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Prenatal airborne polycyclic aromatic hydrocarbon exposure, *LINE1* methylation and child development in a Chinese cohort

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ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAH) are carcinogenic, neurotoxic environmental pollutants generated during incomplete combustion of fossil fuel and other organic material. PAH exposure has been associated with adverse fetal development and epigenetic alterations in cord blood. Several molecular epidemiology studies have established PAH-DNA adducts as biomarkers of PAH exposure.
Objectives: We investigated the relationship between *LINE1* DNA methylation and PAH-DNA adduct levels in cord blood, and with neurodevelopmental outcomes.
Methods: In Tongliang County, China, the current study enrolled two population-based cohorts of nonsmoking pregnant women before (2002) and after (2005) the closure of a local coal-fired power plant in May 2004. We analyzed cord blood samples collected from mothers in the two cohorts ($n = 110$ from 2002 cohort and $n = 107$ from 2005 cohort) for PAH-DNA adducts and genomic *LINE1* DNA methylation. Neurodevelopmental data on children were collected using the Gesell Developmental Scales (GDS) at age 2 and using the Wechsler Intelligence Scale for Children (WISC) at age 5.
Results: A significant inverse relationship was observed between PAH-DNA adducts and *LINE1* DNA methylation ($\beta = -0.010$, $p < 0.038$). A significant, positive association between *LINE1* methylation and scores on WISC full scale and verbal ($\beta = 85.31$, $p < 0.005$; $\beta = 94.36$, $p < 0.003$) but not on the GDS. Mediation analysis did not find *LINE1* to be a direct mediator between PAH-DNA adducts and IQ score.
Conclusion: LINE1 methylation in cord blood DNA was a positive predictor of IQ at age 5 and was decreased at higher levels of prenatal PAH exposure measured by PAH-DNA adducts in cord blood. However, the adverse effects of prenatal exposure to PAH on IQ scores did not appear to be directly mediated by altered *LINE1* methylation.

1. Introduction

PAHs are carcinogenic and neurotoxic environmental pollutants that are released due to incomplete combustion reactions. Exposure to PAH has been associated with genotoxic and epigenetic effects, changes in DNA methylation, and potentially, gene expression (Herbstman et al., 2012; Perera et al., 2009). PAH are metabolized to form phenolic products and reactive epoxides that bind covalently to DNA, forming PAH-DNA adducts (Whyatt et al., 1998; Wood et al., 1976). PAH-DNA adducts have been validated as biomarkers of PAH exposure that represent the biologically effective dose of PAH and are considered an indicator of increased risk of various cancers (Rybicki et al., 2004; Tang et al., 2013; Tang et al., 2001; Tang et al., 1995). PAH-DNA adducts reflect individual variation in exposure, absorption, metabolic activation and DNA repair; and their estimated half-life in total white blood cells is 3–4 months (Dipple, 1983; Mooney et al., 2005; Tang et al., 2008; Tang et al., 2001).

DNA methylation plays a key functional role in development, regulating X-chromosome inactivation, genomic imprinting, chromosome stability, and gene transcription. Methylation of the fifth carbon of cytosine is the most common site of methylation on the DNA and >80% of the CpG dinucleotides in the human genome are methylated (Breiling and Lyko, 2015). Transposable repetitive elements compose 50 to 70% of the mammalian genome and are the most heavily methylated regions (Yang et al., 2004). Long interspersed nuclear elements (*LINE1*) and *Alu* are the best characterized of the repetitive elements and are often used as a proxy for estimating global genomic DNA methylation changes (Hoffmann and Schulz, 2005; Ostertag and Kazazian, 2001). Upon demethylation, repeat elements containing coding regions may be

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expressed and can disrupt gene expression by transposing themselves across the genome (Medstrand et al., 2005). Several lines of evidence suggest that dysregulation of DNA methylation, such as loss of repeat element methylation, can be an early event in carcinogenesis and tumor progression (Brocato and Costa, 2013; Nishida et al., 2013). However, while the effect of aberrant DNA methylation in specific promoter regions have been associated with early cancer development (Dumitrescu, 2012), few studies have explored the role of loss of repeat element methylation in early development and any potential to be involved in neurotoxicity.

Exposure to particulate matter (PM), a component of air pollution, has been associated with decreased global methylation (Baccarelli et al., 2009). This may be due to metals such as lead and nickel on particulate matter, which interact with DNA methyltransferases to inhibit DNA methylation (Takiguchi et al., 2003). Other exposures associated with decreased DNA methylation include airborne PAH (Herbstman et al., 2012) and prenatal tobacco smoke (Breton et al., 2009). Fetal exposure to maternal smoking during pregnancy is associated with reduced methylation in specific sequences including *LINE1* (Flom et al., 2011), although maternal smoking was also associated with increased DNA methylation of other genes (Breton et al., 2009; Breton et al., 2011). In our previous study of women and newborns in New York City, newborns with detectable cord benzo[*a*]pyrene (B[*a*]P)-DNA adducts had higher levels of global genomic methylation than those with nondetectable adducts, measured using an assay to quantify the methylated fraction of DNA from umbilical cord leukocytes (Herbstman et al., 2012).

The Columbia Center for Children's Environmental Health (CCCEH), in collaboration with Chongqing Children's Hospital, conducted two successive prospective cohort studies in Tongliang, China, one in 2002 and the other in 2005. With the closure of a local coal fired power plant in 2004, data from participants were used to assess the impact of environmental exposure from the power plant before and after its closure.

We previously reported a significant inverse association between PAH exposure, measured by PAH-DNA adducts, and neurodevelopment in the Tongliang cohorts. Using the Gesell Developmental Scale (GDS), as a measure of neurodevelopment for ages 0 to 3 years, we found that developmental scores were inversely associated with PAH-DNA adducts (Perera et al., 2008; Tang et al., 2008). The Wechsler Intelligence Scale for Children (WISC), administered at age 5, was inversely associated with PAH-DNA adducts but not significantly. Lack of significance was attributed to insufficient power, given a small sample size, however, a significant interaction between PAH-DNA adducts and environmental tobacco smoke was seen on IQ scores (Perera et al., 2012), indicating an interaction between constituents of tobacco smoke, which include PAHs and other neurotoxicants, with PAH from other environmental sources on neurodevelopment. Results from this and other studies (Vishnevetsky et al., 2015), that found an association between PAH-DNA adducts and IQ at age 5, led us to investigate *LINE1* methylation in the cord blood of the same cohort of children. Here, we evaluated 1) the relationship between PAH-DNA adducts and *LINE1* methylation status; 2) the relationship between *LINE1* methylation and child neurodevelopment; and 3) assessed the role of *LINE1* methylation status as a potential mediator in the relationship between PAH-DNA adducts and childhood neurodevelopmental indices.

2. Methods

The overall approach for this investigation, including populations, design (methods for enrollment and follow up of the cohorts, methods of data collection and analysis), has been previously described (Tang et al., 2006).

2.1. Ethics statement

This study was approved by the Institutional Review Board of Columbia University and Chongqing Medical University. All subjects gave informed written consent by completing a form approved by both the Columbia University Institutional Review Board and Chongqing Medical University.

2.2. Study site

The city of Tongliang has a population of approximately 810,000 and is situated in a small basin approximately 3 km in diameter (Millman et al., 2008). A coal-fired power plant located south of the town center operated during the dry season from 1 December to 31 May each year to compensate for the insufficient hydroelectric power supply during that time period. This plant was the principal local source of air pollution in Tongliang. In 1995, nearly all domestic heating and cooking units had been converted to natural gas, and motor vehicles were not a major pollution source at the time of our research (Chow et al., 2006). In May 2004, the power plant was closed and replaced by the national grid system of electrical energy, supplied through the Three Gorges dam.

2.3. Study subjects

We enrolled 150 pregnant women and their newborns, born between 4 March 2002 and 19 June 2002 (the 2002 cohort) and 158 mother-newborn pairs born between 2 March 2005 and 23 May 2005 (the 2005 cohort). All women gave birth at four hospitals in Tongliang: the Tongliang County Hospital, the Traditional Chinese Medicine Hospital, the Tongliang Maternal Children's Health Hospital, and the Bachuan Hospital. The women were recruited when they checked in for delivery and were screened using a screening questionnaire. Eligibility criteria included current nonsmoking status, ≥20 years of age, and residence within 2.5 km of the Tongliang power plant. Every eligible woman screened agreed to enter the study and gave informed written consent. For analysis, only women and children with measures on all endpoints were included, making the cohort of women and their children included in this analysis 110 for 2002 and 107 for 2005. As reported previously, there were no significant differences between those included and left out of analysis (Tang et al., 2008). The demographic and exposure characteristics of the two cohorts are presented in Table 1.

2.4. Personal interview

A 45-min questionnaire was administered by a trained interviewer after delivery. The questionnaire elicited demographic information, lifetime residential history (location of birth and duration of residence), history of active and passive smoking (including number of household members who smoke), occupational exposure, medication use, alcohol consumption during each trimester of pregnancy, and consumption of PAH-containing meat (frequency of eating fried, broiled, or grilled meat during the last 2 weeks). Socioeconomic data related to income and education was also collected.

2.5. Biological sample collection and adduct analysis

Maternal blood (10 ml) was collected within 1 day postpartum and 40–60 ml of umbilical cord blood was collected at delivery. Samples were transported immediately to the field laboratory at the Tongliang County Hospital for processing. Blood samples, the buffy coat, packed red blood cells, and plasma were separated and stored at −70 °C. Details of laboratory methods for analyzing B[*a*]P-DNA adducts as a measure of PAH-DNA adducts have been described previously (Tang et al., 2008). The detection limit of PAH-DNA adducts was 0.25 adducts/10^8 nucleotides.