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Prenatal urinary polycyclic aromatic hydrocarbon metabolites, global DNA methylation in cord blood, and birth outcomes: A cohort study in China☆

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ABSTRACT

Background: Prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) is a potential risk factor for adverse birth outcomes. Epigenetic mechanisms may play a key role in which PAHs exert its effects.
Objective: Our study aimed to examine whether prenatal PAH exposure was associated with adverse birth outcomes and altered DNA methylation and to explore potential mediating roles of DNA methylation.
Methods: Ten urinary PAH metabolites were measured from 106 pregnant women during late pregnancy in a Chinese cohort study. Cord blood DNA methylation in long interspersed nucleotide element-1 (LINE-1) and Alu repetitive elements as surrogates of global DNA methylation was analyzed by bisulfite pyrosequencing. Multivariable linear regression was used to estimate the associations of urinary PAH metabolites with birth outcomes and DNA methylation, and a mediation analysis was also conducted.
Results: Prenatal urinary 2-hydroxynaphthalene (2-OHNa), ∑OHNa (sum of 1- and 2-OHNa), and sum of monohydroxy-PAH (∑OH-PAHs) were associated with lower birth length (e.g., −0.80%, 95% CI: −1.39%, −0.20% for the third vs. first tertile of 2-OHNa; p for trend = 0.01). Prenatal urinary 2-OHNa and 1-hydroxyphenanthrene (1-OHPh) were associated with lower Alu and LINE-1 methylation (e.g., −1.88%, 95% CI: −3.73%, −0.10% for the third vs. first tertile tertile of 2-OHNa in Alu methylation; p for trend = 0.04). Mediation analysis failed to show a mediator effect of global DNA methylation in the association between prenatal urinary OH-PAHs and birth outcomes.
Conclusions: Prenatal specific PAH exposures are associated with decreased birth length and global DNA methylation. However, global DNA methylation does not mediate the associations of prenatal PAH exposure with birth outcomes. Further studies are needed to confirm the results.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are the ubiquitous environmental chemicals that produced from incomplete combustion reactions of biomass and fossil fuels. General people can be exposed to PAHs via inhalation of contaminated air, ingestion of grilled and charred meats and contaminated food, or dermal absorption from the PAH-containing materials (Larsen and Baker,

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2003; Li et al., 2012; Wang et al., 2011). After exposure, PAHs can be rapidly biotransformed to monohydroxy metabolites and excreted in urine (Jacob and Seidel, 2002; Li et al., 2008). In human study, urinary monohydroxy-PAHs (OH-PAHs) are widely used as proxies of the internal doses to estimate individual PAH exposure (Ramesh et al., 2004). Concerns on human health risks of exposure to PAHs have been raised, as PAHs have been commonly identified to be carcinogenetic, endocrine disrupting property, as well as reproductive and developmental toxicity (Archibong et al., 2002; Bui et al., 1986; Kim et al., 2013; Sievers et al., 2013; Straif et al., 2005).

The developing fetus is known to be more susceptible to toxicological consequences of PAHs because of their physiologic immaturity, weak capability to detoxify toxic chemicals, and deficient immune-response function (Barr et al., 2007; Makri et al., 2004). Previous studies have shown that PAHs can cross through the placenta into the fetus (Wu et al., 2010) and that fetus is 10-fold more susceptible to PAH-induced DNA damage than mother (Perera et al., 2005). Furthermore, epidemiologic evidence reveals that PAH exposure during pregnancy is associated with adverse birth outcomes (Choi et al., 2006, 2008; Dejmek et al., 2000; Lamichhane et al., 2016), such as intrauterine growth retardation and preterm birth (Choi et al., 2008; Singh et al., 2008), as well as decreased birth weight, head circumference, and birth length (Choi et al., 2006; Jedrychowski et al., 2017; Lamichhane et al., 2016). However, the mechanisms by which PAHs exert its effects have not yet been fully characterized.

DNA methylation plays a vital functional role in epigenetic modification of the genome that involves regulation of genome stability and gene expression (Bernstein et al., 2007; Lee and Pausova, 2013). Over 80% of the CpG dinucleotides are methylated in the human genome. Transposable repetitive elements, as the heavily methylated regions, represent a large portion of human genome (Yang, 2004). Among them, Alu and long interspersed nucleotide element-1 (LINE-1) repetitive elements account for up to 50% of human genomic methylation. Measurements of Alu and LINE-1 methylation status have been served as surrogates of global genomic DNA methylation changes (Hoffmann and Schulz, 2005; Yang, 2004). Several studies have also suggested that changes of global DNA methylation may act a regulation in fetal growth and development (Guo et al., 2008; Maccani and Marsit, 2009; McMinn et al., 2006).

Several lines of evidence indicate that DNA methylation is highly vulnerable to environmental exposures (Hou et al., 2012; Kile et al., 2012; Pilsner et al., 2012). *In vivo* and *in vitro* studies have demonstrated that exposure to PAHs can change specific genes DNA methylation and global genomic DNA methylation (Fang et al., 2010; Sadikovic and Rodenhiser, 2006; Teneng et al., 2011; Wilson and Jones, 1983). Human studies have also suggested that PAH exposure is associated with altered global DNA methylation levels (Bollati et al., 2007; Herbstman et al., 2012; Kile et al., 2010; Pavanello et al., 2009). Nevertheless, few studies explored the effect of prenatal exposure to PAHs on global DNA methylation; the role of global DNA methylation between PAH exposure during pregnancy and birth outcomes is still unclear. To fill these gaps, we conducted an exploratory study to investigate 1) whether PAH exposure was associated with adverse birth outcomes; 2) whether PAH exposure was associated with altered Alu and LINE-1 methylation; 3) whether changes of Alu and LINE-1 methylation mediated the associations between PAH exposure and birth outcomes.

2. Methods and materials

2.1. Study population and data collection

Our study is part of an ongoing, longitudinal investigation exploring the effects of exposure to environmental chemicals on birth outcomes between July 2011 and July 2012 in China (Cao et al., 2016). Each participant provided the written informed consent. The research protocol was approved by the ethics committee of Tongji Medical College. In brief, pregnant women who sought for prenatal examination or waited for delivery during the third trimester of pregnancy (≥35 weeks) in the hospital were invited to participate in the study. Pregnant women who had resided in Wuhan at least 1 year, were ≥18 years of age, and had a single gestation viable fetus were eligible for the cohort study. A total of 997 non-smoking pregnant women were included in the cohort study. Of them, 799 subjects provided urine samples for analysis; 115 subjects had the measurements of DNA methylation (Yang et al., 2017a). Because there was insufficient urine volume for the PAH measurements, 106 pregnant women with both the DNA methylation and PAH data were ultimately retained in the current analysis. A flow diagram of study population was presented in Fig. 1. No significant differences were observed between the subjects included in the current analysis and the whole population in demographic characteristics except for weight gain during pregnancy and prenatal body mass index (BMI).

The trained investigators administered a face-to-face questionnaire to each participant. The questionnaire collected general characteristics (e.g., residential address, race/ethnicity, marital status, maternal age, maternal weight and height before pregnancy and delivery, and parity), socio-economic status (e.g., household income and education levels), life style (e.g., passive smoking status and water-use activities), as well as medical history and occupational exposure. Infant birth weight (grams) and birth length (centimeters) were measured using an electronic scale by the midwife in the delivery room. Gestational age (weeks) was based on the interval between the last menstrual period and the date of delivery of the baby.

2.2. Urine collection and PAH analysis

A first morning urine sample was collected with polypropylene cup from all participants when they admitted to the hospital and stored at −20 °C until analysis. Each urine sample was measured for a suite of OH-PAHs: 1-hydroxyphenanthrene (1-OHPh), 2-OHPh, 3-OHPh, 4-OHPh, 9-OHPh, 2-hydroxyfluorene (2-OHFlu), 9-OHFlu, 1-hydroxynaphthalene (1-OHNa), 2-OHNa, and 1-hydroxypyrene (1-OHP). The analysis method has been detailedly represented in our previous study (Yang et al., 2017b). In short, a 2.0-mL urine sample was spiked with β-glucuronidase/sulfatase to hydrolyse at 37 °C for 12 h. Then, liquid-liquid extraction was performed twice with n-hexane. The extracts were evaporated and derived with 100 μL of BSTFA at 45 °C for 90 min. Then, 1 μL of sample was injected on the gas chromatography mass spectrometry system. The average recoveries were in range of 77.67%−115.98%, and the coefficient of variance was less than 10.00%. The limits of detection (LODs) for ten PAH metabolites were in range of 0.03 μg/L to 0.18 μg/L. Urinary creatinine was measured to correct dilution according to our previous study (Yang et al., 2017c). The creatinine adjusted urinary OH-PAH concentrations were expressed as μg/g creatinine.

2.3. DNA methylation analysis

Umbilical cord blood specimens were collected at delivery and transported with ice cooler to the laboratory, then stored in −80 °C until DNA methylation analysis. DNA was isolated from leukocytes in cord blood and their methylation levels were measured by bisulfite pyrosequencing, as detailed in our previous study (Yang et al., 2017a). Briefly, DNA sample (500 ng) was conversed with bisulfite by EpiTect Bisulfite Kit, in which non-CpG cytosine residue