Gene-environment interactions on mental development in African American, Dominican, and Caucasian Mothers and Newborns

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Abstract

The health impact of environmental toxins has gained increasing recognition over the years. Polycyclic aromatic hydrocarbons (PAHs) and environmental tobacco smoke (ETS) are known to affect nervous system development in children, but no studies have investigated how polymorphisms in PAH metabolic or detoxification genes affect child cognitive development following PAH exposure during pregnancy. In two parallel prospective cohort studies of nonsmoking African American and Dominican mothers and children in New York City and of Caucasian mothers and children in Krakow, Poland, we explored the effect of gene-PAH interaction on child mental development index (MDI), as measured by the Bayley Scales of Infant Development-Revised (BSID-II). Genes known to play important roles in the metabolic activation or detoxification of PAHs were selected. Genetic variations in these genes could influence susceptibility to adverse effects of PAHs in polluted air. We explored the effects of interactions between prenatal PAH exposure and 21 polymorphisms or haplotypes in these genes on MDI at 12, 24, and 36 months among 547 newborns and 806 mothers from three different ethnic groups: African Americans, Dominicans, and Caucasians. PAHs were measured by personal air monitoring of mothers during pregnancy. Significant interaction effects between haplotypes and PAHs were observed in mothers and their newborns in all three ethnic groups after Bonferroni correction for multiple comparisons. The strongest and most consistent effect observed was between PAH and haplotype ACCGGC of the *CYP1B1* gene.

Introduction

There is growing evidence that exposure to ambient and indoor air pollutants adversely affects fetal growth and early childhood neural development (Dejmek et al., 2000; Perera et al., 1999; Perera et al., 2003; Perera et al., 2004; Perera et al., 2006). Polycyclic aromatic hydrocarbons (PAHs) are widespread pollutants commonly found in air, food, and drinking water (International Agency for Research on Cancer, 1983). Airborne PAHs (outdoor or indoor) mainly result from combustion of fossil fuels, tobacco, and other organic materials. Emissions from motor vehicles, electricity generation, and residential heating are generally the major source of PAHs in outdoor urban air, whereas environmental tobacco smoke

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(ETS) is a major indoor source (Donnenfeld et al., 1993; Lewtas 1994). Perera et al. recently studied the effect of prenatal exposure to airborne PAHs on child development and found that high prenatal exposure to PAHs was associated with a lower mental development index (MDI) and a higher incidence of developmental delay at age three (Perera et al., 2006). Studies on prenatal exposure to ETS have also associated PAH exposure with reduced fetal growth and cognitive function (Windham et al., 1999; Rauh et al., 2004).

The cytochrome P450 genes *CYP1A1*, *CYP1A2* and *CYP1B1* have been shown to play important roles in the metabolic activation of PAHs, while PAH detoxification is partially controlled by the glutathione *S*-transferase (GST) genes *GSTM1* and *GSTT2* (Kawajiri et al., 1990; Bennett et al., 1999; Abnet et al., 2005). P450 (*CYP1A1*) is involved in conversion of PAH into DNA-binding intermediates (Chisolm 1981; Hanazawa et al., 2000) and is part of an inducible enzyme system that transforms PAH into epoxide-containing metabolites, some of which are mutagenic and carcinogenic (Nebert 1991). *GST* genes participate in antioxidant defense within the airways. Oxidative stress in pregnant women can be modified by heritable polymorphisms in several genes, including GSTM1 (Thomas et al., 1997). To our knowledge, no studies have examined the role of genetic polymorphisms in metabolic activation or detoxification of PAHs on child mental development. Here, we use data from two prospective cohort studies of nonsmoking African American and Dominican mothers and children in New York City and nonsmoking Caucasian mothers and children in Krakow, Poland to assess the association of airborne PAH exposure levels child MDI at 12, 24, and 36 months, measured by Bayley Scales of Infant Development-Revised (BSID-II) (Bayley 1993), with the genotype or haplotype of selected candidate genes.

Materials and Methods

Study populations

Subjects were chosen from two independent, parallel studies. One is currently being conducted in New York City (NYC) and the details on the study design have been previously published (Perera et al., 2003). Nonsmoking, self-identified African American and Dominican women residing in Washington Heights, Central Harlem, NYC, and the South Bronx were recruited through the obstetrical services of New York Presbyterian Hospital, Harlem Hospital, or satellite clinics between February 1998 and February 2003. The women carried a backpack containing a portable personal exposure air monitor during the day and kept it at their bedsides at night during a consecutive 48 hr period during the third trimester of pregnancy for PAH measurements. 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 (Choi et al., 2006). Details on the study design for the Krakow cohort have been previously published (Choi et al., 2006; Jedrychowski et al., 2003). Nonsmoking, pregnant women residing in the Srodmiescie and the Krowodrza-Nowa Huta areas were recruited between November 2000 and March 2003 (Choi et al., 2006). The women carried a backpack containing a portable personal exposure air monitor during the day and kept it at their bedsides at night during a consecutive 48 hr period between the 20th and 30th week of pregnancy for PAH measurements. The study was approved by the ethics committee of the Jagiellonian University, and informed consent was obtained from all subjects.

In Polish study, personal indoor and outdoor PAH exposure was monitored during all three trimesters on a representative subset of 80 women using the same monitoring instrument. High crude correlation coefficients between the second- and third-trimester personal

monitoring values were observed, reflecting the short temporal gap between the monitoring periods (mean, 6 weeks; range, 5–10 weeks). In contrast, lower crude correlation coefficients for the nine PAHs were observed between the first and second trimester due to the longer gap between the two monitoring periods (mean, 19 weeks; range, 17–23 weeks).

The seasonal effect of personal PAH monitoring was further investigated in Choi et al. (2008). In the NYC study, PAH exposure was only monitored during the 3rd trimester for the reasons of cost. However, we have found that the seasonal variation in PAH levels is far smaller in NYC than in Poland. In order to further document prenatal exposure to PAHs and to test whether the prenatal personal air concentrations were correlated with prenatal residential PAH levels, we measured PAH levels of indoor air samples collected in sequential two-week periods from a representative subset of homes over six weeks during the final trimester of pregnancy in a parallel cohort in NYC. The levels of pollutants measured in weeks 1–2 correlated significantly with the levels measured in week 2–6. The indoor air levels of the pollutants over the six weeks of monitoring were significantly associated with the levels measured in the single 48 hour prenatal personal air samples. Thus, our single PAHs/pyrene measurements from prenatal personal air are reasonable indicators of chronic prenatal exposure via inhalation (Choi et al., 2006; Choi et al., 2008).

In both cohort studies, maternal blood (30–35 mL) was collected within 1 day postpartum, and umbilical cord blood (30–60 mL) was collected at delivery.

Subjects included in the present analysis are mothers with genotype data (n=178 African Americans, n=282 Dominicans, and n=381 Polish Caucasians) and their newborns with genotype data (n=116 African Americans, n=167 Dominicans, and n=294 Polish Caucasians) (Table 1). Among those are 98 African American mother-newborn pairs, 138 Dominican mother-newborn pairs, and 275 Caucasian mother-newborn pairs. The subset selected based on genotype availability did not differ significantly from the original cohort with respect to the selected demographic and exposure characteristics. The same cohort was previously investigated to explore the effect of airborne PAH exposure on DNA adducts in mothers and their newborns in the three ethnic groups (Wang et al., 2008).

Selection of Polymorphisms for Genotyping

Twenty-one genetic polymorphisms from genes *CYP1A1*, *CYP1A2*, *CYP1B1*, *GSTM3 GSTM1*, and *GSTT2* were selected from the SNP500Cancer resource (Packer et al., 2006). For *CYP1A1*, *CYP1A2*, and *CYP1B1*, haplotype-tagging SNPs (ht-SNPs) were selected based on the genomic analysis of these genes (seven in *CYP1A1: CYP1A1-78* (rs2198843), *CYP1A1-109* (rs1456432), *CYP1A1-06* (rs4646903), *CYP1A1-15* (rs4646421), *CYP1A1-14* (rs2606345), *CYP1A1-83* (rs7495708), and *CYP1A1-81* (rs2472299); three in *CYP1A2: CYP1A2-03* (rs762551), *CYP1A2-12* (rs2472304), and *CYP1A2-52* (rs4886406); six in *CYP1B1*): *CYP1B1-66* (rs162549), *CYP1B1-06* (rs1056837), *CYP1B1-05* (rs1056836), *CYP1B1-74* (rs162560), *CYP1B1-04* (rs10012), and *CYP1B1-03* (rs2617266)) by the Breast and Prostate Cancer Cohort Consortium (BPC3) (Hunter et al., 2005) using the approach of Stram et al. (2003). Three SNPs were chosen from *GSTT2* (*GSTT2-02* (rs2719), *GSTT2-01* (rs1622002), and *GSTT2-03* (rs140194)), which cover less than 50% of the common genetic variations in this gene. A known nonsynonymous SNP with both functional data and preliminary association in several cancers (Closas et al., 2005) was chosen from *GSTM3* (*GSTM3-01* (rs7483). A real-time PCR assay (TaqMan) was performed for genotyping (Moore et al., 2005).

As in our previous work (Wang et al., 2008), genotyping calls for individual genetic markers with genotyping rates <75% were deleted for quality control (including three African American mothers, 25 African American newborns, one Dominican mother, 56 Dominican

newborns, 14 Polish Caucasian mothers, and 82 Polish Caucasian newborns). All genetic variants had minor allele (defined as the less common allele in the cohort) frequencies greater than 0.05 in all three populations. A goodness-of-fit test for Hardy-Weinberg equilibrium indicated that there were no significant deviations in each population.

Measures of child mental development index (MDI)

The Bayley Scales of Infant Development – Revised (BSID-II) (Bayley, 1993) was used to assess cognitive development at 12, 24, and 36 months of age in all three populations. The BSID-II is the most widely used norm-referenced developmental test for young children. It can be used to diagnose developmental delays and is clinically useful for the identification of children performing in the subnormal range (Burchinal et al., 2000; Sternberg et al, 2001). The BSID-II is known to be sensitive to the developmental effects of toxic exposure. Although cognitive assessments during the first few years of life have limited stability, the predictive power of BSID-II increases after two years. A developmental quotient measured by raw score/chronologic age is yielded with each test, and a mental development index (MDI) is generated based on these data.

Interactions between single genetic markers and PAH on MDI

As previously described (Perera et al., 2004), a composite PAH variable was computed based on 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 were significantly correlated (Geno et al., 1993; Majumdar et al, 1993; Camann et al., 1995). A binary high/low PAH exposure measure was defined by dichotomizing this summed measure at the median (2.45 ng/m^3 for NYC African Americans; 2.30 ng/m^3 for NYC Dominicans; and 16.89 ng/m^3 for Polish Caucasians). Given the significantly higher PAH level in the Polish cohort than in the NYC cohort, the direct comparison of the two cohorts using the continuous PAH measures is not appropriate. In order to examine the genetic effects on MDI at different levels of PAHs within each population, we defined a high PAH and a low PAH exposure group for each ethnic group using the median of the continuous PAH measure. We assessed baby gene-PAH interactions and maternal gene-PAH interactions on MDIs independently at 12, 24, and 36 months, adjusting for significant confounders such as ETS, gender, and whether the mother had high school education, using the following multiple linear regression models:

$$MDI = \beta_0 + \beta_1 ETS + \beta_2 gender + \beta_3 highschool + \beta_4 PAH + \beta_5 Bgeneticma r\ker + \beta_6 Bgeneticma r\ker^* PAH,$$
$$MDI = \beta_0 + \beta_1 ETS + \beta_2 gender + \beta_3 highschool + \beta_4 PAH + \beta_5 Mgeneticma r\ker + \beta_6 Mgeneticma r\ker^* PAH.$$

The possible confounders for which we controlled were independently and significantly associated with MDI measurements, in models not including genetic information, in at least one of the ethnic groups (Supplementary Table S3). We will consider a p-value of <0.00013 as significant for the examination of the 378 single-marker-PAH interactions tested (21 markers, three populations, three outcomes, and maternal and newborn genotypes analyzed separately).

Interactions between haplotype and PAH on MDI

Analysis of haplotypes composed of several genetic markers can sometimes be more powerful than the analysis of single genetic markers for the detection of effects (Akey and Xiong, 2001). We examined haplotypes in *CYP1A1*, *CYP1A2*, *CYP1B1*, and *GSTT2*. Because inferring the most likely pair of haplotypes and treating them as if they were being directly observed results in information loss and biased confidence intervals for parameter estimates (Morris et al., 2004), we modeled the probabilities of all possible haplotype pairs

per subject to account for unphased haplotypes. Linkage disequilibrium (LD) patterns and haplotype structures of *CYP1A1*, *CYP1A2, CYP1B1* and *GSTT2* in African Americans, Dominicans, and Caucasians were first analyzed using Haploview software (http://www.broad.mit.edu/mpg/haploview/) (Barrett et al., 2005), then we used the standardized linkage disequilibrium coefficient D′ to measure pairwise LD between any two genetic markers. Haplotype blocks were defined based on the method of Gabriel et al. (2002). Different haplotype blocks might be defined in different populations, and different haplotype frequencies might be obtained for the same haplotype block in different populations. To ease interpretation of the results, for the analysis of interaction between haplotypes and PAH, we disregarded the haplotype block structures and defined the same reference haplotypes for all three groups. We assessed baby haplotype-PAH interactions and maternal-haplotype-PAH interactions on MDI independently at 12, 24, and 36 months, adjusting for the same set of covariates as in the analysis of interaction between single genetic markers and PAHs using the following generalized linear model (GLM):

$$MDI = \beta_0 + \beta_1 ETS + \beta_2 gender + \beta_3 highschool + \beta_4 PAH + \beta_5 Bhaplotype + \beta_6 Bhaplotye^* PAH,$$
$$MDI = \beta_0 + \beta_1 ETS + \beta_2 gender + \beta_3 highschool + \beta_4 PAH + \beta_5 Mhaplotype + \beta_6 Mhaplotype^* PAH.$$

We will consider a p-value of <0.00038 as significant for the examination of the 132 haplotype-PAH interactions tested (four haplotypes of the *CYP1A1* gene tested in African Americans and Dominicans on three MDIs with mother and newborn haplotypes separately, 2 haplotypes of the *CYP1A1* gene tested in Caucasians on 3 MDIs with mother and newborn haplotypes separately, 5 haplotypes of the *CYP1B1* gene tested in African Americans and Dominicans on 3 MDIs with mother and newborn haplotypes separately, and 2 haplotypes of the *CYP1B1* gene tested in Caucasians on 3 MDIs with mother and newborn haplotypes separately). The analysis was conducted using the R package haplo.stats (Schaid, 2004).

Results

Descriptive Analysis

Demographic and exposure characteristics of mothers and their newborns are summarized in Table 1. The subset analyzed in this study is not significantly different from the total population with respect to the selected demographic and exposure characteristics. Prenatal PAH exposure did not differ significantly between NYC African Americans and Dominicans, but was more than 10-fold higher in Polish Caucasians, consistent with the higher levels of air pollution in Poland (Perera et al., 2003).

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, S2). 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 among the three ethnic groups.

Interactions between single genetic markers and PAH on MDI

Assuming a dominant genetic model (minor allele considered as the risk allele) and with a one degree of freedom (df) association test, we identified a number of significant interactions between maternal genetic markers and PAHs, as well as interactions between newborn genetic markers and PAHs, on MDIs at 12, 24, and 36 months in the three populations separately before adjusting for multiple comparisons with Bonferroni correction. However, little consistency was observed, and no single-marker-PAH interaction remains significant at the 0.05 significance level after Bonferroni correction. The results

highlighted in Table 2 are before Bonferroni correction, and thus are only suggestive. Only one significant gene-PAH interaction (before Bonferroni correction) at marker *GSTT2-02* was observed in both Dominicans and Caucasians on MDI at 24 months and in the same direction. The interaction suggests that MDI at 24 months is lower in newborns whose mothers have a genotype of TT/TG at *GSTT2-02* than those whose mothers have a genotype of GG within the low PAH exposure group, but is higher within the high PAH exposure group (interaction β=8.1, unadjusted-p=0.009, n=227 in Dominicans; interaction β= 7.96, unadjusted p=0.015, n=301 in Caucasians).

We found both maternal markers and newborn markers to interact with PAH on MDIs when there is no genetic main effect. For example, in Caucasians, maternal markers *CYP1A1-14*, *CYP1B1-05* and *CYP1B1-06* significantly interact with PAH on MDI at 12 months, but do not have main effects on MDI at 12 months; maternal markers *GSTT2-02* and *GSTT2-03* significantly interact with PAH on MDI at 24 months, but do not have main effects on MDI at 24 months. We also observed some genetic markers to have main effects on MDIs but do not interact with PAH on MDIs. For example, in Caucasians, maternal markers *CYP1A1-15* and *CYP1B1-66* have significant main effects but do not significantly interact with PAH on MDI at 12 months; newborn marker *CYP1B1-174* has significant main effects but does not significantly interact with PAH on MDI at 36 months. However, as gene-environment interactions are the focus of this study, we do not formally report the genetic main effects here but summarize the main effects of genetic markers in the Supplementary Materials (Supplementary Tables S4 and S5).

Interactions between haplotype and PAH on MDI

Although the analysis of LD structure and haplotype blocks (Supplementary Materials) suggested different haplotype block structures in the three populations, *CYP1A1* and *CYP1B1* were considered to have the same haplotype structure in the haplotype-PAH interaction analysis to facilitate the interpretation of the results. For *CYP1A2* and *GSTT2*, either completely different haplotype blocks were defined or no haplotype block was defined in the three populations. Thus, we did not consider haplotype-PAH interactions for these two genes. Note that the same reference haplotype was selected for all three populations for analysis of *CYP1A1* and *CYP1B1* to facilitate interpretation. Haplotypes frequencies of the *CYP1A1* and *CYP1B1* genes were summarized separately for the three populations (Supplementary Tables S6, S7). Significant effects of interactions between haplotypes of the *CYP1A1* gene (Table 3) or haplotypes of the *CYP1B1* gene (Table 4) and PAHs on MDIs at three years were observed after Bonferroni correction.

To summarize, for the *CYP1A1* gene (Table 3), we found that Caucasian newborn haplotypes significantly interact with PAHs on MDI at 2 year and 3 year after Bonferroni correction and in the same direction; Dominican maternal haplotypes significantly interact with PAHs on MDI at 2 year and 3 year after Bonferroni correction but in the opposite direction as the Caucasian newborn haplotype; African American maternal haplotypes and African American newborn haplotypes significantly interact with PAHs after Bonferroni correction and in the same direction but on different years' MDIs. Note that haplotype GATCTA, which is the most frequent haplotype in Dominican and Caucasian populations but not in African Americans was selected as the reference haplotype for all three populations. Some consistent results of haplotype-PAH interactions were also observed for the *CYP1B1* gene (Table 4). One consistent finding is the haplotype-PAH interactions at the gene *CYP1B1* in African Americans in all three years, where newborns whose mothers have haplotype ACCGGC of the *CYP1B1* gene have higher MDIs than newborns whose mothers have the reference haplotype ATGGCC within the low PAH group and the difference is significantly bigger in high PAH exposure group (with unadjusted p-values <.0001, =.0002, and <.0001 respectively, Figure 1). Note that haplotype ATGGCC, which is the most

frequent haplotype in African American and Dominican populations but not in Caucasians was selected as the reference haplotype for all three populations. Although we picked a common reference haplotype for all three populations to easy the interpretation, we would like to point out that some interesting results might be missed in African Americans for the *CYP1A1* gene, and some interesting results might be missed in Caucasians for the *CYP1B1* gene.

Discussion

Our results indicate that MDI at young ages can be modulated by common genetic variants in the key genes *CYP1A1*, *CYP1A2, CYP1B1*, *GSTT2,* and *GSTM1*. Interaction effects between single genetic markers (ht-SNPs) and PAHs were observed in African American, Dominican, and Caucasian mothers and their newborns before Bonferroni correction for multiple comparisons, with more gene-PAH interaction effects observed in African Americans and Dominicans than in Caucasians. However, little consistency was observed across ethnic groups and across different MDI ages. No single marker-PAH interaction remains significant after Bonferroni correction. The significant interactions between single genetic markers and PAHs in Table 2 are thus only suggestive.

Pairwise LD analysis suggests that the same haplotype block with six SNPs (*CYP1A1-78*, *CYP1A1-109*, *CYP1A1-06*, *CYP1A1-15*, *CYP1A1-14*, and *CYP1A1-83*) was defined in the three populations for the *CYP1A1* gene (Supplementary Figure S1), for which similar LD patterns were observed in both mothers and newborns. The LD structure of the *CYP1A2* gene and *CYP1B1* gene differed substantially in the three populations (Supplementary Figures S2 and S3), which is not surprising because of the differences in population genetic histories and because of the possible selection bias of the chosen SNPs based on the Caucasians in the Breast and Prostate Cancer Cohort Consortium (BPC3) (Hunter et al., 2005). No haplotype block was defined for the gene *GSTT2* in the three populations (Supplementary Figure S4). Despite different haplotype blocks defined in the three populations, *CYP1A1* and *CYP1B1* were considered to have the same haplotype structure in the haplotype-PAH interaction analysis to facilitate interpretation of the results.

We observed more consistent and stronger interaction effects between haplotypes and PAHs than between single markers and PAHs in African American, Dominican, and Caucasian mothers and their newborns. This is consistent with what has been observed by researchers. Clark (2004) in a review paper discussed the role of haplotypes in candidate gene studies and concluded that there were three possible reasons why the phased haplotype should be an improvement over single genetic markers. First, the folding and other properties of the polypeptide chains from the protein products of the candidate genes may depends on particular combination of amino acid. Second, variation in population is inherently structured into haplotypes. Third, the statistical power of association tests with phased haplotypes is likely to be improved because of the reduction in dimension. The consistent findings on interactions between *CYP1A1* and *CYP1B1* haplotypes and PAHs in all three populations may suggest that *CYP1A1* and *CYP1B1* are important effect modifiers for MDIs at early ages. Our previous study on DNA adducts (Wang et al, 2008) also suggested that *CYP1A1* and *CYP1B1* might be important effect modifiers in Caucasians. Savas et al. (1997) have found that *CYP1B1* might be an important contributor to activation of PAHs particularly in extra hepatic tissues that are susceptible to cancer where *CYP1B1* in contrast to *CYP1A1* is constitutively expressed. Notably, we observed that more interactions between maternal genetic markers/haplotypes and PAH had effects on MDIs than did interactions between newborn genetic markers/haplotypes and PAH. At this point, we have no compelling explanation for this difference, since in theory both maternal and fetal genotypes could be important in determining the metabolic fate and toxicity of PAH.

We note that the study examines a modest sample of subjects, which limited the study power in detecting gene-environment interactions. Moreover, the relationships observed for low-income minority women might be different for women of other races or ethnic, cultural, or socioeconomic backgrounds. Furthermore, it is possible that high PAH levels may be associated with living near an exposure source, such as a bus route or garage. This may lead to some confounding socioeconomic factors that were uncontrolled even within our low-income population. A strength of the study is the ability to explore gene-PAH interactions and haplotype-PAH interactions using ht-genetic-markers from genes known to be involved in metabolism and detoxification of PAHs and are relevant to multiple effects of the same pollutants, MDI and environmental monitoring. Another strength of the study is the examination of the possible confounding variables such as dietary PAH and maternal ETS which makes the effect of the prenatal PAHs in the air more accurate. More research efforts are needed to examine these relationships in replication studies. These studies should include fine mapping to identify variables whose causality can be tested by corroborative laboratory analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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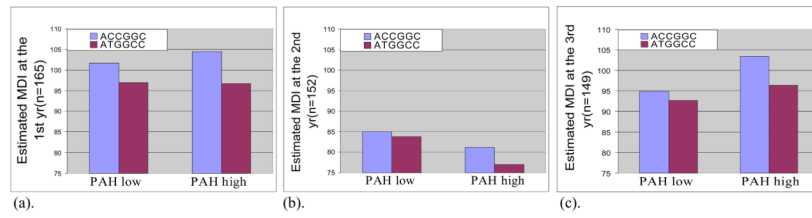
Figure 1.
Interaction effect between maternal haplotype ACCGGC of the *CYP1B1* gene and PAHs in African Americans on (a) MDI at 12 months; (b) MDI at 24 months; and (c) MDI at 36 months. More specifically, newborns whose mothers have haplotype ACCGGC have higher MDIs than newborns whose mother have haplotype ATGGCC within the low PAH exposure group and the difference is bigger in high PAH exposure group (interaction $\beta = 2.94$, unadjusted-p=<0.0001, n=165 on MDI at 12 months; interaction $\beta = 3.10$, unadjusted-p=0.0002, n=152 on MDI at 24 months; and interaction $\beta = 4.90$, unadjusted-p=<.0001, n=149 on MDI at 36 months).

Table 1

Exposure, biomarker and demographic characteristics of the study population that have maternal or baby genotyped.[*][†]

	AA[#]	D[#]	C[#]	P-value AA vs. D	P-value AA vs. C	P-value D vs. C
Prenatal PAHs in air (ng/m³)[a]	3.32 ± 3.50(n=166)	3.48 ± 4.10(n=255)	36.75 ± 46.19(n=328)	0.58[i]	<.001[i]	<.001[i]
Maternal age (year)	24.21 ± 4.98(n=166)	25.45 ± 5(n=260)	27.95 ± 3.72(n=345)	0.006[iv]	<.001[iv]	<.001[iv]
Dietary PAH²	46.62 ± 9.63(n=172)	39.29 ± 6.75(n=257)	42.54 ± 5.97(n=345)	<.001[i]	<.001[i]	<.001[i]
Maternal ETS[b]	49% (n=175)	27% (n=279)	23% (n=345)	<.001[iii]	<.001[iii]	0.25[iii]
Maternal BMI	27.13 ± 6.72(n=159)	25.03 ± 5.85(n=218)	21.41 ± 3.14(n=345)	0.003[i]	<.001[i]	<.001[i]
>=High school[c]	56% (n=178)	64% (n=281)	90% (n=345)	0.09[iii]	<.001[iii]	<.001[iii]
Gender[d]	57% (n=116)	56% (n=167)	49%(n=263)	0.87[iii]	0.15[iii]	0.16[iii]
MDI_12[e]	96.30 ± 10.03(n=111)	96.52 ± 9.58(n=147)	100.61 ± 9.95(n=284)	0.86[ii]	<.001[ii]	<.001[ii]
MDI_24[e]	88.53 ± 12.28(n=106)	84.46 ± 11.96(n=143)	101.04 ± 12.84(n=271)	0.009[ii]	<.001[ii]	<.001[ii]
MDI_36[e]	94.22 ± 11.91(n=93)	88.79 ± 10.38(n=138)	103.52 ± 10.58(n=255)	<.001[ii]	<.001[ii]	<.001[ii]

[*] Subjects included in the present analysis are those with genotype data in mothers (178 AA, 282 D, and 381 C) or in their newborns (116 AA, 167 D, and 294 C). Among those, there are 98 mother-baby pairs in AA, 138 mother-baby pairs in D, and 275 mother-baby pairs in C.

[†] No significant difference between the subset and the total population with respect to selected demographic and exposure characteristic.

[#] AA: African American; D: Dominican; C: Caucasian;

[a] mean± sd (n);

[b] Maternal ETS (Environmental Tobacco Smoke): percentage who report a smoker in household;

[c] >=High school: percent of mothers with >=12 years of education;

[d] Gender: percentage of female;

[e] MDI_12, MDI_24, MDI_36: mental development index at months 12, 24, and 36.

[i] two sample t test after natural log transformation;

[ii] two sample t test with original scale;

[iii] two sample z test;

[iv]Wilcoxon signed-rank test;

[2]Dietary PAH is measured through questionnaire on meat consumptions.

Table 2

Significant single-marker-PAH interactions on three year MDIs[†]

Genetic marker	AA				D				C			
	Minor allele(%)	β^a	P-value	N	Minor allele(%)	β^a	P-value	N	Minor allele(%)	β^a	P-value	N
Part I: Interaction of PAHs and *maternal genetic markers* on MDIs												
Interaction of PAHs and *maternal genetic markers* on MDI_12												
CYP1A1-14	T (14%)	2.57	0.49	164	T(36%)	−2.58	0.333	231	G(32%)	**−4.98**	**0.036**	316
CYP1A1-78	G (44%)	4.26	0.211	163	C(36%)	**5.5**	**0.038**	231	C(12%)	−2.76	0.32	316
CYP1A1-109	A(46%)	5.38	0.121	161	G(36%)	**6**	**0.021**	231	G(12%)	−2.58	0.356	315
CYP1B1-05	C(27%)	1.25	0.693	163	C(41%)	1.53	0.576	230	G(43%)	**−5.5**	**0.033**	315
CYP1B1-06	C(29%)	1.51	0.628	165	C(42%)	2.08	0.446	232	T(43%)	**−5.62**	**0.03**	313
GSTT2-03	A(6%)	**−9.6**	**0.046**	161	A(17%)	4.78	0.094	221	A(19%)	2.59	0.306	313
Interaction of PAHs and *maternal genetic markers* on MDI_24												
GSTT2-02	T(12%)	1.18	0.797	149	T(33%)	**8.1**	**0.009**	227	G(49%)	**7.96**	**0.015**	301
GSTT2-03	A(6%)	−11	0.091	148	A(17%)	2.85	0.42	217	A(19%)	**6.86**	**0.023**	298
Interaction of PAHs and *maternal genetic markers* on MDI_36 (none)												
Part II: Interaction of PAHs and *newborn genetic markers* on MDIs												
Interaction of PAHs and *newborn genetic marker* on MDI_12												
GSTM3-01	A(8%)	**12.5**	**0.041**	99	A (24%)	−4.39	0.195	123	A(31%)	−3.6	0.179	215
Interaction of PAHs and *newborn genetic marker* on MDI_24												
CYP1A1-83	G(49%)	**−15**	**0.02**	51	G(34%)	−2.38	0.633	93	G(15%)	1.05	0.824	131
CYP1B1-03	T(23%)	−4.6	0.34	92	T(20%)	**−9**	**0.038**	123	T(32%)	−0.5	0.889	227
GSTM3-01	A(8%)	3.91	0.63	95	A(24%)	**−9.3**	**0.021**	121	A(31%)	4.22	0.231	205
GSTT2-03	A(6%)	NA[*]	NA	NA	A(15%)	**10.5**	**0.029**	122	A(19%)	0.75	0.829	227
Interaction of PAHs and *newborn genetic marker* on MDI_36												
CYP1B1-66	T(18%)	1.75	0.747	90	T(14%)	3.44	0.407	123	T(19%)	**5.72**	**0.041**	217
CYP1A1-06	C(26%)	**10**	**0.035**	87	C(19%)	**−8.6**	**0.023**	124	C(9%)	−2.2	0.568	215
CYP1A1-15	T(37%)	6.27	0.196	90	T(25%)	**−7.8**	**0.029**	123	T(9%)	−2.4	0.521	217
CYP1A1-81	T(41%)	−6.2	0.21	89	T(43%)	**8**	**0.031**	125	T(33%)	−1.6	0.549	213

Genetic marker	AA				D				C			
	Minor allele(%)	β^a	P-value	N	Minor allele(%)	β^a	P-value	N	Minor allele(%)	β^a	P-value	N
CYP1A2-03	C(42%)	−5.3	0.294	88	C(42%)	**8.5**	**0.023**	123	C(33%)	−0.8	0.77	211
CYP1B1-03	T(23%)	−5.6	0.254	91	T(20%)	**−8.3**	**0.027**	122	T(32%)	1.3	0.641	215
GSTM3-01	A(8%)	**17.6**	**0.009**	84	A(24%)	−1.77	0.639	115	A(31%)	3.48	0.231	194
GSTT2-03	A(6%)	NA*	NA	NA	A(15%)	**10.4**	**0.011**	121	A(19%)	−4.2	0.137	216

[†]Unadjusted p-values are listed; the highlighted ones are the significant ones BEFORE Bonferroni correction (the Bonferroni adjusted significance level is 0.00013, which adjusted for number of single markers tested, number of populations tested, and number of outcomes tested, please refer to the test for more detail).

[a]Regression coefficients of the gene-PAH interactions from the linear regression models.

[*]No estimation can be found because of collinearity between genetic polymorphism and other covaraites in the model.

Table 3

Significant interactions between haplotypes of *CYP1A1* gene and PAHs on three year MDIs [*][†]

Part I: Interaction between PAHs and ***maternal*** haplotypes of *CYP1A1* gene on MDIs

Haplotype	AA			D			C		
	β^a	P-value	N	β^a	P-value	N	β^a	P-value	N
MDI_12									
CGCTGG	−2.60	0.20	165	4.42	0.00145	233	−3.49	<.0001	318
GATCGA	−1.35	0.46	165	2.12	0.16	233	−4.45	<.0001	318
CGTTGG	**−10.64**	<.0001	165	1.66	0.046	233	NA§	NA	NA
MDI_24									
CGCTGG	−1.24	0.53	152	**5.75**	<.0001	229	2.24	0.0041	303
MDI_36									
CGCTGG	−2.68	0.25	149	**3.57**	<.0001	225	−1.11	0.069	289
GATCGA	−3.23	0.15	149	**3.62**	<.0001	225	0.30	0.61	289
CGTTGG	**−7.51**	<.0001	149	1.89	0.053	149	NA	NA	NA
CGTCGG	−7.40	0.0021	149	**6.68**	<.0001	225	NA	NA	NA

Part II: Interaction between PAHs and ***newborn*** haplotypes of *CYP1A1* gene on MDIs

Haplotype	AA			D			C		
MDI_12									
CGTTGG	−7.67	0.038	106	**5.78**	<.0001	133	NA	NA	NA
CGTCGG	−0.31	0.91	106	**9.89**	<.0001	131	NA	NA	NA
MDI_24									
CGCTGG	2.00	0.50	101	−4.81	0.14	131	**−5.32**	<.0001	230
CGTTGG	**−6.90**	<.0001	101	−0.64	0.80	131	NA	NA	NA
MDI_36									
CGCTGG	11.95	0.00062	91	−5.29	0.08	126	**−4.24**	<.0001	219
CGTCGG	1.10	0.78	91	−1.06	0.54	126	**−4.17**	<.0001	219

[†] Unadjusted p-values are listed; the highlighted ones are the significant ones AFTER Bonferroni correction (the Bonferroni adjusted significance level is 0.00036, which adjusted for number of haplotypes tested, number of populations tested, and number of outcomes tested, please refer to the test for more detail).

[*] Analyses were restricted to haplotypes with frequencies greater or equal to 0.05; the reference haplotye is GATCTA.

[a] Regression coefficients of haplotype-PAH interactions from the linear regression models.

[§] Haplotype does not exist in Caucasian cohort.

Table 4

Significant interactions between PAHs and haplotypes of *CYP1B1* gene and PAHs on three year MDIs *†

Haplotype	AA			D			C		
	β^a	P-value	N	β^a	P-value	N	β^a	P-value	N
Part I: Interaction between PAHs and *maternal* haplotypes of *CYP1B1* gene on MDIs									
MDI_12				MDI_12			MDI_12		
TTGACC	**−4.41**	**<.0001**	165	−1.26	0.044	233	**−1.87**	**<.0001**	318
ATGGGT	−2.14	0.0025	165	**−8.6**	**<.0001**	233	NA§	NA	NA
ACCGGC	**2.94**	**<.0001**	165	NA§	NA	NA	NA§	NA	NA
MDI_24				MDI_24			MDI_24		
TTGACC	1.88	1	152	**4.95**	**<.0001**	229	**−4.50**	**<.0001**	303
ACCGGT	**−5.06**	**<.0001**	152	−0.30	0.73	229	0.93	0.16	303
ATGGGT	**−4.018**	**0.0001**	152	3.49	0.0001	229	NA§	NA	NA
ACCGGC	**3.10**	**0.0002**	152	NA§	NA	NA	NA§	NA	NA
MDI_36				MDI_36			MDI_36		
TTGACC	**−5.97**	**<.0001**	149	**5.69**	**<.0001**	225	1.23	0.0036	289
ACCGCC	NA§	NA	NA	**6.45**	**<.0001**	225	−1.06	0.017	289
ACCGGT	**5.53**	**<.0001**	149	−1.90	0.027	225	0.96	0.077	289
ATGGGC	**−7.13**	**<.0001**	149	**4.87**	**<.0001**	225	NA§	NA	NA
ATGGGT	**−6.90**	**<.0001**	149	−1.05	0.21	225	NA§	NA	NA
ACCGGC	**4.90**	**<.0001**	149	NA§	NA	NA	NA§	NA	NA
Part II: Interaction between PAHs and *newborn* haplotypes of gene on MDIs *CYP1B1*									
MDI_12				MDI_12			MDI_12		
ATGGGT	**−5.26**	**<.0001**	106	3.95	0.0012	133	NA§	NA	NA
MDI_24				MDI_24			MDI_24		
ATGGGT	**−8.30**	**<.0001**	101	**−8.94**	**<.00001**	131	NA§	NA	NA
MDI_36				MDI_36			MDI_36		
TTGACC	0.84	0.83	91	3.35	0.21	126	**5.56**	**<.0001**	219

	AA		D			C	
ATGGGT	−12.54	1	91	**−9.26**	**<.00001**	126	NA§	NA	NA

†Unadjusted p-values are listed; the highlighted ones are the significant ones AFTER Bonferroni correction (the Bonferroni adjusted significance level is 0.00036, which adjusted for number of haplotypes tested, number of populations tested, and number of outcomes tested, please refer to the test for more detail).

*Analyses were restricted to haplotypes with frequencies greater or equal to 0.05; the reference haplotye is ATGGCC.

^{a}Regression coefficients of haplotype-PAH interactions from the linear regression models.

§Haplotype does not exist in Caucasian cohort or haplotype frequency is less than 0.05 in African American and Dominican cohorts.