Environment International 98 (2017) 198–203

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Identifying sensitive windows for prenatal particulate air pollution exposure and mitochondrial DNA content in cord blood

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ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form 12 October 2016
Accepted 4 November 2016
Available online 11 November 2016

Keywords:
Particulate matter
Mitochondrial DNA
Distributive lag models
Prenatal exposure

ABSTRACT

Introduction: Changes in mitochondrial DNA (mtDNA) can serve as a marker of cumulative oxidative stress (OS) due to the mitochondria's unique genome and relative lack of repair systems. *In utero* particulate matter ≤2.5 μm ($PM_{2.5}$) exposure can enhance oxidative stress. Our objective was to identify sensitive windows to predict mtDNA damage experienced in the prenatal period due to $PM_{2.5}$ exposure using mtDNA content measured in cord blood.

Material and methods: Women affiliated with the Mexican social security system were recruited during pregnancy in the Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) study. Mothers with cord blood collected at delivery and complete covariate data were included (n = 456). Mothers' prenatal daily exposure to $PM_{2.5}$ was estimated using a satellite-based spatio-temporally resolved prediction model and place of residence during pregnancy. DNA was extracted from umbilical cord leukocytes. Quantitative real-time polymerase chain reaction (qPCR) was used to determine mtDNA content. A distributive lag regression model (DLM) incorporating weekly averages of daily $PM_{2.5}$ predictions was constructed to plot the association between exposure and OS over the length of pregnancy.

Results: In models that included child's sex, mother's age at delivery, prenatal environmental tobacco smoke exposure, birth year, maternal education, and assay batch, we found significant associations between higher $PM_{2.5}$ exposure during late pregnancy (35–40 weeks) and lower mtDNA content in cord blood.

Conclusions: Increased $PM_{2.5}$ during a specific prenatal window in the third trimester was associated with decreased mtDNA content suggesting heightened sensitivity to PM-induced OS during this life stage.

1. Introduction

Prenatal exposure to particulate matter <2.5 μm in diameter ($PM_{2.5}$) has been associated with a number of adverse fetal outcomes including reductions in birth weight and pre-term birth (Fleisch et al., 2015; Hyder et al., 2014; Lakshmanan et al., 2015; Lamichhane et al. 2015; Morello-Frosch et al., 2010). The underlying mechanism through which exposure to ambient air pollution leads to adverse fetal outcomes has not been completely elucidated, although oxidative stress (OS) is thought to play a central role (Slama et al., 2008). Because mitochondrial DNA (mtDNA) lacks protective histones and has diminished DNA

Abbreviations: DLM, distributive lag models; mitochondrial DNA, mtDNA; $PM_{2.5}$, particulate matter <2.5 μm in diameter; qPCR, quantitative real time polymerase chain reaction; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.envint.2016.11.007
0160-4120/

repair capacity compared to nuclear DNA, it is particularly prone to oxidative damage (Shaughnessy et al., 2014). In humans, mitochondria contain multiple copies of maternally-inherited double stranded, circular mitochondrial DNA (mtDNA). To maintain optimal physiological functions, mtDNA content (also referred to as mtDNA copy number) is kept within a relatively stable range. Mitochondria are both major intracellular sources of and primary targets of reactive oxygen species (ROS), and are especially susceptible to even small increases in systemic OS (Lee and Wei, 2000; Lee and Wei, 2005; Sinha et al., 2013). Mitochondria may respond to increased energy demands by increasing copy number (Lee et al., 2010; Lee and Wei, 2005). However, when compensatory mechanisms are overwhelmed, mtDNA content may decrease (Lee et al., 2010; Lee and Wei, 2005). Also, mtDNA mutations and their resulting biochemical defects accumulate over time. As such, altered mtDNA content may provide a record of past environmental exposures to pro-oxidant chemicals. Therefore, measurement of mtDNA content in cord blood may also serve to assess a particular vulnerable period in which the fetus is susceptible because of rapid development but also due to immature detoxifying enzyme systems (Wells et al., 2009).

Exposure to particulate matter has been associated with changes in mitochondrial DNA content in non-pregnant adults (Hou et al., 2010; Hou et al., 2013) as well as in cord blood and placenta reflecting *in utero* exposures (Clemente et al., 2015; Janssen et al., 2012). Measurement of this biomarker in cord blood may provide a source of integrated molecular information of fetal exposures over pregnancy. Studies are starting to emerge that link prenatal pro-oxidant environmental exposures to mtDNA content at birth. A study on prenatal smoking reported decreased placental mtDNA content related to increasing number of cigarettes smoked per day (Bouhours-Nouet et al., 2005). Other pro-oxidant exposures such as outdoor air pollutants (*e.g.*, particulate matter) have also been associated with changes in mtDNA content in the placenta (Clemente et al., 2015; Janssen et al., 2012). Moreover, there may be periods of time in which individuals are more sensitive to pro-oxidant exposure, such as periods of rapid growth when mitosis is highly active. Exposure to pro-oxidant chemicals during these life stages might induce a cellular response, such as reduced mtDNA copy numbers, that is more prominent than when exposure occurs at other times. For example, development occurs as a cascade of gene expression changes that vary at different life stages. Because the developmental processes and sets of genes expressed differ by developmental stage, potential clues to the underlying biological processes can be inferred simply by understanding what life stages are more sensitive to exposure.

We leveraged daily prenatal $PM_{2.5}$ measures available over pregnancy to more precisely identify sensitive windows in relation to mtDNA content measured in cord blood. We combined these approaches with distributive lag models (DLM) that allow us to statistically model and visualize the exposure timing-dependent pattern of associations. Because there is evidence to suggest sex-specific effects of air pollution exposure during pregnancy (Chiu et al., 2016; Hsu et al., 2015; Lakshmanan et al., 2015) we also examined sex stratified associations.

2. Material and methods

2.1. Study population

Pregnant women who were receiving prenatal care through the Mexican Social Security System (Instituto Mexicano del Seguro Social–IMSS) between July 2007 and February 2011 were recruited in the Programming Research in Obesity, Growth, Environment and Social Stressors (PROGRESS) study. The IMSS provides healthcare to affiliated private sector employees, the majority low- to middle-income workers and their families. Eligibility criteria were as follows: <20 weeks gestation, at least18 years old, planning to stay in Mexico City for the next 3 years, had access to a telephone, had no medical history of heart or kidney disease, did not consume alcohol daily, and did not use any steroid or anti-epilepsy medications. Procedures were approved by institutional review boards at the Harvard School of Public Health, Icahn School of Medicine at Mount Sinai, and the Mexican National Institute of Public Health. Women provided written informed consent.

2.2. Prenatal $PM_{2.5}$ levels

Our group has developed an improved satellite based method to estimate daily $PM_{2.5}$ levels across Mexico City for the years 2004–2014 (Just et al., 2015). Ultrasounds were not routinely performed as standard of care; therefore gestational age was based on last menstrual period (LMP) and by a standardized physical examination to determine gestational age at birth (Capurro et al., 1978). If the physical examination assessment of gestational age differed by >3 weeks from the gestational age based on LMP, the physical exam was used instead of the gestational age determined by LMP. Daily exposure to $PM_{2.5}$ were then estimated for each cohort participant during pregnancy (*i.e.*, individual-level exposure estimates) using a novel spatio-temporal model that incorporates Moderate Resolution Imaging Spectroradiometer (MODIS) satellite-derived Aerosol Optical Depth (AOD) measurements at a 1×1 km spatial resolution (Just et al., 2015). These remote sensing data are calibrated with municipal ground level monitors of $PM_{2.5}$, land use regression (LUR) variables, and meteorological data to yield estimates of daily residential $PM_{2.5}$ levels for each participant. The model was run using day-specific calibrations of AOD data calibrated against ground $PM_{2.5}$ measurements from 12 monitoring stations covering Mexico City and LUR and meteorological variables (roadway density, temperature, relative humidity, planetary boundary layer and daily precipitation). As in previous studies, mixed effect models with spatial and temporal predictors and day-specific random effects were used to account for temporal variations in the $PM_{2.5}$–AOD relationship. For days without AOD data, the model was fit with a seasonal smooth function of latitude and longitude and time-varying average incorporating local monitoring. Model performance was assessed using monitor-level leave one-out cross-validation; the model performed well with an R^2 of 0.724. Due to day to day variation, daily $PM_{2.5}$ measures were averaged into weekly measurements as in prior work (Chiu et al., 2016; Hsu et al., 2015). To compare the DLM approach to traditional windows, we also calculated the average PM over clinically defined trimesters (1st trimester: 1–13 weeks, 2nd trimester: 14–27 weeks, 3rd trimester: 28 weeks-delivery).

2.3. Mitochondrial DNA content

Venous umbilical cord blood was obtained at the time of delivery for 531 of the 948 infants born into the study. Most missing samples were due to births occurring late at night or in the very early morning hours or mothers not reporting the start of labor to the study workers. The first 260 whole blood samples were stored in PAXgene™ Blood DNA Tubes (PreAnalytiX GmbH, Hombrechtikon Switzerland) and extracted using a QIAamp DNA Blood Kit (QIAGEN). The DNA was then stored at −80 °C prior to analysis. The next 271 samples were extracted by conventional phenol–chloroform method after red cell lysis by a second laboratory. The second laboratory stored the DNA at 4 °C. Multiplex quantitative real-time polymerase chain reaction (qPCR) was used to determine mtDNA copy number/content. The copy number was calculated by simultaneously measuring the abundance of two gene targets - one specific to mtDNA 12S ribosomal RNA and one to nuclear DNA (nDNA) - and calculated as the ratio of the abundance of these two genes. The primers for qPCR analysis of mtDNA were: mtF805 5′-CCACGGGAAACAGCAGTGATT-3'and mtR927 5′-CTATTGACTTGGGTTAATCGTGTGA-3′. We used a commercial kit to quantify nDNA (TaqMan RNase P Control Reagents Kit, Applied Biosystems); because this is a commercial kit, the information on primers and probe are protected Quantitative real-time PCR was