| α-Tocopherol (high versus low) | 0.49 | 0.01 to 0.97 | 1.64 | 1.01–2.64 | 0.044 |

95% CI, 95% confidence interval.

RIGHTSLINK

tobacco use, cumulative tobacco use, or cotinine levels. Although slightly larger proportions of African-Americans started smoking before the age of 15 years and smoked more than one pack per day currently, the differences were not statistically significant. We found no association between smoking-related variables and adduct levels, perhaps because most of the study participants were heavy smokers and because most were relatively young: only 17% were aged 50 years or older. Another limitation of this study was the relatively small number of subjects within each race/ethnic group. This implies that the power for detecting effect modification of the relationship between adduct levels and *GSTM1* status by ethnicity was limited; this finding should be pursued in future studies with larger sample sizes.

The distribution of PAH–DNA adduct levels presents another limitation to this analysis. In addition to being right-skewed, there is an accumulation of non-detectable levels at the lower tail. While the logarithmic transformation served to make the distribution of detectable values more symmetric (and, therefore, more closely normal), it could not correct the lower tail problem. Furthermore, we know of no continuous data transformation that could correct this problem. Concern remains, therefore, regarding the impact of non-normality on the results from the linear regression analysis. To address this issue, we conducted a confirmatory logistic regression analysis in which outcome (PAH–DNA level) was defined as 'detectable' versus 'non-detectable'. Although the power of this analysis is presumably lower than the power of the continuous scale analysis, we were able to demonstrate that the directions of association and approximate significance levels for the covariates considered were consistent across the two approaches (data not shown). This finding diminishes concern over the non-normality of the log-transformed PAH–DNA levels.

No prior studies have assessed the association of DNA adduct levels with race/ethnicity. In a previous study in children where limited amounts of blood were available, we investigated PAH–albumin adducts as a surrogate for DNA adducts (Crawford *et al*. 1994). Adduct levels were significantly higher in African-American than in Hispanic children. 3- and 4-Aminobiphenyl haemoglobin adducts have also been measured in different groups, with the highest levels of adducts found in Caucasians, intermediate levels in African-Americans and the lowest levels in Asians (Yu *et al*. 1994). This order is in agreement with rates of bladder cancer, which is highest in Caucasians and lowest in Asians.

In the present study, a lower prevalence of *GSTM1* deletion was observed among African-Americans (26.1%) than among Caucasians (50.0%) or Hispanics (45.2%). Prior case-control studies of *GSTM1* and lung cancer have found similar associations between *GSTM1* deletion prevalence and race (London *et al*. 1995, Kelsey *et al*. 1997, Woodson *et al*. 1999, Ford *et al*. 2000). *GSTM1* deletion rates among Caucasian controls ranged from 48.6% among clinic controls (Woodson *et al*. 1999) to 52.4% among motor vehicle controls (London *et al*. 1995). Three case-control studies have included African-American participants. The prevalence of *GSTM1* deletions among African-American controls was 22.2% in a convenience sample (Kelsey *et al*. 1997), 20.0% among cancer-free patients from pulmonary clinics (Ford *et al*. 1997), and 27.1% among motor vehicle registrants (London *et al*. 1995). Only one study evaluated the relationship between *GSTM1*

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

RIGHTSLINK

deletion rates and lung cancer among Mexican-Americans; 40% of the controls had a *GSTM1* deletion (Kelsey *et al*. 1997).

Studies of the relationship between *GST* genotype and DNA adduct levels have produced conflicting results. For *GSTM1*, a positive association between gene deletion and PAH–DNA adducts measured by $^{32}$P-postlabelling was reported for mononuclear cells (Butkiewicz *et al*. 1998) but not for white blood cells (WBCs) (Ichiba *et al*. 1995, Hemminki *et al*. 1997, Binkova *et al*. 1998, Peluso *et al*. 1998). However, two studies found that coke-oven workers with a *GSTM1* deletion had higher adduct levels in WBCs than *GSTM1*-positive workers (Brescia *et al*. 1999, Rojas *et al*. 2000). In contrast, among smokers newly diagnosed with lung cancer, the *GSTM1* genotype was not associated with adducts of benzo[*a*]pyrene measured by gas chromatography in mononuclear cells (Pastorelli *et al*. 1998). Our own study of the relationship between the *GSTM1* genotype and PAH–DNA adduct levels in the mononuclear cells of smokers, measured by ELISA, found the highest adduct levels in subjects with the null genotype, but no statistically significant difference in mean adduct levels between those with and those without the gene; however, our sample size was small ($n = 63$) (Santella *et al*. 1994). Another study using a similar ELISA also failed to find an effect of *GSTM1* genotype on WBC PAH–DNA adduct level ($n = 47$) (Rothman *et al*. 1995). Mononuclear cells have a longer lifespan than granulocytes, the predominante cell type in assays of total WBCs. Higher levels of adducts have been detected in mononuclear cells compared with granulocytes (Savela and Hemminki 1991, Godschalk *et al*. 1998). However, it is also known that blood cells are only a marker of exposure in the past few months (Mooney *et al*. 1995).

Several studies have investigated the effect of the *GSTP1* genotype on adduct formation (Viezzer *et al*. 1999, Butkiewicz *et al*. 2000, Grzybowska *et al*. 2000, Zhang *et al*. 2000). No effect of the *GSTP1* genotype alone was found, but *GSTM1* deletion in combination with *GSTP1 AG* or *GG* resulted in higher adducts than other combinations (Butkiewicz *et al*. 2000). The conflicting data on the relationship between *GST* genotypes and adducts may be the result of the small sample size in many studies, different methods for the determination of DNA adduct levels, the use of total WBCs containing many short-lived granulocytes versus longer lived mononuclear cells, and very different levels of PAH exposure.

Sociological and environmental exposures probably account for some of the race/ethnic group differences we observed in PAH–DNA adduct levels. Sociological factors, such as age at smoking initiation, fruit and vegetable consumption, substance abuse, access to and utilization of health care, and physical activity levels differ by race/ethnicity (Syme and Balfour 1998). In general, except for age at smoking initiation, African-Americans fare worse in terms of a healthy lifestyle than their Caucasian and Latino counterparts (Healthy People Consortium Meeting 2000). Environmental and occupational exposures probably also differ by race/ethnicity (US Environmental Protection Agency 1997). Taken together these results suggest that it will be difficult to determine simple relationships between single genes and DNA damage levels.

Most prior studies have found an inverse association between plasma antioxidant micronutrient levels and adduct levels. In our data, the association

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

RIGHTSLINK

was positive, although only marginally statistically significant. Among those who contacted us in response to our recruitment fliers, many were ineligible because they were currently taking antioxidant supplements in order to protect themselves from the adverse health effects of smoking. It is possible that the heavier smoking study participants also tried to compensate by modifying their diet.

PAH–DNA adduct levels are markers of exposure and may also serve to identify individuals at higher than average risk for lung cancer. It is likely that genetic and other (including behavioural) factors modify the effects of environmental exposures on adduct levels. Future studies should be conducted in larger samples to evaluate the interactions between environmental, occupational and behavioural factors together, with other genes that metabolize tobacco carcinogens in addition to GSTs, such as cytochrome P450 and *N*-acetyltransferase 1 and 2 (Vineis *et al*. 1999), genes that influence smoking addiction (Lerman *et al*. 1999) and socio-cultural factors. In addition, investigation of lung cancer by histological type and lobular subsite, which are associated with cigarette composition and certain smoking behaviours, may contribute to a better understanding of lung carcinogenesis.

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

## Acknowledgements

This work was supported by grants from the American Institute for Cancer Research, the National Cancer Institute (CA73330), the National Center for Research Resources (RR0045), and the National Institute for Environmental Health Sciences (ES09089).

## References

Bell, D. A., Taylor, J. A., Paulson, D. F., Robertson, C. N., Mohler, J. L. and Lucier, G. W. 1993, Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione *S*-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. *Journal of the Nattional Cancer Institute*, **85**, 1159–1164.

Binkova, B., Topinka, J., Mrackova, G., Gajdosova, D., Vidova, P., Stavkova, Z., Peterka, V., Pilcik, T., Rimar, V., Dobias, L., Farmer, P. B. and Sram, R. J. 1998, Coke oven workers study: the effect of exposure and GSTM1 and NAT2 genotypes on DNA adduct levels in white blood cells and lymphocytes as determined by $^{32}$P-postlabelling. *Mutation Research*, **416**, 67–84.

Bonassi, S., Merlo, F., Pearce, N. and Puntoni, R. 1989, Bladder cancer and occupational exposure to polycyclic aromatic hydrocarbons. *International Journal of Cancer*, **44**, 648–651.

Brescia, G., Celotti, L., Clonfero, E., Neumann, G. H., Forni, A., Foa, V., Pisoni, M., Ferri, G. M. and Assennato, G. 1999, The influence of cytochrome P450 1A1 and glutathione *S*-transferase M1 genotypes on biomarker levels in coke-oven workers. *Archives of Toxicology*, **73**, 431–439.

Butkiewicz, D., Grzybowska, E., Hemminki, K., Ovrebo, S., Haugen, A., Motykiewicz, G. and Chorazy, M. 1998, Modulation of DNA adduct levels in human mononuclear white blood cells and granulocytes by CYP1A1, CYP2D6 and GSTM1 genetic polymorphisms. *Mutation Research*, **415**, 97–108.

Butkiewicz, D., Gryzbowska, E., Phillips, D. H., Hemminiki, K. and Chorazy, M. 2000, Polymorphisms of the GSTP1 and GSTM1 genes and PAH-DNA adducts in human mononuclear white blood cells. *Environmental and Molecular Mutagenesis*, **35**, 99–105.

Crawford, F. G., Mayer, J., Santella, R. M., Cooper, T. B., Ottman, R., Tsai, W. Y., Simon-Cereijido, G., Wang, M., Tang, D. L. and Perera, F. P. 1994, Biomarkers of environmental tobacco smoke in preschool children and their mothers. *Journal of the National Cancer Institute*, **86**, 1398–1402.

Degawa, M., Stern, S. J., Martin, M. V., Guengerich, F. P., Fu, P. P., Ilett, K. F., Kaderlik, R. K. and Kadlubar, F. F. 1994, Metabolic activation and carcinogen–DNA adduct detection in human larynx. *Cancer Research*, **54**, 4915–4919.

RIGHTSLINK

FORD, J. G., LI, Y., O'SULLIVAN, M. M., DEMOPOULOS, R., GARTE, S., TAIOLI, E. and BRANDT-RAUF, P. W. 2000, Glutathione *S*-transferase M1 polymorphism and lung cancer risk in African-Americans. *Carcinogenesis*, **21**, 1971–1975.

GODSCHALK, R. W., MAAS, L. M., VAN ZANDWIJK, N., VAN'T VEER, L. J., BREEDIJK, A., BORM, P. J., VERHAERT, J., KLEINJANS, J. C. and VAN SCHOOTEN, F. J. 1998, Differences in aromatic–DNA adduct levels between alveolar macrophages and subpopulations of white blood cells from smokers. *Carcinogenesis*, **19**, 819–825.

GRZYBOWSKA, E., BUTKIEWICZ, D., MOTYKIEWICZ, G. and CHORAZY, M. 2000, The effect of the genetic polymorphisms of CYP1A1, CYP2D6, GSTM1 and GSTP1 on aromatic DNA adduct levels in the population of healthy women. *Mutation Research*, **469**, 271–277.

HARRIES, L. W., STUBBINS, M. J., FORMAN, D., HOWARD, G. C. and WOLF, C. R. 1997, Identification of genetic polymorphisms at the glutathione *S*-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis*, **18**, 641–644.

HEALTHY PEOPLE CONSORTIUM MEETING. 2000, *Healthy People 2010 Special Edition CD-Rom*. American Public Health Association, Washington, DC.

HECHT, S. S. 1999, Tobacco smoke carcinogens and lung cancer. *Journal of the National Cancer Institute*, **91**, 1194–1210.

HEMMINKI, K., DICKEY, C., KARLSSON, S., BELL, D., HSU, Y., TSAI, W. Y., MOONEY, L. A., SAVELA, K. and PERERA, F. P. 1997, Aromatic DNA adducts in foundry workers in relation to exposure, life style and CYP1A1 and glutathione transferase M1 genotype. *Carcinogenesis*, **18**, 345–350.

ICHIBA, M., HAGMAR, L., RANNUG, A., HOGSTEDT, B., ALEXANDRIE, A. K., CARSTENSEN, U. and HEMMINKI, K. 1995, Aromatic DNA adducts, micronuclei and genetic polymorphism for CYP1A1 and GST1 in chimney sweeps. *Carcinogenesis*, **16**, 1347–1351.

JACOBSON, J. S., BEGG, M. D., WANG, L. W., WANG, Q., AGARWAL, M., NORKUS, E. P., SINGH, V. N., YOUNG, T. L., YANG, D. and SANTELLA, R. M. 2000, Effects of a six-month intervention on DNA damage in heavy smokers. *Cancer Epidemiology Biomarkers and Prevention*, **9**, 1303–1311.

KELSEY, K. T., SPITZ, M. R., ZUO, Z. F. and WIENCKE, J. K. 1997, Polymorphisms in the glutathione *S*-transferase class mu and theta genes interact and increase susceptibility to lung cancer in minority populations (Texas, United States). *Cancer Causes and Control*, **8**, 554–559.

LERMAN, C., CAPORASO, N. E., AUDRAIN, J., MAIN, D., BOWMAN, E. D., LOCKSHIN, B., BOYD, N. R. and SHIELDS, P. G. 1999, Evidence suggesting the role of specific genetic factors in cigarette smoking. *Health Psychology*, **18**, 14–20.

LONDON, S. J., DALY, A. K., COOPER, J., NAVIDI, W. C., CARPENTER, C. L. and IDLE, J. R. 1995, Polymorphism of glutathione *S*-transferase M1 and lung cancer risk among African-Americans and Caucasians in Los Angeles County, California. *Journal of the National Cancer Institute*, **87**, 1246–1253.

MOONEY, L. A., SANTELLA, R. M., COVEY, L., JEFFREY, A. M., BIGBEE, W., RANDALL, M. C., COOPER, T. B., OTTMAN, R., TSAI, W. Y. and WAZNEH, L. 1995, Decline of DNA damage and other biomarkers in peripheral blood following smoking cessation. *Cancer Epidemiology Biomarkers and Prevention*, **4**, 627–634.

PASTORELLI, R., GUANCI, M., CERRI, A., NEGRI, E., LAVECCHIA, C., FUMAGALLI, F., MEZZETTI, M., CAPPELLI, R., PANIGALLI, T., FANELLI, R. and AIROLDI, L. 1998, Impact of inherited polymorphisms in glutathione *S*-transferase M1, microsomal epoxide hydrolase, cytochrome P450 enzymes on DNA, and blood protein adducts of benzo[*a*]pyrene-diolepoxide. *Cancer Epidemiology Biomarkers and Prevention*, **7**, 703–709.

PELUSO, M., AIROLDI, L., ARMELLE, M., MARTONE, T., CODA, R., MALAVEILLE, C., GIACOMELLI, G., TERRONE, C., CASETTA, G. and VINEIS, P. 1998, White blood cell DNA adducts, smoking, and NAT2 and GSTM1 genotypes in bladder cancer: a case-control study. *Cancer Epidemiology Biomarkers and Prevention*, **7**, 341–346.

PEREZ-STABLE, E. J., HERRERA, B., JACOB, P. and BENOWITZ, N. L. 1998, Nicotine metabolism and intake in black and white smokers. *JAMA*, **280**, 152–156.

ROJAS, M., CASCORBI, I., ALEXANDROV, K., KRIEK, E., AUBURTIN, G., MAYER, L., KOPP-SCHNÉIDER, A., ROOTS, I. and BARTSCH, H. 2000, Modulation of benzo[*a*]pyrene diolepoxide–DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. *Carcinogenesis*, **21**, 35–41.

ROTHMAN, N., SHIELDS, P. G., POIRIER, M. C., HARRINGTON, A. M., FORD, D. P. and STRICKLAND, P. T. 1995, The impact of glutathione *S*-transferase M1 and cytochrome P450 1A1 genotypes on white-blood-cell polycyclic aromatic hydrocarbon–DNA adducts levels in humans. *Molecular Carcinogenesis*, **14**, 63–68.

SANTELLA, R. M., GRINBERG-FUNES, R. A., YOUNG, T. L., DICKEY, C., SINGH, V. N., WANG, L. W. and PERERA, F. P. 1994, Cigarette smoking related polycyclic aromatic hydrocarbon-DNA adducts in peripheral mononuclear cells. *Carcinogenesis*, **13**, 2041–2045.

SAVELA, K. and HEMMINKI, K. 1991, DNA adducts in lymphocytes and granuloctyes of smokers and nonsmokers detected by the $^{32}$P-postlabeling assay. *Carcinogenesis*, **12**, 503–508.

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

RIGHTSLINK

SHAKOOR-ABDULLAH, B., KOTCHEN, J. M., WALKER, W. E., CHELIUS, T. H. and HOFFMANN, R. G. 1997, Incorporating socio-economic and risk factor diversity into the development of an African-American community blood pressure control program. *Ethnicity and Disease*, 7, 175–183.

SHIELDS, P. G. 2000, Epidemiology of tobacco carcinogenesis. *Current Oncology Reports*, **2**, 257–262.

SYME, S. L. and BALFOUR, J. L. 1998, Social determinants of disease. In *Public Health and Preventive Medicine*, edited by R. B. WALLACE (Stamford: Appelton & Lange), pp. 795–810.

SZYFTER, K., HEMMINKI, K., SZYFTER, W., SZMEJA, Z., BANASZEWSKI, J. and YANG, K. 1994, Aromatic DNA adducts in larynx biopsies and leukocytes. *Carcinogenesis*, **15**, 2195–2199.

TANG, D. L., RUNDLE, A., WARBURTON, D., SANTELLA, R. M., TSAI, W. Y., CHIAMPRASERT, S., ZHOU, J. Z., SHAO, Y. Z., HSU, Y. Z. and PERERA, F. P. 1998, Associations between both genetic and environmental biomarkers and lung cancer: evidence of a greater risk of lung cancer in women smokers. *Carcinogenesis*, **19**, 1949–1954.

THURNHAM, D. I., DAVIES, J. A., CRUMP, B. J., SITUNAYAKE, R. D. and DAVIS, M. 1986, The use of different lipids to express serum tocopherol:lipid ratios for the measurement of vitamin E status. *Annals of Clinical Biochemistry*, **23**, 514–520.

US ENVIRONMENTAL PROTECTION AGENCY (1997) *National Air Quality and Trends Report* (Washington DC: Office of Air and Radiation, EPA).

VIEZZER, C., NORPPA, H., CLONFERO, E., GABBANI, G., MASTRANGELO, G., HIRVONEN, A. and CELOTTI, L. 1999, Influence of GSTM1, GSTT1, GSTP1, and EPHX gene polymorphisms on DNA adduct level and HPRT mutant frequency in coke-oven workers. *Mutation Research*, **431**, 259–269.

VINEIS, P., MALATS, N., LANG, M., D'ERRICO, A., CAPORASO, N., CUZICK, J. and BOFFETA, P. 1999, *Metabolic Polymorphisms and Susceptibility to Cancer* (Lyon: International Agency for Research on Cancer).

WAGENKNECHT, L. E., CUTTER, G. R., HALEY, N. J., SIDNEY, S., MANOLIO, T. A., HUGHES, G. H. and JACOBS, D. R. 1990, Racial differences in serum cotinine levels among smokers in the Coronary Artery Risk Development in (Young) Adults study. *American Journal of Public Health*, **80**, 1053–1056.

WILLIAMS, D. R. 1996, Race/ethnicity and socioeconomic status: measurement and methodological issues. *International Journal of Health Services*, **26**, 483–505.

WOODSON, K., STEWART, C., BARRETT, M., BHAT, N. K., VIRTAMO, J., TAYLOR, P. R. and ALBANES, D. 1999, Effect of vitamin intervention on the relationship between GSTM1, smoking, and lung cancer risk among male smokers. *Cancer Epidemiology Biomarkers and Prevention*, **8**, 965–970.

WYNDER, E. L. and MUSCAT, J. E. 1995, The changing epidemiology of smoking and lung cancer histology. *Environmental Health Perspectives*, **103** (Supplement 8), 143–148.

YU, M. C., SKIPPER, P. L., TAGHIZADEH, K., TANNENBAUM, S. R., CHAN, K. K., HENDERSON, B. E. and ROSS, R. K. 1994, Acetylator phenotype, aminobiphenyl–hemoglobin adduct levels, and bladder cancer risk in white, black, and Asian men in Los Angeles, California. *Journal of the National Cancer Institute*, **86**, 712–716.

ZHANG, J., ICHIBA, M., FENG, Y., PAN, G., HANAOKA, T., YAMANO, Y., HARA, K., TAKAHASHI, K. and TOMOKUNI, K. 2000, Aromatic DNA adducts in coke-oven workers, in relation to exposure, lifestyle and genetic polymorphism of metabolic enzymes. *International Archives of Occupational and Environmental Health*, **73**, 127–135.

Biomarkers Downloaded from informahealthcare.com by Huazhong University of Science & Technology on 11/18/12
For personal use only.

RIGHTSLINK