Assessment of Interactions between PAH Exposure and Genetic Polymorphisms on PAH-DNA Adducts in African American, Dominican, and Caucasian Mothers and Newborns

Shuang Wang[1], **Stephen Chanock**[3], **Deliang Tang**[2], **Zhigang Li**[1], **Wieslaw Jedrychowski**[2], and **Frederica P. Perera**[2]

[1]Department of Biostatistics, Columbia University, New York, New York [2]Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York, New York [3]National Cancer Institute, Bethesda, Maryland

Abstract

Polycyclic aromatic hydrocarbons (PAH) are widespread pollutants commonly found in air, food, and drinking water. Benzo[*a*]pyrene is a well-studied representative PAH found in air from fossil fuel combustion and a transplacental carcinogen experimentally. PAHs bind covalently to DNA to form DNA adducts, an indicator of DNA damage, and an informative biomarker of potential cancer risk. Associations between PAH-DNA adduct levels and both cancer risk and developmental deficits have been seen in previous experimental and epidemiologic studies. Several genes have been shown to play an important role in the metabolic activation or detoxification of PAHs, including the cytochrome *P*450 genes *CYP1A1* and *CYP1B1* and the glutathione *S*-transferase (GST) genes *GSTM*1, and *GSTT2*. Genetic variation in these genes could influence susceptibility to adverse effects of PAHs in polluted air. Here, we have explored interactions between prenatal PAH exposure and 17 polymorphisms in these genes (*rs2198843, rs1456432, rs4646903, rs4646421, rs2606345, rs7495708, rs2472299, rs162549, rs1056837, rs1056836, rs162560, rs10012, rs2617266, rs2719, rs1622002, rs140194,* and gene deletion *GSTM1-02*) and haplotypes on PAH-DNA adducts in cord blood of 547 newborns and in maternal blood of 806 mothers from three different self-described ethnic groups: African Americans, Dominicans, and Caucasians. PAHs were measured by personal air monitoring of mothers during pregnancy. Significant interactions ($p < 0.05$) were observed between certain genetic polymorphisms and CYP1A1 haplotype and PAHs in mothers and their newborns in the three ethnic groups. However, with our limited sample size, the current findings are suggestive only, warranting further study.

Introduction

Polycyclic aromatic hydrocarbons (PAH) are widespread pollutants commonly found in air, food, and drinking water (1). Airborne PAHs (outdoor or indoor) mainly result from combustion of fossil fuels, tobacco, and other organic materials. Generally, emissions from motor vehicles, electricity generation, and residential heating are the major source of PAHs in outdoor urban air, whereas environmental tobacco smoke (ETS) is a major indoor source (2, 3). Benzo[*a*]pyrene (B[*a*]P) is a well-studied representative PAH found in environmental

mixtures, including air pollution (4). B[*a*]P is a well-known human carcinogen and has been found to be a transplacental carcinogen in experimental bioassays and produces tumors in the liver, lung, lymphatic tissues, and nervous system of the offspring (5–7).

PAHs bind covalently to DNA to form DNA adducts. As an indicator of DNA damage, carcinogen-DNA adducts represent a critical step in the carcinogenic pathways and thus can be considered an informative biomarker of cancer risk. Associations between PAH-DNA adduct levels and cancer risks have been reported in both experimental and epidemiologic research (8–14). PAH-DNA adducts have also been implicated in neuro-developmental deficits in children with exposure. Both experimental and epidemiologic studies have shown that fetal levels of PAH-DNA adducts were generally higher than expected, per estimated transplacental dose of PAHs, suggesting increased susceptibility to deleterious outcomes (15–19). The adverse outcomes linked previously to PAH exposure or PAH-DNA adducts in the cohorts under study in the present report include disorders in fetal growth and child development, childhood asthma, and cancer (20–25).

CYP1A1 and *CYP1B1* (26, 27) have been shown to play important roles in the metabolic activation of PAHs, whereas PAH detoxification is partially affected by the glutathione *S*-transferase (GST) genes *GSTM1*, and *GSTT2*. Figure 1 displays a diagram that shows the mechanistic relationship between the genes and the formation of the adducts. There are conflicting data on the contribution of genetic variants in these genes and susceptibility to lung cancer partly due to underpowered studies (13–19, 26–32). Still, common genetic polymorphisms in these genes could affect individual susceptibility to adverse effects of environmental air pollution. Previous studies have not explored possible gene-environment interactions in mothers and their newborns in underrepresented groups, such as African Americans and Dominicans. The purpose of this study was to explore gene-environment interactions by assessing the association of DNA adducts with airborne PAH exposure levels among mothers and their newborns in three different ethnic groups with different genotypes (common genetic variants in *CYP1A1, CYP1B1, GSTM1*, and *GSTT2*). Only when no gene-environment interaction was observed did we examine possible main effects of genotypes on DNA adducts.

Materials and Methods

Study Populations

Subjects were chosen from two independent, parallel studies. One currently is being conducted in New York City (NYC) and the details on the study design have been published previously (20). Study subjects included nonsmoking African American and Dominican women residing in Washington Heights, Central Harlem, NYC, and the South Bronx, who were recruited through the obstetrical services of New York Presbyterian Hospital, Harlem Hospital, or satellite clinics between February 1998 and February 2003. Ethnicity was self-identified. The institutional review board of New York Presbyterian Medical Center approved the study, and informed consent was obtained from all study participants. The second cohort study is being conducted in Krakow, Poland, a region known to have higher levels of air pollution than NYC presumably due to coal burning and vehicle emission (21). Details on study design for the Krakow cohort have been published previously (21). Nonsmoking, pregnant women residing in the Srodmiescie and the Krowodrza-Nowa Huta areas were recruited between November 2000 and March 2003 (21). Informed consent was obtained from all subjects, and the study was approved by the ethics committee of the Jagiellonian University.

In both cohorts, pregnant women were eligible if they were not currently smoking, registered at prenatal health care clinics, had lived at the present address for at least a year

before the initial interview, were ages ≥18 years, had no history of illicit drug use, pregnancy-related diabetes, or hypertension, and had a valid estimate of gestational age. During the second or third trimesters pregnancy, the women carried a backpack containing a portable personal exposure air monitor during the day and kept it near the bed at night during a consecutive 48-h period for PAH measurements (20, 21).

Subjects included in the present analysis are mothers with genotype data ($n = 160$ African Americans, $n = 265$ Dominicans, and $n = 381$ Polish Caucasians) and their newborns with genotype data ($n = 99$ African Americans, $n = 154$ Dominicans, and $n = 294$ Polish Caucasians; Table 1). The subset did not differ significantly from the total population with respect to selected demographic and exposure characteristics.

Maternal blood (30–35 mL) was collected within 1 day postpartum, and umbilical cord blood (30–60 mL) was collected at delivery (33). B[*a*]P-DNA adducts were analyzed in all maternal and umbilical cord blood samples with sufficient DNA quantities. B[*a*]P is widely used as a representative PAH because concentrations of individual PAHs in the urban setting are highly correlated (33). B[*a*]P-DNA adducts can serve as a proxy for PAH-DNA adducts (34). B[*a*]P-DNA adducts in extracted WBC DNA were analyzed using the high-performance liquid chromatography-fluorescence method of Alexandrov et al. (35), which detects B[*a*]P tetraols. The method has a coefficient of variation of 12% and a lower limit of detection of 0.25 adducts per 10^8 nucleotides. As in prior analyses, samples below the limit of detection were assigned a value midway between the limit of detection and zero (0.125 adducts per 10^8 nucleotides).

Selection of Polymorphisms for Genotyping

Sixteen single nucleotide polymorphisms (SNP) from *CYP1A1, CYP1B1*, and *GSTT2* were selected from the SNP500Cancer resource (36). For the first two genes, haplotype-tagging SNPs were selected based on the genomic analysis of these genes (7 in *CYP1A1* and 6 in *CYP1B1*) by the Breast and Prostate Cancer Cohort Consortium (37) using the approach of Stram et al. (38). Three SNPs were chosen from *GSTT2*, which cover less than 50% of the common genetic variation in this gene. A real-time TaqMan assay for homozygous or heterozygous deletions of *GSTM1* was done (39).

For quality-control assessment, results on individual genetic markers with genotyping rates of <75% were removed (including 3 African American mothers, 25 African American newborns, 1 Dominican mother, 56 Dominican newborns, 14 Polish Caucasian mothers, and 82 Polish Caucasian newborns). All tested genetic variants had minor allele (defined as the less common allele in the cohort) frequencies greater than 0.05. A goodness-of-fit test to test for Hardy-Weinberg equilibrium indicated that there were no significant deviations in each population.

Interaction between Genetic Markers and PAHs on DNA Adducts

As described previously (33), the eight PAH air concentration measures: benz[*a*]anthracene, chrysene, benzo[*b*]fluroanthene, benzo[*k*]fluroanthene, B[*a*]P, indeno[1,2,3-*cd*]pyrene, disbenz[*a,h*]anthracene, and benzo[*g,h,i*]perylene (40–42) were significantly correlated; thus, a composite PAH variable was computed. This summed measure was dichotomized at the median (2.45 ng/m^3 for NYC African Americans, 2.3 ng/m^3 for NYC Dominicans, and 16.89 ng/m^3 for Polish Caucasians) to obtain a binary PAH exposure, defined as PAH high or PAH low. We first assessed genetic marker × PAH interactions on PAH-DNA adducts. The adduct data were logarithmic transformed to be consistent with the previous studies and to better normalize the distribution. We used multiple linear regression models:

$$ln(\text{DNA adduct})=\beta_0+\beta_1 \text{ETS}+\beta_2 \text{PAH}+\beta_3 \text{ genetic marker}+\beta_4 \text{ genetic marker}\times\text{PAH}.$$

Although ETS was not significantly associated with DNA adducts, it was included in the model as a potential confounder. Other covariates, such as maternal age, dietary PAH, and maternal BMI, were not significantly associated with DNA adducts at the significance level of $P \leq 0.05$ and were not included in the model. For genetic markers that did not show significant interactions ($P \leq 0.05$), main effects of genotype on adducts were examined, also with multiple linear regression models:

$$ln(\text{DNA adduct})=\beta_0+\beta_1 \text{ETS}+\beta_2 \text{PAH}+\beta_3 \text{ genetic marker}.$$

Because the study was limited by the small sample size, we did not routinely correct for multiple comparisons given the fact we tested many interaction effects. Therefore, the findings are suggestive only.

Linkage Disequilibrium Analysis

We analyzed linkage disequilibrium (LD) patterns and haplotype structures of *CYP1A1, CYP1B1*, and *GSTT2* genes using software Haploview (ref. 43, http://www.broad.mit.edu/mpg/haploview/) in African Americans, Dominicans, and Caucasians, respectively. Pairwise LD between any two genetic markers was measured by standardized LD coefficient D' and visualized through Haploview. Haplotype blocks were defined based on the method of Gabriel et al. (44), where a pair of genetic markers is defined to be in "strong LD" if the one-sided upper 95% confidence bound on D' is 0.98 and the lower bound is above 0.7. Haplotype frequencies of defined haplotype blocks were estimated also using Haploview.

Interaction between Haplotypes and PAHs on DNA Adducts

Because haplotypes composed of genetic markers (with or without function) can sometimes provide greater power for detection of effect than the analysis of single-genetic marker, we examined haplotypes in *CYP1A1* and *CYP1B1* (45). As haplotype phase is usually unknown, inferring the most likely pair of haplotypes and then treating them as if they could be directly observed would result in information loss and biased confidence intervals for variable estimates (46). To account for unphased haplotypes, we modeled the probabilities of the possible haplotype pairs per subject using the generalized linear model. The haplotype analyses adjusted for environmental covariates were conducted with the R package haplo.stats (47). We first assessed haplotype × PAH interactions on logarithm-transformed PAH-DNA adduct levels; when no interaction was observed, we tested main effects of haplotypes.

Results

Descriptive Analysis

Table 1 provides demographic and exposure characteristics of mothers and their newborns. There were no significant differences between the subset analyzed in this study and the total population with respect to the selected demographic and exposure characteristics. Maternal and newborn PAH-DNA adducts and prenatal PAH exposure were not significantly different between African Americans and Dominicans. Polish Caucasians had more than 10-fold higher PAH exposure than NYC African Americans and Dominicans, consistent with the higher levels of air pollution in Poland (20). Figure 2 displays scatter plots to show the

correlations between the DNA adduct levels of the mothers and their newborns in three ethnic groups separately. Significant correlations were observed in all three ethnic groups. The correlations between the maternal or newborn DNA adduct levels and the continuous PAH exposure levels are significant in Caucasians (correlation = 0.205 between the newborn DNA adducts and the continuous PAH with $P < 0.001$ and correlation = 0.116 between the maternal DNA adducts and the continuous PAH with $P = 0.030$) but not in African Americans and Dominicans, which is consistent with the previous studies.

The genotype frequency distributions of the tested common genetic variants are summarized for African Americans, Dominicans, and Caucasians for mothers and newborns separately (Supplementary Tables S1 and S2).[4] Note that, for some genetic markers, different ethnic groups have different minor alleles. Supplementary Table S2 shows that the genotype frequency distributions of most genetic markers differ significantly between the three ethnic groups.

Interaction between Genetic Markers and PAHs on DNA Adducts

Assuming a dominant genetic model (minor allele considered as the risk allele) and with a 1 *df* association test, we identified several significant interactions between maternal genetic markers and PAHs on maternal PAH-DNA adducts, interactions between maternal genetic markers and PAHs on newborn PAH-DNA adducts. No interaction was observed between newborn genetic markers and PAHs on newborn PAH-DNA adducts in the three populations separately (Table 2).

In African Americans, significant interactions between maternal genotypes of *CYP1B1-06* (*rs4646903*) and *GSTT2-01* (*rs1622002*) and PAHs were observed on maternal PAH-DNA adducts. The interaction effect between *GSTT2-01* and PAHs is plotted in Fig. 3A for illustration purposes. It shows that maternal adducts are lower in mothers with genotypes *AA/AG* at *GSTT2-01* than in mothers with wild-type homozygote *GG* within the high PAH exposure group, but adducts are higher within the low PAH exposure group (interaction coefficient $\beta = -0.44$; $P = 0.01$; $n = 127$).

In Dominicans, a significant interaction was observed between maternal genotype *CYP1B1-03* (*rs2617266*) and PAHs (interaction coefficient $\beta = -0.30$; $P = 0.04$; $n = 165$) on maternal PAH-DNA adducts (Fig. 3B). Maternal adducts were lower in mothers with genotypes *TT/TC* at *CYP1B1-03* than in mothers with homozygous wild-type *CC* within the high PAH exposure group but were higher within the low PAH exposure group.

More genetic marker × PAH interactions were observed in Caucasians. Interestingly, among Caucasians, [*CYP1A1-14* (rs2606345), was observed in both mother and newborn to interact with PAHs on newborn PAH-DNA adducts and in the same direction. The interaction effect between newborn *CYP1A1-14* and PAHs (interaction coefficient $\beta = 0.29$; $P = 0.020$; $n = 288$) on newborn adducts is displayed in Fig. 3C. The newborn adducts were higher in newborns with genotypes GG/GT at *CYP1A1-14* than newborns with homozygous wild-type TT within the high PAH exposure group but were lower within the low PAH exposure group. Moreover, among Caucasians, maternal *CYP1A1-83* and another maternal SNPs of *CYP1B1* gene [*CYP1B1-74* (rs162560)] were observed to interact with PAHs on maternal PAH-DNA adducts. No genetic markers × PAH interaction remained significant after correction for multiple comparisons among three populations.

---

[4]Supplementary materials for this article are available at Cancer Epidemiology, Biomakers & Prevention Online (http://cebp.aacrjournals.org/).

Table 3 displays main effects for those genetic markers that did not show genetic marker × PAH interactions. In African Americans, mothers with genotypes +/+ or +/− at genetic marker *GSTM1-02* have significantly higher PAH-DNA adducts than mothers with genotypes −/−. African American newborns whose mothers had genotypes *TT/TA* at *CYP1B1-66* or genotypes *AA/AG* at *CYP1B1-74* had significantly higher newborn PAH-DNA adducts, whereas Dominicans newborns whose mothers had genotypes TT/TA at *CYP1B1-66* or genotypes AA/AG at *CYP1B1-74* had significantly lower PAH-DNA adducts. In Caucasians, newborns with genotypes CC/CT at *CYP1A1-06* or genotypes TT/ TC at *CYP1A1-15* had significantly higher newborn PAH-DNA adducts.

LD Analysis

Pairwise LD analysis shows that the same haplotype block with six *CYP1A1* SNPs was defined in the three populations for *CYP1A1* gene (Supplementary Fig. S1). Similar LD patterns were observed in mothers and newborns in the three populations. The estimated haplotype frequencies of the defined haplotype block of the *CYP1A1* gene for mothers and newborns in each population are displayed in Table 4. The LD structure of the *CYP1B1* gene differed substantially between Caucasians and African Americans and Dominicans but this is not surprising because of the differences in population genetic histories of the different groups and the selection bias of choosing SNPs based on the Caucasians in Breast and Prostate Cancer Cohort Consortium (37). Pairwise LD analysis suggests that four *CYP1B1* SNPs [*CYP1B1-66* (*rs162549*), *CYP1B1-06* (*rs1056837*), *CYP1B1-05* (*rs1056836*), and *CYP1B1-74* (*rs162560*)] could form one haplotype block ($D' > 0.9$) in African Americans, whereas three *CYP1B1* SNPs (*CYP1B1-06, CYP1B1-05,* and *CYP1B1-74*) could form one haplotype block in Dominicans. Two *CYP1B1* SNPs [*CYP1B1-04* (*rs10012*) and *CYP1B1-03* (*rs2617266*)] formed another haplotype block in both African Americans and Dominicans. Among Caucasians, all six *CYP1B1* SNPs defined one haplotype block (Supplementary Fig. S2). The estimated haplotype frequencies of each defined haplotype block of the *CYP1B1* gene are displayed in Table 5. No strong haplotype block was defined for the *GSTT2* gene (Supplementary Fig. S3) in any population.

Interaction between Haplotypes and PAHs on DNA Adducts

A significant interaction between maternal haplotypes of the defined haplotype block of the *CYP1A1* gene and PAHs on newborn PAH-DNA adducts was observed in Caucasians (P = 0.03). More specifically, for Caucasians, newborns whose mothers had haplotype CGTCGG had higher PAH-DNA adducts than those whose mothers had the most common haplotype *GATCTA* within the high PAH exposure group, but newborns whose mothers had haplotype *CGTCGG* had lower PAH-DNA adducts than whose mothers had the most common haplotype *GATCTA* within the low PAH exposure group. A significant main effect of maternal haplotype on maternal PAH-DNA adducts was observed in Dominicans (P = 0.02). Dominican mothers with haplotype *CGTTGG* had higher PAH-DNA adducts than Dominican mothers with the most common haplotype *GATCTA*. Based on the regression model described above (47), among Dominican mothers with high PAH exposure level and mean ETS level, those that had haplotype *CGTTGG* had an average DNA adduct level of 0.203, whereas those with the common haplotype *GATCTA* had an average DNA adduct level of 0.160 (P = 0.02). A significant main effect of newborn haplotype on newborn PAH-DNA adducts was observed in Caucasians (P = 0.03). Caucasian newborns with haplotype CGTCGG had lower PAH-DNA adducts than Caucasians newborns with the most common haplotype GATCTA.

Discussion

Our findings are notable because it appears that PAH-DNA adduct formation could be modulated by common genetic variants in key genes, *CYP1A1, CYP1B1, GSTT2*, and *GSTM1*. Interaction effects between genetic markers and PAH were observed in African American, Dominican, and Caucasian mothers and their newborns, but more interaction effects were observed in Caucasians than in African Americans and Dominicans (Table 2). In Fig. 3, we graphically presented some of the interactions observed in the respective ethnic groups. In African Americans, maternal adducts were lower in mothers with genotypes *AA/ AG* at *GSTT2-01* than in mothers with homozygous wild-type *GG* within the high PAH exposure group but were higher within the low PAH exposure group. In Dominicans, maternal adducts were lower in mothers with genotypes *TT/TC* at *CYP1B1-03* than in mothers with homozygous wild-type CC within the high PAH exposure group but were higher within the low PAH exposure group. In Caucasians, newborn adducts were higher in newborns whose mothers have genotypes *CC/CT* at *CYP1A1-14* than in newborns with homozygous wild-type *TT* within the high PAH exposure group but were lower within the low PAH exposure group. A haplotype by PAH interaction effect PAH × CYP1A1 haplotype was also observed but in Caucasians only. Although genetic polymorphisms were detected to interact with PAHs on PAH-DNA adducts in the three ethnic groups, with the limited sample sizes, we consider the current findings as suggestive only and warranting further study.

Interactions between PAHs and genetic polymorphisms in *CYP1A1* were observed only in Caucasians. This may be due to the much larger sample size and the much higher PAH exposure levels in the Caucasian group. Among Caucasians, maternal CYP1A1-14 significantly interacted with PAH exposure on both maternal and newborn adducts and maternal *CYP1A1-83* significantly interacted with PAHs on maternal DNA adducts. These observations suggest that the *CYP1A1* gene may be an important effect modifier in Caucasians. Whether a similar conclusion can be drawn in other ethnic groups needs to be investigated by independent studies with bigger sample sizes. We note that the *CYP* genotypes would in theory be less predictive for the adduct levels than the enzyme levels measured in the blood. However, we did not obtain data on enzyme levels. We also note that in several cases different interaction effects were observed on maternal DNA adducts and newborn DNA adducts when the same genetic markers were investigated. This may be due to the fact that the mothers and babies have different genotypes. Moreover, the enzyme systems responsible for metabolism, detoxification, and DNA repair are immature in the fetus so that one might not expect to see the same effect of the genotype in mothers and newborns. More research efforts are needed to examine these relationships.

Main effects of the genetic polymorphisms on PAH-DNA adducts were also observed. One noticeable finding is the protective effect of *GSTM1-02* deletion in African Americans, which is contradictory to some reports that *GSTM1-02* deletion increases cancer risk (48). More research effort is needed to replicate this finding in African Americans. A strength of the study is the ability to explore gene-environment interaction using haplotype-tagging genetic markers, adducts, and environmental monitoring. However, it is possible that the effects we observed in this pilot study could be due to other variants that were untested but are in strong LD with the tested variants or, alternatively, chance. Moreover, because of the difference in population genetic histories of the different groups and the selection bias of choosing SNPs based on the Caucasians in Breast and Prostate Cancer Cohort Consortium (37), further work is required to confirm these findings in replication studies and, if replicated, fine mapping to determine variants that could be causally defined by corroborative laboratory analyses.

Our results are also notable because we interrogated common genetic variants in candidate genes in three distinct populations and observed different underlying genetic structures. For instance, the frequencies of the genetic polymorphisms among Caucasians are significantly different from those in African Americans and Dominicans. LD analysis also shows that heterogeneity in LD structures among the three ethnic groups is present, especially for *CYP1B1* gene. Although the haplotype structure is similar in the three ethnic groups for *CYP1A1* gene, the haplotype frequencies are different. Thus, in looking at genetic main effects or gene-environment interactions, researchers should be aware of the heterogeneity between ethnic groups and try to perform studies within relatively homogeneous populations to avoid spurious associations from population stratification (49). In conclusion, further studies in distinct populations will not only help to identify loci in different LD structures that are associated with gene-environment interactions but also to highlight possible variants that could confer increased risk according to population structure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. IARC. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Lyon (France): IARC; 1983. Polynuclear aromatic compounds. Part l. Chemical, environmental, and experimental data; p. 1-453.

2. Donnenfeld AE, Pulkkinen A, Palomaki GE, Knight GJ, Haddow JE. Simultaneous fetal and maternal cotinine levels in pregnant women smokers. Am J Obstet Gynecol. 1993; 168:781–2. [PubMed: 8456879]

3. Lewtas J. Human exposure to complex mixtures of air pollutants. Toxicol Lett. 1994; 72:163–9. [PubMed: 8202929]

4. De Raat WK, Kooijman SA, Gielen JW. Concentrations of polycyclic hydrocarbons in airborne particles in The Netherlands and their correlation with mutagenicity. Sci Total Environ. 1987; 66:95–114. [PubMed: 3317821]

5. Bulay OM, Wattenberg LW. Carcinogenic effects of polycyclic hydrocarbocarcinogens administered to mice during pregnancy on the progeny. J Natl Cancer Inst. 1971; 46:397–402. [PubMed: 5115907]

6. Rice JM, Ward JM. Age dependence of susceptibility to carcinogenesis in the nervous system. Ann N Y Acad Sci. 1982; 381:274–89. [PubMed: 6953795]

7. Vesselinovitch SC, Kyriazis AP, Mihailovich N, Rao KV. Conditions modifying development of tumors in mice at various sites by benzo(*a*)pyrene. Cancer Res. 1975; 35:2948–53. [PubMed: 1182688]

8. Bartsch H, Hietanen E. The role of individual susceptibility in cancer burden related to environmental exposure. Environ Health Perspect. 1996; 104 (Suppl 3):569–77. [PubMed: 8781385]

9. Poirier MC, Beland FA. DNA adduct measurements and tumor incidence during chronic carcinogen exposure in animal models: implications for DNA adduct-based human cancer risk assessment. Chem Res Toxicol. 1992; 5:749–55. [PubMed: 1489923]

10. Stowers SJ, Anderson MW. Formation and persistence of benzo[*a*-]pyrene metabolite-DNA adducts. Environ Health Perspect. 1985; 62:31–9. [PubMed: 4085435]

11. Rybicki BA, Rundle A, Savera AT, Sankey SS, Tang D. Polycyclic aromatic hydrocarbon-DNA adducts in prostate cancer. Cancer Res. 2004; 64:8854–9. [PubMed: 15604244]

12. Tang D, Santella RM, Blackwood A, et al. A molecular epidemiological case control study of lung cancer. Cancer Epidemiol Biomarkers Prev. 1995; 4:341–6. [PubMed: 7655328]

13. Tang D, Cho S, Rundle A, et al. Polymorphisms in the DNA repair enzyme XPD are associated with increased levels of PAH-DNA adducts in a case-control study of breast cancer. Breast Cancer Res Treat. 2002; 75:159–66. [PubMed: 12243508]

14. Veglia F, Matullo G, Vineis P. Bulky DNA adducts and risk of cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2003; 12:157–60. [PubMed: 12582026]

15. Lu LJ, Disher RM, Reddy MV, Randerath K. 32P-postlabeling assay in mice of transplacental DNA damage induced by the environmental carcinogens safrole, 4-aminobiphenyl and benzo(*a*)pyrene. Cancer Res. 1986; 46:3046–54. [PubMed: 3698023]

16. Lu LJ, Wang MY. Modulation of benzo(*a*)pyrene-induced covalent DNA modification in adult and fetal mouse tissues by gestation stage. Carcinogenesis. 1990; 11:1367–72. [PubMed: 2387022]

17. Wang MY, Lu LJ. Differential effect of gestation stage on benzo(*a*)-)pyrene-induced micronucleus formation and/or covalent DNA modifications in mice. Cancer Res. 1990; 50:2146–51. [PubMed: 2317806]

18. Whyatt RM, Jedrychowski W, Hemminki K, et al. Biomarkers of polycyclic aromatic hydrocarbon-DNA damage and cigarette smoke exposures in paired maternal and newborn blood samples as a measure of differential susceptibility. Cancer Epidemiol Biomarkers Prev. 2001; 10:581–8. [PubMed: 11401906]

19. Perera FP, Tang D, Whyatt RM, Lederman SA, Jedrychowski W. DNA damage from polycyclic aromatic hydrocarbons measured by benzo[*a*]pyrene-DNA adducts in mothers and newborns from northern Manhattan, the World Trade Center area, Poland, and China. Cancer Epidemiol Biomarkers Prev. 2005; 14:709–14. [PubMed: 15767354]

20. Perera FP, Rauh V, Tsai WY, et al. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ Health Perspect. 2003; 111:201–5. [PubMed: 12573906]

21. Choi H, Jedrychowski W, Spengler J, et al. International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. Environ Health Perspect. 2006; 114:1744–50. [PubMed: 17107862]

22. Perera FP, Tang D, Rauh V, et al. Relationships among polycyclic aromatic hydrocarbon-DNA adducts, proximity to the World Trade Center, and effects on fetal growth. Environ Health Perspect. 2005; 115:1062–7. [PubMed: 16079080]

23. Perera FP, Rauh V, Whyatt RM, et al. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. Environ Health Perspect. 2005; 114:1287–92. [PubMed: 16882541]

24. Miller RL, Garfinkel R, Horton M, et al. Polycyclic aromatic hydrocarbons, environmental tobacco smoke, and respiratory symptoms in an inner-city birth cohort. Chest. 2004; 126:1071–8. [PubMed: 15486366]

25. Bocskay KA, Tang D, Orjuela MA, Liu X, Warburton DP, Perera FP. Chromosomal aberrations in cord blood are associated with prenatal exposure to carcinogenic polycyclic aromatic hydrocarbons. Cancer Epidemiol Biomarkers Prev. 2005; 14:506–11. [PubMed: 15734979]

26. Kawajiri K, Nakachi K, Imai K, Hayashi S, Watanabe J. Individual differences in lung cancer susceptibility in relation to polymorphisms of P-450IA1 gene and cigarette dose. Princess Takamatsu Symp. 1990; 21:55–61. [PubMed: 2134690]

27. Bennett WP, Alavanja MCR, Blomeke B, et al. Environmental tobacco smoke, genetic susceptibility, and risk of lung cancer in never-smoking women. J Int Cancer Inst. 1999; 91:2009–14.

28. McWilliams JE, Sanderson BJ, Harris EL, Richert-Boe KE, Henner WD. Glutathione *S*-transferase M1 (GSTM1) deficiency and lung cancer risk. Cancer Epidemiol Biomarkers Prev. 1995; 4:589–94. [PubMed: 8547824]

29. Ketterer B, Harris JM, Talaska G, et al. The human glutathione *S*-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. Environ Health Perspect. 1992; 98:87–94. [PubMed: 1486868]

30. Kihara M, Noda K. Risk of smoking for squamous and small cell carcinomas of the lung modulated by combinations of CYP1A1 and GSTM1 gene polymorphisms in a Japanese population. Carcinogenesis. 1995; 16:2331–6. [PubMed: 7586131]

31. Nakachi K, Imai K, Hayashi S, Watanabe J, Kawajiri K. Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. Cancer Res. 1991; 51:5177–80. [PubMed: 1655248]

32. Saarikoski ST, Voho A, Reinikainen M, et al. Combined effect of polymorphic GST genes on individual susceptibility to lung cancer. Int J Cancer. 1998; 77:516–21. [PubMed: 9679751]

33. Perera FP, Tang D, Tu YT, et al. Biomarkers in maternal and newborn blood indicate heightened fetal susceptibility to procarcinogenic DNA damage. Environ Health Perspect. 2004; 112:1133–6. [PubMed: 15238289]

34. Lederman SA, Rauh V, Weiss L, et al. The effects of the World Trade Center event on birth outcomes among term deliveries at three lower Manhattan hospitals. Environ Health Perspect. 2004; 112:1772–8. [PubMed: 15579426]

35. Alexandrov K, Rojas M, Geneste O, et al. An improved fluorometric assay for dosimetry of benzo(*a*)pyrene diol-epoxide-DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. Cancer Res. 1992; 52:6248–53. [PubMed: 1423269]

36. Packer BR, Yeager M, Burdett L, et al. marker500Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. Nucleic Acids Res. 2006; 34:617–21.

37. Hunter DJ, Riboli E, Haiman CA, et al. A candidate gene approach to searching for low-penetrance breast and prostate cancer genes. Nat Rev Cancer. 2005; 5:977–85. [PubMed: 16341085]

38. Stram DO, Haiman CA, Hirschhorn JN, et al. Choosing haplotype-tagging markers based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the multiethnic cohort study. Hum Hered. 2003; 55:27–36. [PubMed: 12890923]

39. Moore LE, Huang WY, Chatterjee N, et al. GSTM1, GSTT1, and GSTP1 polymorphisms and risk of advanced colorectal adenoma. Cancer Epidemiol Biomarkers Prev. 2005; 14:1823–7. [PubMed: 16030123]

40. Camann, DE.; Harding, HJ.; Clothier, JM.; Kuchibhatla, RV.; Bond, AE. Dermal and in-home exposure of the farm family to agricultural pesticides. Measurement of toxic and related air pollutants: proceedings of an international symposium; Research Triangle Park (NC): Air & Waste Management Association; 1995. p. 548-54.

41. Geno, PW.; Camann, DE.; Villalobos, K.; Lewis, RG. Analytical methods for assessing the exposure of farmers and their families to pesticides. Measurement of toxic and related air pollutants: proceedings of the 1993 U.S. EPA/A&WMA International Specialty Conference; Research Triangle Park (NC): Air & Waste Management Association; 1993. p. 698-705.

42. Majumdar, TK.; Camann, DE.; Geno, PW. Analytical method for the screening of pesticides and polynuclear aromatic hydrocarbons from house dust: proceedings of the 1993 U.S. EPA/A&WMA International Specialty Conference; Research Triangle Park (NC): Air & Waste Management Association; 1993. p. 685-90.

43. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–5. [PubMed: 15297300]

44. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science. 2002; 296:2225–9. [PubMed: 12029063]

45. Akey J, Xiong M. Haplotypes vs. single marker linkage disequilibrium tests: what do we gain? Eur J Hum Genet. 2001; 9:291–300. [PubMed: 11313774]

46. Morris AP, Whittaker JC, Balding DJ. Little loss of information due to unknown phase for fine scales linkage mapping with single-nucleotide-polymorphism genotype data. Am J Hum Genet. 2004; 74:945–53. [PubMed: 15077198]

47. Schaid DJ. Evaluating associations of haplotypes with traits. Genet Epidemiol. 2004; 27:348–64. [PubMed: 15543638]

48. García-Closas M, Malats N, Silverman D, et al. *NAT2* slow acetylation and *GSTM1* null genotypes increase bladder cancer risk: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet. 2005; 366:649–59. [PubMed: 16112301]

49. Lander ES, Schork NJ. Genetic dissection of complex traits. Science. 1994; 265:2037–48. [PubMed: 8091226]
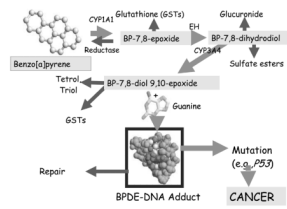
Figure 1.
Diagram of the mechanistic relationship between the genes and formation of the adducts.
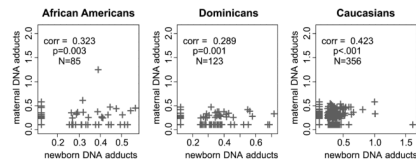
Figure 2.
Scatter plots that show the correlations between maternal and newborn DNA adducts in the three ethnic groups and the corresponding *P* values.
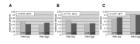
Figure 3.
A. Interaction effect between maternal *GSTT2-01* and PAHs on maternal DNA adducts in African Americans (*AA*). More specifically, for African Americans, maternal adducts are lower in mothers with genotypes *AA/AG* at *GSTT2-01* within the high PAH exposure group but are higher within the low PAH exposure group (interaction coefficient $\beta = -0.44$, $P = 0.01$; $n = 127$). **B.** Interaction effect between maternal *CYP1B1-03* and PAHs on maternal DNA adducts in Dominicans. More specifically, for Dominicans, maternal adducts are lower in mothers with genotypes *TT/TC* at *CYP1B1-03* within the high PAH exposure group but are higher within the low PAH exposure group (interaction coefficient $\beta = -0.30$, $P = 0.04$, $n = 165$). **C.** Interaction effect between newborn *CYP1A1-14* and PAHs on newborn DNA adducts in Caucasians (*C*). More specifically, for Caucasians, newborn adducts are higher in newborns whose mothers have genotypes *CC/CT* at *CYP1A1-14* within the high PAH exposure group but are lower within the low PAH exposure group (interaction coefficient $\beta = 0.29$, $P = 0.020$, $n = 288$).

Table 1

Exposure, biomarker, and demographic characteristics of the study population with mother and/or newborn genotypes [*n* (mean ± SD)]

	African Americans	Dominicans	Caucasians
Prenatal PAHs in air (ng/m^3)[*]	155 (3.36 ± 3.60)	250 (3.49 ± 4.22)	328 (36.75 ± 46.19)
Newborn B[*a*]P-DNA adducts[*,†]	76 (0.24 ± 0.15)	104 (0.23 ± 0.15)	255 (0.27 ± 0.17)
Maternal B[*a*]P-DNA adducts[*,†]	132 (0.20 ± 0.15)	176 (0.19 ± 0.10)	339 (0.27 ± 0.16)
Maternal age (y)[‡]	159 (24.21 ± 4.94)	264 (25.52 ± 5)	345 (27.95 ± 3.72)
Dietary PAH[‡,§]	151 (23.71 ± 3.59)	236 (21.03 ± 2.67)	345 (42.54 ± 5.97)
Maternal ETS[//,¶]	158 (0.46 ± 0.50)	262 (0.26 ± 0.44)	345 (0.23 ± 0.42)
Maternal BMI[‡]	152 (27.05 ± 6.99)	249 (25.02 ± 5.29)	345 (21.41 ± 3.14)

NOTE: Subjects included in the present analysis are those with genotype data in mothers (160 African Americans, 265 Dominicans, and 381 Caucasians) or in their newborns (99 African Americans, 154 Dominicans, and 294 Caucasians). No significant difference between the subset and the total population with respect to the selected demographic and exposure characteristics.

[*] African Americans and Dominicans are not significantly different, but both are significantly different from Caucasians.

[†] Adducts per 10^8 nucleotides.

[‡] African Americans, Dominicans, and Caucasians are significantly different pairwisely.

[§] PAH from dietary consumption of smoked meat, cheese, and fish.

[//] Dominicans and Caucasians are not significantly different, but both are significantly different from African Americans.

[¶] Percentage who report a smoker in household.

Table 2

Significant genetic marker × PAH interactions on PAH-DNA adducts

Genetic marker	African Americans			Dominicans			Caucasians		
	β^*	P	n	β^*	P	n	β^*	P	n
Interaction of PAHs and maternal genetic marker on PAH-DNA adducts in maternal blood									
CYP1A1-14 (GG/GT vs TT)[†]							0.26	0.027	320
CYP1A1-83 (GG/GA vs AA)[‡]							0.37	0.009	298
CYP1B1-03 (TT/TC vs CC)				−0.30	0.04	165			
CYP1B1-06 (CC/CT vs TT)[§]	0.41	0.02	129						
CYP1B1-74 (AA/AG vs GG)							−0.25	0.035	321
GSTT2-01 (AA/AG vs GG)	−0.44	0.01	127						
Interaction of PAHs and maternal genetic marker on PAH-DNA adducts in newborn blood									
CYP1A1-14 (GG/GT vs TT)[†]							0.29	0.020	288
Interaction of PAHs and newborn genetic marker on PAH-DNA adducts in newborn blood									

NOTE: Significant ($P \le 0.05$, without correction for multiple comparisons) genetic marker × PAH interactions are displayed. Blanks mean no significant genetic marker × PAH interactions ($P > 0.05$).

*
Regression coefficients of the genetic marker × PAH interactions from the multiple linear regression models.

[†]Minor allele: African Americans (allele T), Dominicans (allele T), and Caucasians (allele G); displayed reflects Caucasians.

[‡]Minor allele: African Americans (allele A), Dominicans (allele G), and Caucasians (allele G); displayed reflects Dominicans and Caucasians.

[§]Minor allele: African Americans (allele C), Dominicans (allele C), and Caucasians (allele T); displayed reflects African Americans and Dominicans.

Table 3

Significant main effects of genetic markers on PAH-DNA adducts

Genetic marker	African Americans			Dominicans			Caucasians		
	β^*	P	n	β^*	P	n	β^*	P	n
Main effect of maternal genetic-marker on PAH-DNA adducts in maternal blood									
GSTM1-02 (+/+ or +/− vs −/−)	0.28	0.01	107						
Main effect of maternal genetic-marker on PAH-DNA adducts in newborn blood									
CYP1B1-66 (TT/TA vs AA)	0.26	0.04	84	−0.28	0.01	112			
CYP1B1-74 (AA/AG vs GG)	0.34	0.005	83	−0.23	0.04	112			
Main effect of newborn genetic-marker on PAH-DNA adducts in newborn blood									
CYP1A1-06 (CC/CT vs TT)							0.21	0.029	236
CYP1A1-15 (TT/TC vs CC)							0.20	0.041	238

NOTE: These did not show interaction on PAH and therefore were analyzed for main effects. Significant ($P \leq 0.05$, without correction for multiple comparisons) main effects are displayed. Blanks mean no significant main effects of the genetic markers ($P > 0.05$).

*
Regression coefficients of the genetic markers from the multiple linear regression models.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 4

Haplotype frequencies of *CYP1A1* gene within each population

Haplotypes of gene *CYP1A1*	African Americans		Dominicans		Caucasians	
	Mother	Newborn	Mother	Newborn	Mother	Newborn
*GATCGA****	0.311	0.308	0.273	0.265	0.184	0.171
GATCTA†	0.144	0.138	0.361	0.359	0.686	0.686
*CGCTGG****	0.231	0.234	0.191	0.188	0.079	0.095
CGTCGG‡	0.186	0.193	0.071	0.076	NA	NA
CGTTGG	0.101	0.101	0.066	0.071	NA	NA

NOTE: Haplotypes with frequency more than 5% are displayed. Haplotype frequencies among the three populations were compared pairwisely using two-sample *z* test to compare the equality of two binomial proportions.

* Caucasians is different from African Americans and Dominicans for both mothers and newborns.

† All three populations are significantly different from each other for both mothers and newborns (*P* < 0.05).

‡ African Americans and Dominicans are different for both mothers and newborns.

Table 5

Haplotype frequencies of *CYP1B1* gene within each population

African Americans	Haplotype (block 1)	Mother	Newborn	Haplotype (block 2)	Mother	Newborn
	ATGG	0.544	0.524	CC*	0.439	0.445
	ACCG	0.239	0.260	GC*	0.308	0.305
	TTGA	0.183	0.183	GT	0.253	0.250
Dominicans	Haplotype (block 1)	Mother	Newborn	Haplotype (block 2)	Mother	Newborn
	TGG	0.410	0.407	CC*	0.659	0.654
	CCG	0.405	0.419	GC*	0.131	0.127
	TGA	0.176	0.167	GT	0.208	0.217
Caucasians	Haplotype	Mother	Newborn			
	ACCGGT	0.307	0.308			
	ACCGCC	0.262	0.245			
	ATGGCC	0.220	0.229			
	TTGACC	0.200	0.208			

NOTE: Haplotypes with frequency more than 5% are displayed. Haplotype frequencies of block 2 in African Americans and Dominicans were compared pairwisely using two-sample z test to compare the equality of two binomial proportions.

*
African Americans and Dominicans are different for both mothers and newborns.