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Prenatal particulate matter exposure and mitochondrial dysfunction at the maternal-fetal interface: Effect modification by maternal lifetime trauma and child sex

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2017.12.020>

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Abstract

Background—Prenatal ambient fine particulate matter (PM_{2.5}) and maternal chronic psychosocial stress have independently been linked to changes in mitochondrial DNA copy number (mtDNAcn), a marker of mitochondrial response and dysfunction. Further, overlapping research shows sex-specific effects of PM_{2.5} and stress on developmental outcomes. Interactions among PM_{2.5}, maternal stress, and child sex have not been examined in this context.

Methods—We examined associations among exposure to prenatal PM_{2.5}, maternal lifetime traumatic stressors, and mtDNAcn at birth in a sociodemographically diverse pregnancy cohort ($N = 167$). Mothers' daily exposure to PM_{2.5} over gestation was estimated using a satellite-based spatio-temporally resolved prediction model. Lifetime exposure to traumatic stressors was ascertained using the Life Stressor Checklist-Revised; exposure was categorized as high vs. low based on a median split. Quantitative real-time polymerase chain reaction (qPCR) was used to determine mtDNAcn in placenta and cord blood leukocytes. Bayesian Distributed Lag Interaction regression models (BDLIMs) were used to statistically model and visualize the PM_{2.5} timing-dependent pattern of associations with mtDNAcn and explore effect modification by maternal lifetime trauma and child sex.

Results—Increased PM_{2.5} exposure across pregnancy was associated with decreased mtDNAcn in cord blood (cumulative effect estimate = -0.78 ; 95% CI $-1.41, -0.16$). Higher maternal lifetime trauma was associated with reduced mtDNAcn in placenta ($\beta = -0.33$; 95% CI $-0.63, -0.02$). Among women reporting low trauma, increased PM_{2.5} exposure late in pregnancy (30–38 weeks gestation) was significantly associated with decreased mtDNAcn in placenta; no significant association was found in the high trauma group. BDLIMs identified a significant 3-way interaction between PM_{2.5}, maternal trauma, and child sex. Specifically, PM_{2.5} exposure between 25 and 40 weeks gestation was significantly associated with increased placental mtDNAcn among boys of mothers reporting high trauma. In contrast, PM_{2.5} exposure in this same window was significantly associated with decreased placental mtDNAcn among girls of mothers reporting low trauma. Similar 3-way interactive effects were observed in cord blood.

Conclusions—These results indicate that joint exposure to PM_{2.5} in late pregnancy and maternal lifetime trauma influence mtDNAcn at the maternal-fetal interface in a sex-specific manner. Additional studies will assist in understanding if the sex-specific patterns reflect distinct pathophysiological processes in addition to mitochondrial dysfunction.

Keywords

Mitochondria; Air pollution; Pregnancy; Psychosocial stress; Bioenergetics

1. Introduction

Gestational exposure to ambient fine particulate matter (PM_{2.5}) has been linked to correlates of chronic disease risk (e.g., low birth weight, preterm delivery) (Malmqvist et al., 2011; Lamichhane et al., 2015) and adverse child health outcomes (e.g., poorer cognition, asthma)

(Brunst et al., 2015; Basagana et al., 2016). Similarly, maternal psychosocial stress has been associated with preterm birth, low birth weight, and adverse child respiratory and neurodevelopmental outcomes (Henrichs et al., 2010; van de Loo et al., 2016; Grote et al., 2010; Seng et al., 2011; Hohwu et al., 2014; Scheinost et al., 2016). Maternal trauma, in particular, has been shown to have a lasting impact on fetal development even if occurring remote from pregnancy (Sternthal et al., 2009). While mechanisms involved in the toxicity of PM and maternal psychosocial stress/trauma are complex, evolving epidemiological and biological evidence suggests both exposures trigger a cellular stress response and may result in oxidative damage (Romieu et al., 2008; Traboulsi et al., 2017; Yang et al., 2017; Colaïanna et al., 2013; Jorgensen, 2013; Aschbacher et al., 2013; Irie et al., 2000; Gidron et al., 2006).

Mitochondria facilitate cellular energy delivery through the production of adenosine-5'-triphosphate (ATP) via oxidative phosphorylation. Mitochondrial function is critical to maintaining appropriate energy supply (i.e., ATP), cell functions/signaling, and fetal vitality. Cells contain numerous mitochondria, each containing multiple copies of mitochondrial DNA (mtDNA). The number of mtDNA copies in a cell can be used as a biomarker of mitochondrial response and dysfunction in the presence of oxidative damage. Changes in energy demands can trigger or reduce mitochondrial biogenesis, hallmarked by increases or decreases in mtDNA level, respectively (Shaughnessy et al., 2014; Carelli et al., 2015; Meyer et al., 2017). Thus, the process of regulating mtDNAcn is very dynamic and little is known about other potential upstream regulators of mtDNAcn (Shaughnessy et al., 2014; Carelli et al., 2015).

Biomarkers of mitochondrial dysfunction at the maternal-fetal interface that correlate with in utero environmental exposures can provide insight into the underlying involvement of mitochondrial bioenergetics in chronic disease programming. Changes in mtDNAcn as a result of prenatal PM have been observed in both umbilical cord blood (a marker more accurately reflecting the state of the fetus) and placenta (a key regulator of the external environmental and maternal fetal signaling (Nugent and Bale, 2015)) with some studies observing sex-specific effects (Clemente et al., 2016; Janssen et al., 2012; Rosa et al., 2017a). Further, emerging data suggest that timing of PM exposure during pregnancy may be a key factor in triggering a mitochondrial response. Recent studies demonstrate that increased exposure to PM_{2.5} during the third trimester (35–40 weeks gestation) of pregnancy was associated with decreased mtDNAcn in cord blood (Janssen et al., 2012; Rosa et al., 2017a). Studies examining associations between stress and stress correlates (e.g., psychological functioning) and mtDNAcn in maternal-fetal biomarkers remain sparse and have shown conflicting results (Wang et al., 2017; Brunst et al., 2017). Moreover, while ambient PM_{2.5} and maternal psychosocial stress can have synergistic effects on developmental outcomes (Islam et al., 2011; Shankardass et al., 2009; Rosa et al., 2017b), this is the first study to examine the combined effects of in utero PM_{2.5} and maternal psychosocial stress exposure on mtDNAcn assessed at birth.

We leveraged daily prenatal PM_{2.5} exposure estimates over pregnancy in the PProgramming of Intergenerational Stress Mechanisms (PRISM) study and implemented Bayesian Distributed Lag Interaction models (BDLIMs) (Wilson et al., 2017a) to statistically examine

and visualize the PM_{2.5} time-dependent pattern of associations with mtDNA_{cn} in placenta and cord blood. These models also allowed for the assessment of interactive effects with maternal lifetime trauma and child sex.

2. Methods

2.1. Sample

Between March 2011 and August 2012, $N = 167$ women were recruited (26.9 ± 8.1 weeks gestation) from prenatal clinics at the Beth Israel Deaconess Medical Center (BIDMC). Eligibility criteria included: (a) English- or Spanish-speaking; (b) age ≥ 18 years at enrollment; and (c) singleton pregnancies. Mothers who endorsed drinking ≥ 7 alcoholic drinks/week prior to pregnancy or any alcohol following pregnancy recognition were excluded given prior association with child health problems of interest to the study (Testa et al., 2003; Patra et al., 2011). Procedures were approved by the relevant institutions' human studies committees; written consent was obtained in the participant's primary language.

2.2. Placenta and cord blood collection

Among the 167 women, 147 cord blood samples were collected prior to clotting and 162 provided acceptable placenta samples collected within 30–60 min after birth [72% ($n = 121$) of participants had both placenta and cord blood samples collected]. Placenta samples ($\sim 1\text{--}2\text{ cm}^3$) were taken on the fetal side approximately 4 cm from the cord insertion site in four quadrants, taking care to avoid large vessels as per a published protocol (De Carli et al., 2017). The deciduas and fetal membranes were removed, the sample was rinsed in a cold PBS bath, cut into smaller pieces ($\sim 0.1\text{ cm}^3$), and placed into 1 ml of RNA_{later}TM RNA Stabilization Reagent (Qiagen). Samples in RNA_{later} were placed at -4°C for 24 h; excess RNA_{later} was then removed and samples were placed at -80°C until DNA extraction. Cord blood samples were collected at delivery in EDTA-tubes, centrifuged to obtain buffy coat fraction, and stored at -20°C until DNA extraction.

2.3. Mitochondrial DNA copy number

Placenta and cord blood DNA extraction was conducted using the Maxwell 16 automated DNA extraction system (Promega – Madison, WI, USA). Relative mtDNA_{cn} was measured using quantitative real-time-PCR (Andreu et al., 2009) which simultaneously measured the abundance of two gene targets- a mitochondrial gene (mt 12S) and a nuclear gene (RNAse P). Relative mtDNA_{cn} was calculated as the ratio of abundance of these two genes (Zhong et al., 2016). Samples were run in triplicate and averaged; the CV was 6% and the interplate variation was 3%.

2.4. Maternal lifetime trauma exposure

Our group has previously shown that increased maternal lifetime stress/trauma exposure, as compared with a measure of current life events in pregnancy, was a better predictor of decreased mtDNA_{cn} in placenta (Brunst et al., 2017). Given this finding, we chose to look at the effects of exposure to maternal lifetime trauma on mitochondrial function in placenta and cord blood. Within two weeks of enrollment, women completed the Life Stressor Checklist-Revised (LSC-R) which is available in English and Spanish and has established

reliability and validity in a diverse population of women (McHugo et al., 2005; Wolfe and Kimerling, 1997). The LSC-R assesses exposure to 30 potentially traumatic events with a special focus on events relevant to women (e.g. sexual assault, abortion, interpersonal violence). For each endorsed event, women were asked whether she thought that she or someone close to her could have been seriously harmed or killed during the event; thus accounting for whether the events met the Diagnostic and Statistical Manual of Mental Disorders—5th Edition (DSM-V) posttraumatic stress disorder (PTSD) Criterion A for classifying an event as traumatic (Association AP, 2013). A maternal lifetime trauma score was derived from the number of events for which the mother endorsed and reported the potential for serious harm to herself or someone close to her. Mothers were then categorized into high trauma and low trauma groups based on the median value of the maternal lifetime trauma score (i.e., 2 vs. 0–1).

2.5. Prenatal ambient fine particulate matter exposure

Geocoding of residential address history was conducted by a Geographic Information Systems specialist using Arc Geographic Information Systems (ArcGIS). Records of relocation were documented and geocoding was done for all address histories if a participant changed location. The daily PM_{2.5} estimate, for participants that moved, was based on location and time at each address. To validate our geocoding we randomly sampled 15% of the data we geocoded and evaluated accuracy by visually examining the locations on a map using established map services like the Environmental Systems Research Institute (ESRI) ArcGIS street datasets. Initial completeness was approximately 90% and the erroneous few addresses were then geocoded manually. Daily PM_{2.5} levels were estimated using an exposure model assessing temporally- and spatially-resolved PM_{2.5} exposures, as detailed previously (Kloog et al., 2014). Moderate Resolution Imaging Spectroradiometer (MODIS) satellite-derived Aerosol Optical Depth (AOD) measurements based on the Multi-Angle Implementation of Atmospheric Correction (MAIAC) algorithm were used at a 1 km × 1 km grid spatial resolution to predict daily PM_{2.5} levels across New England. For each study participant, the hybrid model combined remote sensing data with spatio-temporal predictors that represent within-grid variation to yield residence-specific estimates of PM_{2.5} exposures. The model was run using day-specific calibrations of AOD data using ground PM_{2.5} measurements from 161 monitoring stations and land use regression (LUR) (e.g., traffic density, point sources, etc.) and meteorological (e.g., temperature, wind speed, visibility, elevation, distance to major roads, percent open space, point emissions and area emissions) variables using mixed models with day-specific random intercepts, and fixed and random AOD and temperature slopes. Generalized additive mixed models were used to estimate exposures on days when AOD measures were not available (due to cloud coverage, snow, etc.). The residuals from the final model for each monitor then were regressed against the local spatial and temporal variables at each monitoring site to derive 200 m localized predictions. The mean cross validation R² for daily values was 0.88. To reduce noise from the day-to-day PM_{2.5} variation, weekly PM_{2.5} levels were calculated by averaging daily PM_{2.5} levels over each week of pregnancy. Given the interquartile range of PM_{2.5} averaged across pregnancy was 0.9 µg/m³, results are presented for a 1 µg/m³ increase in PM_{2.5}.

2.6. Potential covariates

Initial covariate selection was based on published literature. Maternal age (in years), race (White, Black/Haitian, Hispanic, Other/multi-race), maternal smoking during pregnancy (yes/no) and education status (high school degree, some college or college degree) were ascertained at enrollment by questionnaire, and child sex was reported postnatally. Gestational age was also considered as a potential covariate.

2.7. Data analysis

The analytic sample sizes, for placenta and cord blood analyses, included those with cord blood and/or placenta samples who also had data on prenatal PM_{2.5} and maternal lifetime traumatic stress. This resulted in an analytic sample size for placenta analyses of $m = 140$ and $n = 126$ for cord blood analyses. The analytic samples did not differ from the total sample recruited ($N = 167$) based on key covariates including maternal age, race, and education or child sex (Table S1).

First, linear regression was used to assess the relationship between maternal lifetime trauma exposure and mtDNAcn in placenta and cord blood. Effect modification by child sex was tested by including a cross-product term in each model predicting mtDNAcn in cord blood and placenta.

To examine associations between prenatal PM_{2.5} and cord blood/placenta mtDNAcn and modification by maternal lifetime trauma exposure and child sex, we used Bayesian distributed lag interaction models (BDLIMs). BDLIMs extend the traditional constrained distributed lag model (DLM) framework that identifies critical windows (Hsu et al., 2015a) as it can detect effect modifications (Wilson et al., 2017b). The BDLIMs were first implemented to examine the main effect of PM_{2.5} exposure over pregnancy on mtDNAcn in placenta and cord blood. We next examined effect modification by maternal lifetime trauma exposure and child sex. In addition to critical windows, BDLIMs can estimate the cumulative effect of PM_{2.5} exposure over pregnancy and for each trauma-sex combination, accounting for critical windows and within-window effects. BDLIM partitions the distributed lag function into two components: 1) the weights that identify critical windows of susceptibility, and 2) the coefficients that identify the magnitude of the within-window effects. For example, each maternal lifetime trauma and/or child sex combination can have either the same, or different, sensitive windows (weights) and within-window effects (magnitude of effects) which are not allowed in the standard DLM framework (Wilson et al., 2017b). When the weights are constant over time, the BDLIM is equivalent to a model with mean exposure interacted with maternal lifetime trauma group and/or child sex. When the weights vary by time, the model identifies time periods with greater weight (i.e., potential sensitive windows) that will graphically appear as a bump during which exposure is significantly associated with the mtDNAcn outcome. The model quantifies the likelihood of each pattern of heterogeneity and estimates the association between exposure and outcome under the effect modification pattern that is best supported by the data. Normalized posterior densities are interpreted as a probability for determining which model (i.e., the pattern of effect modification) best fits the data.

While being able to examine the interaction, the BDLIM incorporates the data from all exposure time points simultaneously and assumes that the association between the outcome and exposure varies smoothly as a function of time while controlling for exposure at all other time points. Including exposures at all time-points in the analysis, as done here, has been shown to reduce bias compared to selecting an arbitrary window (e.g., a single trimester analysis) (Wilson et al., 2017b).

All models were adjusted for maternal characteristics including education, age, and race/ethnicity; models not examining effect modification by child sex also included sex as a covariate. Models predicting mtDNAcn in cord blood were also adjusted for estimated proportions of nucleated red blood cells, granulocytes, monocytes, natural killer cells, B cells, CD4(+)T cells, and CD8(+)T cells (Bakulski et al., 2016). The inclusion of maternal smoking during pregnancy and gestational age into the main effect models did not significantly alter the results. Thus maternal smoking during pregnancy and gestational age were excluded from the final models to avoid overfitting the regression models.

3. Results

3.1. Descriptive statistics

Maternal and child characteristics for the analytic samples are presented in Table 1 and are compared to the recruitment sample size ($N = 167$) in the supplement (Table S1). Women were primarily racial/ethnic minorities (Black/Haitian, Hispanic, or multi-racial) and had greater than a high school degree. The prevalence of smoking during pregnancy was 15%. Nearly all children were born full term (92%).

3.2. Main effects of maternal trauma and child sex interactions using linear regression

Using linear regression, adjusting for maternal age, education, race/ethnicity, maternal lifetime trauma, child sex, and cell types (the latter was considered for cord blood analyses only), maternal lifetime trauma exposure was significantly associated with reduced mtDNAcn in placenta ($\beta = -0.33$; 95%CI $-0.63, -0.02$) but not cord blood ($\beta = 0.09$; 95%CI $-1.16, 1.35$). We did not observe differences based on child sex in placenta ($P_{\text{trauma} * \text{sex}} = 0.83$) or cord blood ($P_{\text{trauma} * \text{sex}} = 0.52$).

3.3. Main effects of prenatal PM_{2.5} and child sex interactions using BDLIM

The time-varying associations per $\mu\text{g}/\text{m}^3$ increase in prenatal PM_{2.5} and mtDNAcn placenta and cord blood were examined in separate BDLIMs, adjusting for maternal age, education, race/ethnicity, maternal lifetime trauma, child sex, and cell types (the latter was considered for cord blood analyses only). In the overall analysis, the BDLIM identified a statistically significant cumulative effect of increased PM_{2.5} exposure across pregnancy on decreased mtDNAcn in cord blood (cumulative effect estimate = -0.78 ; 95%CI $-1.41, -0.16$). The cumulative effect is the expected change in mtDNAcn associated with a one $\mu\text{g}/\text{m}^3$ increase in PM_{2.5} at every time point (i.e., week) in pregnancy. While in the same direction, the cumulative effect of PM_{2.5} on placental mtDNAcn did not reach significance (cumulative effect estimate = -0.07 ; 95%CI $-0.23, 0.04$) (Fig. 1). BDLIMs did not identify significant

two-way interactions between prenatal PM_{2.5} and child sex in relation to mtDNAcn in cord blood or placenta (Table S2, Fig. S1).

3.4. Effect modification by maternal lifetime trauma using BDLIM

The BDLIM did suggest an interaction between PM_{2.5} and maternal lifetime trauma exposure on mtDNAcn in placenta (Fig. 2). Specifically, a statistically significant window for the effect of increased PM_{2.5} exposure during 30–38 weeks gestation on decreased mtDNAcn in placenta was observed among women exposed to low trauma; no statistically significant window was found in the high trauma group (Fig. 2). The estimated cumulative effect over the entire pregnancy was significantly negative for the low maternal trauma group (cumulative effect estimate = −0.16; 95% CI −0.39, −0.004) and remained nonsignificant for the high maternal trauma group (cumulative effect estimate = 0.03; 95% CI −0.16, 0.23). The BDLIM did not detect a significant interaction between PM_{2.5} exposure and maternal lifetime trauma in analyses of cord blood mtDNAcn (Table S2).

3.5. Effect modification by maternal lifetime trauma and child sex on placenta mtDNAcn using BDLIM

In models predicting mtDNAcn in placenta (Fig. 3), the BDLIM detected a significant 3-way interaction identifying a similar sensitive window (25–40 weeks gestation) for boys and girls but with varying magnitude/direction of effects of prenatal PM_{2.5} exposure. Specifically, we observed a significant positive association between prenatal PM_{2.5} exposure at 25–40 weeks gestation and placental mtDNAcn among boys of mothers reporting high trauma, but a significant negative association at the same window among girls of mothers reporting low trauma (Fig. 3). The combined cumulative effect of time-varying prenatal PM_{2.5} exposure and maternal exposure to high trauma on increased placental mtDNAcn was significant in boys (cumulative effect estimate = 0.21; 95%CI 0.03, 0.37), but not statistically significant in girls (cumulative effect estimate = −0.06; 95%CI −0.33, 0.18). On the other hand, the estimated cumulative effect of prenatal PM_{2.5} exposure was significantly associated with reduced placental mtDNAcn among girls born to mothers with low trauma (cumulative effect estimate = −0.31; 95% CI −0.50, −0.06); the effect estimate was in the same direction among boys born to mothers with low trauma although it did not quite reach statistical significance (cumulative effect estimate = −0.10; 95%CI −0.20, 0.01).

3.6. Effect modification by maternal lifetime trauma and child sex on cord blood mtDNAcn using BDLIM

Similar 3-way interactive effects were observed in the cord blood model (Fig. 4). The time-varying association between prenatal PM_{2.5} and cord blood mtDNAcn was overall positive with a significant window at 32–37 weeks gestation among boys born to mothers experiencing high trauma, whereas prenatal PM_{2.5} was overall associated with reduced cord blood mtDNAcn with a significant window at 32–37 weeks gestation among girls born to mothers with low trauma. The cumulative effect estimate over the pregnancy was also significantly positive among boys born to mothers with high trauma (cumulative effect estimate = 1.94; 95%CI 0.27, 3.41), whereas it was significantly negative among girls born to mothers reporting low trauma (cumulative effect estimate = −1.50; 95%CI −2.54, −0.38). We did not observe any statistically significant window or cumulative effect over the

pregnancy among boys born to mothers reporting low trauma or girls born to mothers reporting high trauma.

All normalized posterior densities (i.e., model probabilities) for BDLIMs can be found in Table S2 of the online data supplement.

4. Discussion

The goal of this study was to determine the effect of prenatal exposure to fine particulate matter and maternal lifetime exposure to psychosocial trauma on placenta and cord blood mtDNAcn, a marker of mitochondrial response and dysfunction. Our findings suggest that 1) there is an overall effect of prenatal PM_{2.5} exposure on decreased mtDNAcn in cord blood, 2) there is an overall effect of maternal trauma exposure on decreased placenta mtDNAcn, 3) prenatal exposure to PM_{2.5} during a specific window in late pregnancy (30–38 weeks) is associated with lower mtDNAcn in placenta among women reporting low trauma exposure, and 4) the magnitude and direction of the combined effect (i.e., PM_{2.5} × maternal trauma interaction) varies by child sex. Specifically, a positive association between prenatal PM_{2.5} exposure and placental mtDNAcn was observed among boys of mothers reporting high lifetime trauma. In contrast, a negative association between PM_{2.5} and placental mtDNAcn was observed among girls of mothers reporting low trauma. Similar patterns were observed in analyses of cord blood.

Many studies have reported associations between ambient air pollution and mtDNAcn; however, the direction of effects has varied. Generally speaking, the mitochondria's response to short term (days) or low dose exposures is to produce more copies of the mitochondrial genome (i.e., biogenesis) in an attempt to repair or dilute the damaged mtDNA; this process can be overwhelmed by higher doses of exposure or longer durations of exposure. However, little is known about the process of regulating mtDNAcn in response to ambient air pollution. With this in mind, there are likely many factors contributing to the varying effects observed across studies. For instance, in a cross-sectional study of occupational workers, exposure to particulate matter (PM₁₀ and PM₁) and low-dose benzene was associated with higher mtDNAcn in blood (Hou et al., 2010; Carugno et al., 2012); however, in a repeated measure analysis of exposure to PM or elemental carbon, researchers observed increases in exposure to be associated with lower mtDNAcn (Hou et al., 2013). Similarly, long-term PM_{2.5} exposure was associated with decreased mtDNAcn in the Normative Aging Study (Nwanaji-Enwerem et al., 2017). Only two studies have investigated the impact of prenatal exposure to PM on mtDNAcn in cord blood (Janssen et al., 2012; Rosa et al., 2017a); one of these studies also reported results in placenta (Janssen et al., 2012). Janssen et al. observed PM₁₀ exposure during the third trimester of pregnancy to be associated with lower levels of mtDNAcn in placenta but not cord blood (Janssen et al., 2012). The second study, utilizing a traditional distributed lag model, also demonstrated that prenatal exposure to PM_{2.5} during a specific window in late pregnancy (35–40 weeks gestation) was associated with lower mtDNAcn in cord blood, particularly among boys (Rosa et al., 2017a). Despite using a similar statistical approach (e.g., DLM vs. BDLIM), we did not identify a significant window of PM_{2.5} exposure nor effect modification by child sex on mtDNAcn in cord blood. We did, however, observe an overall cumulative effect of higher PM_{2.5} during pregnancy and

decreased mtDNAcn in cord blood but not placenta. It is worth noting that racial differences in placental mtDNA copy number have been observed in studies of other exposures capable of triggering a mitochondrial response such as chronic maternal lifetime stress (Brunst et al., 2017). Thus, it is possible the differences in population demographics among the three cohorts (i.e., mostly Europeans of higher SES vs. Mexican women of lower SES vs. multi-ethnic U.S. population with mixed SES backgrounds) as well as differences in level and timing of exposure contributed to the lack of replication of the independent effects of PM_{2.5} on cord blood and placenta mtDNAcn.

We corroborate a previous analysis using different methods (Brunst et al., 2017) showing that mothers reporting more traumatic events over their lifecourse had lower placental mtDNAcn. This finding supports the notion that psychosocial stress experienced in childhood can impact mitochondrial biogenesis in adulthood (Tyrka et al., 2016). In addition, we observed a significant interaction between prenatal PM_{2.5} and maternal lifetime trauma. Specifically, we found that increased PM_{2.5} exposure late in pregnancy (30–38 weeks gestation) was associated with decreased mtDNAcn in placenta among women exposed to fewer traumatic events. This finding was unexpected, as we had hypothesized that the effect of PM_{2.5} would be more robust (i.e. more negative) among those women exposed to more traumatic events, but the opposite was observed. In addition, we did not observe the interaction between PM_{2.5} and trauma in cord blood where we observed the significant main effect of PM_{2.5} on mtDNAcn. While the relationship is likely complex, there are a few reasons that may account for these differences. First, it may be the case that additional exposure to prenatal PM_{2.5} does not lead to further reduction in biogenesis or more mitochondrial dysfunction in an already compromised fetal environment (i.e. a maternal history of lifetime trauma). Similar scenarios have been observed when examining environmental tobacco smoke exposure as an effect modifier in air pollution studies investigating fetal growth and child asthma outcomes (Clemens et al., 2017; Rabinovitch et al., 2011). A second possibility is that the effects of maternal lifetime trauma on mtDNAcn are not linear and vary over pregnancy. For instance, Bersani et al. report an inverted U-shaped relationship between combat PTSD symptom severity and mtDNAcn in blood (Bersani et al., 2016). However, there was no evidence of a curvilinear relationship in our study (i.e., a non-significant quadratic term in the models). Third, perhaps the placenta is more susceptible to mtDNAcn changes occurring due to psychosocial stress than to PM_{2.5} exposure and/or different biological response mechanisms are triggered for each exposure. For example, increased placental exposure to glucocorticoids, hormones that produce an array of effects in response to psychosocial stress, has been shown to impact the pro-oxidant/antioxidant balance of the placenta (Stark et al., 2011). On the other hand, PM_{2.5} exposure during preconception and pregnancy has been associated with intrauterine inflammation, based partially on placental pathology (Nachman et al., 2016). Thus, it is possible that altered mtDNAcn resulting from combined exposure to prenatal PM_{2.5} and maternal lifetime trauma may reflect differing pathophysiological responses that have different consequences on mitochondrial function. Lastly, it is possible that the combined effect of PM and high trauma is triggering rather than inhibiting mitochondrial biogenesis. Thus, since the independent effect of PM is negative in cord blood and showing trends of negativity in placenta, it would appear as though there was no effect in the high trauma group upon

examining the interaction. Although not significant, we do see a shift towards increased mtDNAcn in the high trauma group compared to the low trauma group (Fig. 2).

This study also reports that the combined effects of prenatal PM_{2.5} and maternal lifetime trauma exposure on mtDNAcn vary by child sex in magnitude and direction but within the same sensitive window. We observed a negative association between PM_{2.5} exposure in the second half of pregnancy and mtDNAcn in both tissues (i.e., placenta and cord blood) among girls of mothers reporting low lifetime trauma exposure; conversely, a positive association at the same critical window was observed among boys of mothers reporting high trauma. Sex differences of mitochondrial biogenesis in response to various environmental stimuli have been reported. Hypoxic conditions have been shown to induce sex-specific effects on mitochondrial biogenesis in mice, with males exhibiting increased mtDNAcn and opposing differences with respect to the expression of key genes associated with mitochondrial biogenesis compared to females (i.e., male mice showed increased expression, and female mice showed reduced or no change in expression) (Sharma et al., 2014). Placental mitochondrial biogenesis also appears to be influenced by fetal sex among diabetic mothers, potentially explaining the enhanced risk for future metabolic diseases in males (Jiang et al., 2017). Interestingly, these differences have been reported to start at a very young age leading to differences in reactive oxygen species (ROS) homeostasis in fundamental organs such as the heart and brain (Khalifa et al., 2017). It may also be helpful to turn to the cancer literature to help explain sex-specific effects. Similar to cancer cells, the placenta relies heavily on aerobic glycolysis (i.e., Warburg Effect) to produce cellular energy (i.e., ATP); this is to prevent oxygen from being consumed by the cells and to ensure that it remains available for the fetus (Burton and Fowden, 2015). Therefore, despite their many differences, their methods for energy production are similar. Interestingly, in a study of mtDNA variation across many types of cancers, researchers found that increases in mtDNAcn were consistently associated with increased mitochondrial respiratory gene expression; in opposition, decreases in mtDNAcn were associated with increased expression of immune response and cell-cycle genes (Reznik et al., 2016). Thus, the directionality of the sex-specific effects may provide insight into the different response mechanisms triggered in girls (e.g., decreased mtDNAcn associated with changes in immune response) and boys (e.g., increased mtDNAcn associated with mitochondrial respiration metabolism) when they are exposed to prenatal PM_{2.5} and/or the lasting physiological effects of maternal lifetime trauma. It is also possible that boys exhibit a more pronounced compensatory response (i.e., increased biogenesis) as a result of combined exposure to high PM and trauma which is typically seen in mitochondrial disorders characterized gross mitochondrial dysfunction (Lee et al., 2000; Picard et al., 2014). In contrast, girls exposed to high PM and low trauma appear to be exhibiting a response typical of cellular aging (i.e., reduced mtDNAcn/biogenesis) as has been observed in aging diseases such as Parkinson's (Pyle et al., 2016), Huntington's Disease (Petersen et al., 2014), and Alzheimer's (Rice et al., 2014). While studies have yet to report on the predictive ability of placenta and cord blood mtDNAcn on child health outcomes, studies have shown sex-specific effects of both prenatal psychosocial stress and PM on child asthma and neurodevelopment, suggesting different biological responses to stress and PM might be involved (Lee et al., 2017; Chiu et al., 2016; Hsu et al., 2015b; Lee et al., 2016).

The strengths of this study include the ability to examine the joint effects of ambient particulate matter and lifetime maternal stress, utilization of BDLIMs to better delineate the role of exposure timing in air pollution health effects and to enhance power to detect complex interactive effects (Wilson et al., 2017a), and assessment of mtDNAcn in both placenta and cord blood. We also acknowledge some limitations. First, mtDNA copy number is a biomarker of mitochondrial response and dysfunction, not a specific marker of oxidative stress. Thus, it may be informative to simultaneously measure biomarkers of oxidative stress and cellular aging, such as telomere integrity/length, given the reciprocal pathways between mtDNAcn, oxidative damage and telomeres as regulators of cellular and tissue function and their role in environmentally-induced complications (Geronimus et al., 2015; Sahin et al., 2011). Second, our sample size is relatively small, which might have affected our ability to detect significant associations and interactions in some models. Third, we were unable to adjust for cell-type proportions in analyses considering placental mtDNAcn. We were not able to collect information on the distribution of cell types, and to our knowledge, placental reference-based estimates are not yet available. It should be noted that the proportion of each cell type did not vary across maternal trauma exposure groups nor were the proportions strongly correlated with average prenatal PM_{2.5} exposure. Fourth, we are aware that other socioeconomic factors, such as neighborhood SES, or other traffic-related pollutants such as NO₂ could be potential sources of unmeasured confounding and should be considered in future analyses. Lastly, it is not clear at what level of PM_{2.5} exposure mitochondrial (i.e., cellular) function will substantially be affected. For instance, with mitochondrial disorders there is usually a threshold effect where 80% of the patient's mitochondria must be dysfunctional before symptoms arise and they are diagnosed with disease (Stewart and Chinnery, 2015).

In conclusion, these results indicate that joint exposure to PM_{2.5} in late pregnancy and maternal lifetime trauma influence mtDNAcn at the maternal-fetal interface in a sex-specific manner. Identifying such complex interactions provides insight as to why certain subgroups are differentially vulnerable to a range of developmental outcomes previously linked to PM and/or chronic stress exposures. Additional studies will assist in understanding if this pattern reflects distinct pathophysiological processes across groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Andreu AL, Martinez R, Marti R, Garcia-Arumi E. Quantification of mitochondrial DNA copy number: pre-analytical factors. *Mitochondrion*. 2009; 9(4):242–246. [PubMed: 19272467]
- Aschbacher K, O'Donovan A, Wolkowitz OM, Dhabhar FS, Su Y, Epel E. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology*. 2013; 38(9):1698–1708. [PubMed: 23490070]
- Association, AP. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. American Psychiatric Association; Washington, DC: 2013.
- Bakulski KM, Feinberg JI, Andrews SV, et al. DNA methylation of cord blood cell types: applications for mixed cell birth studies. *Epigenetics*. 2016; 11(5):354–362. [PubMed: 27019159]

- Basagana X, Esnaola M, Rivas I, et al. Neurodevelopmental deceleration by urban fine particles from different emission sources: a longitudinal observational study. *Environ. Health Perspect.* 2016; 124(10):1630–1636. [PubMed: 27128166]
- Bersani FS, Morley C, Lindqvist D, et al. Mitochondrial DNA copy number is reduced in male combat veterans with PTSD. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry.* 2016; 64:10–17.
- Brunst KJ, Ryan PH, Brokamp C, et al. Timing and duration of traffic-related air pollution exposure and the risk for childhood wheeze and asthma. *Am. J. Respir. Crit. Care Med.* 2015; 192(4):421–427. [PubMed: 26106807]
- Brunst KJ, Sanchez Guerra M, Gennings C, et al. Maternal lifetime stress and prenatal psychological functioning are associated with decreased placental mitochondrial DNA copy number in the PRISM study. *Am. J. Epidemiol.* 2017; 186(11):1227–1236. [PubMed: 28595325]
- Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 2015; 370(1663):20140066. [PubMed: 25602070]
- Carelli V, Maresca A, Caporali L, Trifunov S, Zanna C, Rugolo M. Mitochondria: biogenesis and mitophagy balance in segregation and clonal expansion of mitochondrial DNA mutations. *Int. J. Biochem. Cell Biol.* 2015; 63:21–24. [PubMed: 25666555]
- Carugno M, Pesatori AC, Dioni L, et al. Increased mitochondrial DNA copy number in occupations associated with low-dose benzene exposure. *Environ. Health Perspect.* 2012; 120(2):210–215. [PubMed: 22005026]
- Chiu YH, Hsu HH, Coull BA, et al. Prenatal particulate air pollution and neurodevelopment in urban children: examining sensitive windows and sex-specific associations. *Environ. Int.* 2016; 87:56–65. [PubMed: 26641520]
- Clemens T, Turner S, Dibben C. Maternal exposure to ambient air pollution and fetal growth in North-East Scotland: a population-based study using routine ultrasound scans. *Environ. Int.* 2017; 107:216–226. [PubMed: 28753483]
- Clemente DB, Casas M, Vilahur N, et al. Prenatal ambient air pollution, placental mitochondrial DNA content, and birth weight in the INMA (Spain) and ENVIRONAGE (Belgium) birth cohorts. *Environ. Health Perspect.* 2016; 124(5):659–665. [PubMed: 26317635]
- Colaïanna M, Schiavone S, Zotti M, et al. Neuroendocrine profile in a rat model of psychosocial stress: relation to oxidative stress. *Antioxid. Redox Signal.* 2013; 18(12):1385–1399. [PubMed: 23320850]
- De Carli MM, Baccarelli AA, Trevisi L, et al. Epigenome-wide cross-tissue predictive modeling and comparison of cord blood and placental methylation in a birth cohort. *Epigenomics.* 2017; 9(3):231–240. [PubMed: 28234020]
- Geronimus AT, Pearson JA, Linnenbringer E, et al. Race-ethnicity, poverty, urban stressors, and telomere length in a Detroit community-based sample. *J. Health Soc. Behav.* 2015; 56(2):199–224. [PubMed: 25930147]
- Gidron Y, Russ K, Tissarchondou H, Warner J. The relation between psychological factors and DNA-damage: a critical review. *Biol. Psychol.* 2006; 72(3):291–304. [PubMed: 16406268]
- Grote NK, Bridge JA, Gavin AR, Melville JL, Iyengar S, Katon WJ. A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. *Arch. Gen. Psychiatry.* 2010; 67(10):1012–1024. [PubMed: 20921117]
- Henrichs J, Schenk JJ, Roza SJ, et al. Maternal psychological distress and fetal growth trajectories: the Generation R Study. *Psychol. Med.* 2010; 40(4):633–643. [PubMed: 19656431]
- Hohwu L, Li J, Olsen J, Sorensen TI, Obel C. Severe maternal stress exposure due to bereavement before, during and after pregnancy and risk of overweight and obesity in young adult men: a Danish National Cohort Study. *PLoS One.* 2014; 9(5):e97490. [PubMed: 24828434]
- Hou L, Zhu ZZ, Zhang X, et al. Airborne particulate matter and mitochondrial damage: a cross-sectional study. *Environ. Health.* 2010; 9:48. [PubMed: 20696069]
- Hou L, Zhang X, Dioni L, et al. Inhalable particulate matter and mitochondrial DNA copy number in highly exposed individuals in Beijing, China: a repeated-measure study. *Part. Fibre Toxicol.* 2013; 10:17. [PubMed: 23628000]

- Hsu HL, Chiu YM, Coull BA, et al. Prenatal particulate air pollution and asthma onset in urban children: identifying sensitive windows and sex differences. *Am. J. Respir. Crit. Care Med.* 2015a; 192(9):1052–1059. [PubMed: 26176842]
- Hsu HH, Chiu YH, Coull BA, et al. Prenatal particulate air pollution and asthma onset in urban children. Identifying sensitive windows and sex differences. *Am. J. Respir. Crit. Care Med.* 2015b; 192(9):1052–1059. [PubMed: 26176842]
- Irie M, Asami S, Nagata S, Miyata M, Kasai H. Classical conditioning of oxidative DNA damage in rats. *Neurosci. Lett.* 2000; 288(1):13–16. [PubMed: 10869804]
- Islam T, Urman R, Gauderman WJ, et al. Parental stress increases the detrimental effect of traffic exposure on children's lung function. *Am. J. Respir. Crit. Care Med.* 2011; 184(7):822–827. [PubMed: 21700914]
- Janssen BG, Munters E, Pieters N, et al. Placental mitochondrial DNA content and particulate air pollution during in utero life. *Environ. Health Perspect.* 2012; 120(9):1346–1352. [PubMed: 22626541]
- Jiang S, Teague AM, Tryggstad JB, Aston CE, Lyons T, Chernausk SD. Effects of maternal diabetes and fetal sex on human placenta mitochondrial biogenesis. *Placenta.* 2017; 57:26–32. [PubMed: 28864016]
- Jorgensen A. Oxidatively generated DNA/RNA damage in psychological stress states. *Dan. Med. J.* 2013; 60(7):B4685. [PubMed: 23809980]
- Khalifa AR, Abdel-Rahman EA, Mahmoud AM, et al. Sex-specific differences in mitochondria biogenesis, morphology, respiratory function, and ROS homeostasis in young mouse heart and brain. *Phys. Rep.* 2017; 5(6)
- Kloog I, Chudnovsky AA, Just AC, et al. A new hybrid spatio-temporal model for estimating daily multi-year PM_{2.5} concentrations across northeastern USA using high resolution aerosol optical depth data. *Atmos. Environ.* 2014; 95:581–590.
- Lamichhane DK, Leem JH, Lee JY, Kim HC. A meta-analysis of exposure to particulate matter and adverse birth outcomes. *Environ. Health Toxicol.* 2015; 30:e2015011. [PubMed: 26796890]
- Lee HC, Yin PH, Lu CY, Chi CW, Wei YH. Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. *Biochem. J.* 2000; 348(Pt 2):425–432. [PubMed: 10816438]
- Lee A, Mathilda Chiu YH, Rosa MJ, et al. Prenatal and postnatal stress and asthma in children: temporal- and sex-specific associations. *J. Allergy Clin. Immunol.* 2016; 138(3):740–747. e743. [PubMed: 26953156]
- Lee A, Leon Hsu HH, Mathilda Chiu YH, Bose S, Rosa MJ, Kloog I, Wilson A, Schwartz J, Cohen S, Coull BA, Wright RO, Wright RJ. Prenatal fine particulate exposure and early childhood asthma: Effect of maternal stress and fetal sex. *J. Allergy Clin. Immunol.* 2017. . pii: S0091-6749(17)31273-3, [Epub ahead of print] PubMed PMID: 28801196
- van de Loo KF, van Gelder MM, Roukema J, Roeleveld N, Merkus PJ, Verhaak CM. Prenatal maternal psychological stress and childhood asthma and wheezing: a meta-analysis. *Eur. Respir. J.* 2016; 47(1):133–146. [PubMed: 26541526]
- Malmqvist E, Rignell-Hydborn A, Tinnerberg H, et al. Maternal exposure of air pollution and birth outcomes. *Environ. Health Perspect.* 2011; 119:553–558. [PubMed: 21212043]
- McHugo GJ, Casp Y, Kammerer N, et al. The assessment of trauma history in women with co-occurring substance abuse and mental disorders and a history of interpersonal violence. *J. Behav. Health Serv. Res.* 2005; 32:113–127. [PubMed: 15834262]
- Meyer JN, Leuthner TC, Luz AL. Mitochondrial fusion, fission, and mitochondrial toxicity. *Toxicology.* 2017; 391:42–53. [PubMed: 28789970]
- Nachman RM, Mao G, Zhang X, et al. Intrauterine inflammation and maternal exposure to ambient PM_{2.5} during preconception and specific periods of pregnancy: the Boston birth cohort. *Environ. Health Perspect.* 2016; 124(10):1608–1615. [PubMed: 27120296]
- Nugent BM, Bale TL. The omniscient placenta: metabolic and epigenetic regulation of fetal programming. *Front. Neuroendocrinol.* 2015; 39:28–37. [PubMed: 26368654]

- Nwanaji-Enwerem JC, Colicino E, Dai L, et al. Impacts of the mitochondrial genome on the relationship of long-term ambient fine particle exposure with blood DNA methylation age. *Environ. Sci. Technol.* 2017; 51(14):8185–8195. [PubMed: 28636816]
- Patra J, Bakker R, Irving H, Jaddoe VW, Malini S, Rehm J. Dose-response relationship between alcohol consumption before and during pregnancy and the risks of low birthweight, preterm birth and small for gestational age (SGA)-a systematic review and meta-analyses. *BJOG.* 2011; 118(12): 1411–1421. [PubMed: 21729235]
- Petersen MH, Budtz-Jorgensen E, Sorensen SA, et al. Reduction in mitochondrial DNA copy number in peripheral leukocytes after onset of Huntington's disease. *Mitochondrion.* 2014; 17:14–21. [PubMed: 24836434]
- Picard M, Juster RP, McEwen BS. Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids. *Nat. Rev. Endocrinol.* 2014; 10(5):303–310. [PubMed: 24663223]
- Pyle A, Anugraha H, Kurzawa-Akanbi M, Yarnall A, Burn D, Hudson G. Reduced mitochondrial DNA copy number is a biomarker of Parkinson's disease. *Neurobiol. Aging.* 2016; 38:216–216. e217–216, e210.
- Rabinovitch N, Silveira L, Gelfand EW, Strand M. The response of children with asthma to ambient particulate is modified by tobacco smoke exposure. *Am. J. Respir. Crit. Care Med.* 2011; 184(12): 1350–1357. [PubMed: 21868505]
- Reznik E, Miller ML, Senbabaoglu Y, et al. Mitochondrial DNA copy number variation across human cancers. *elife.* 2016; 5
- Rice AC, Keeney PM, Algarzae NK, Ladd AC, Thomas RR, Bennett JP Jr. Mitochondrial DNA copy numbers in pyramidal neurons are decreased and mitochondrial biogenesis transcriptome signaling is disrupted in Alzheimer's disease hippocampi. *J. Alzheimers Dis.* 2014; 40(2):319–330. [PubMed: 24448779]
- Romieu I, Castro-Giner F, Kunz N, Sunyer J. Air pollution, oxidative stress and dietary supplementation: a review. *Eur. Respir. J.* 2008; 31:179–196. [PubMed: 18166596]
- Rosa MJ, Just AC, Guerra MS, et al. Identifying sensitive windows for prenatal particulate air pollution exposure and mitochondrial DNA content in cord blood. *Environ. Int.* 2017a; 98:198–203. [PubMed: 27843010]
- Rosa MJ, Just AC, Kloog I, et al. Prenatal particulate matter exposure and wheeze in Mexican children: effect modification by prenatal psychosocial stress. *Ann Allergy Asthma Immunol.* 2017b; 119(3):232–237. [PubMed: 28757229]
- Sahin E, Colla S, Liesa M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature.* 2011; 470(7334):359–365. [PubMed: 21307849]
- Scheinost D, Sinha R, Cross SN, et al. Does prenatal stress alter the developing connectome? *Pediatr. Res.* 2016; 81:214–226. [PubMed: 27673421]
- Seng JS, Low LK, Sperlich M, Ronis DL, Liberzon I. Post-traumatic stress disorder, child abuse history, birthweight and gestational age: a prospective cohort study. *BJOG.* 2011; 118(11):1329–1339. [PubMed: 21790957]
- Shankardass K, McConnell R, Jerrett M, Milam J, Richardson J, Berhane K. Parental stress increases the effect of traffic-related air pollution on childhood asthma incidence. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106(30):12406–12411. [PubMed: 19620729]
- Sharma J, Johnston MV, Hossain MA. Sex differences in mitochondrial biogenesis determine neuronal death and survival in response to oxygen glucose deprivation and reoxygenation. *BMC Neurosci.* 2014; 15:9. [PubMed: 24410996]
- Shaughnessy DT, McAllister K, Worth L, et al. Mitochondria, energetics, epigenetics, and cellular responses to stress. *Environ. Health Perspect.* 2014; 122(12):1271–1278. [PubMed: 25127496]
- Stark MJ, Hodyl NA, Wright IM, Clifton VL. Influence of sex and glucocorticoid exposure on preterm placental pro-oxidant-antioxidant balance. *Placenta.* 2011; 32(11):865–870. [PubMed: 21903264]
- Sternthal MJ, Enlow MB, Cohen S, et al. Maternal interpersonal trauma and cord blood IgE levels in an inner-city cohort: a life-course perspective. *J. Allergy Clin. Immunol.* 2009; 124(5):954–960. [PubMed: 19748657]
- Stewart JB, Chinnery PF. The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease. *Nat. Rev. Genet.* 2015; 16(9):530–542. [PubMed: 26281784]

- Testa M, Quigley BM, Eiden RD. The effects of prenatal alcohol exposure on infant mental development: a meta-analytical review. *Alcohol Alcohol*. 2003; 38(4):295–304. [PubMed: 12814894]
- Traboulsi H, Guerrina N, Iu M, Maysinger D, Ariya P, Bagloli CJ. Inhaled pollutants: the molecular scene behind respiratory and systemic diseases associated with ultrafine particulate matter. *Int. J. Mol. Sci*. 2017; 18(2)
- Tyrka AR, Parade SH, Price LH, et al. Alterations of mitochondrial DNA copy number and telomere length with early adversity and psychopathology. *Biol. Psychiatry*. 2016; 79(2):78–86. [PubMed: 25749099]
- Wang X, Sundquist K, Rastkhani H, Palmer K, Memon AA, Sundquist J. Association of mitochondrial DNA in peripheral blood with depression, anxiety and stress- and adjustment disorders in primary health care patients. *Eur. Neuropsychopharmacol*. 2017; 27(8):751–758. [PubMed: 28647451]
- Wilson A, Chiu YM, Hsu HL, Wright RO, Wright RJ, Coull BA. Bayesian distributed lag interaction models to identify perinatal windows of vulnerability in children's health. *Biostatistics*. 2017a; 18(3):537–552. [PubMed: 28334179]
- Wilson A, Chiu YM, Hsu HL, Wright RO, Wright RJ, Coull BA. Potential for bias when estimating critical windows for air pollution in children's health. *Am. J. Epidemiol*. 2017b; 186(11):1281–1289. [PubMed: 29206986]
- Wolfe J, Kimerling R. Gender issues in the assessment of posttraumatic stress disorder. In: Wilson JP, Keane TM, editors *Assessing Psychological Trauma and PTSD*. Guildford; New York: 1997. 192–238.
- Yang L, Hou XY, Wei Y, Thai P, Chai F. Biomarkers of the health outcomes associated with ambient particulate matter exposure. *Sci. Total Environ*. 2017; 579:1446–1459. [PubMed: 27908628]
- Zhong J, Cayir A, Trevisi L, et al. Traffic-related air pollution, blood pressure, and adaptive response of mitochondrial abundance. *Circulation*. 2016; 133(4):378–387. [PubMed: 26660284]

Fig. 1. Associations between weekly $PM_{2.5}$ levels over gestation and mtDNAcn in placenta (panel A) and in cord blood (panel B)

This figure demonstrates the association between $PM_{2.5}$ exposure over pregnancy and change in mtDNAcn in: (A) placenta, and (B) cord blood, using BDLIM assuming week-specific effects. The estimated time-varying effects weekly $PM_{2.5}$ exposures are shown on the left-hand side of each panel, and the estimated cumulative effects across the entire pregnancy are shown on the right-hand side of each panel; estimates correspond to a $\mu g/m^3$ increase in $PM_{2.5}$. The y-axis represents the change in mtDNAcn; the x-axis is gestational age in weeks. Solid lines show the predicted change in mtDNAcn. Gray areas indicate 95% confidence intervals (CIs). A sensitive window is identified for the weeks where the

estimated pointwise 95% CI (shaded area) does not include zero. The placenta model was adjusted for maternal age, education, race/ethnicity, maternal lifetime trauma, child sex; the cord blood model was additionally adjusted for cell type proportions.

Fig. 2. Associations between weekly $PM_{2.5}$ levels over gestation and mtDNAcn in placenta: interaction by maternal lifetime trauma

This figure demonstrates the effect modification by maternal lifetime trauma on the association between $PM_{2.5}$ exposure over pregnancy and mtDNAcn in placenta, using BDLIM assuming week-specific effects. The model was adjusted for maternal age, education, race/ethnicity, and child sex. The y-axis represents the time-varying change in mtDNAcn corresponding to a $\mu g/m^3$ increase in $PM_{2.5}$; the x-axis is gestational age in weeks. Solid lines show the predicted change in mtDNAcn. Gray areas indicate 95% confidence intervals (CIs). A sensitive window is identified for the weeks where the estimated pointwise 95% CI (shaded area) does not include zero.

Fig. 3. Associations between weekly PM_{2.5} levels over gestation and mtDNAcn in placenta: interaction by maternal lifetime trauma and child sex

This figure demonstrates the effect modification by maternal trauma and child sex on the association between PM_{2.5} exposure over pregnancy and mtDNAcn in placenta, using BDLIM assuming week-specific effects. The model was adjusted for maternal age, education, and race/ethnicity. The y-axis represents the time-varying change in mtDNAcn corresponding to a $\mu\text{g}/\text{m}^3$ increase in PM_{2.5}; the x-axis is gestational age in weeks. Solid lines show the predicted change in mtDNAcn. Gray areas indicate 95% confidence intervals (CIs). A sensitive window is identified for the weeks where the estimated pointwise 95% CI (shaded area) does not include zero.

Fig. 4. Associations between weekly PM_{2.5} levels over gestation and mtDNAcn in cord blood: interaction by maternal lifetime trauma and child sex

This figure demonstrates the effect modification by maternal trauma and child sex on the association between PM_{2.5} exposure over pregnancy and mtDNAcn in cord blood, using BDLIM assuming week-specific effects. The model was adjusted for maternal age, education, and race/ethnicity as well as cell type proportions. The y-axis represents the time-varying change in mtDNAcn corresponding to a $\mu\text{g}/\text{m}^3$ increase in PM_{2.5}; the x-axis is gestational age in weeks. Solid lines show the predicted change in mtDNAcn. Gray areas indicate 95% confidence intervals (CIs). A sensitive window is identified for the weeks where the estimated pointwise 95% CI (shaded area) does not include zero.

Table 1

Sample characteristics by mitochondrial DNA biomarker source.

Characteristic (s)	Placenta (n = 140)	Cord blood (n = 126)
Maternal age at enrollment in years [mean (SD)]	31.5 (5.15)	31.5 (5.03)
Maternal education no. (%)		
High school degree	18 (13)	15 (12)
Missing	2 (1)	1 (0.8)
Race/ethnicity no. (%)		
White	67 (48)	59 (47)
Black	49 (35)	47 (37)
Hispanic	11 (8)	8 (6)
Mixed/Other	13 (9)	12 (10)
Child sex no. (%)		
Male	73 (52)	63 (50)
Smoking during pregnancy	23 (16)	24 (19)
Preterm births (< 37 weeks gestation)	9 (6)	6 (5)
Placental mtDNA copy number [mean (SD)]	2.17 (0.93)	–
Cord Blood mtDNA copy number [mean (SD)]	–	4.64 (1.69)
Average prenatal PM _{2.5} exposure (µg/m ³) [mean (SD)]	7.95 (0.67)	7.96 (0.69)
High maternal lifetime trauma exposure no. (%)	46 (33)	43 (34)

Differences in continuous variables among groups were examined using Kruskal-Wallis ANOVA; differences in proportions were examined using chi-square test. There were no significant differences between placenta and cord blood groups with respect to descriptive statistics (all *p*-values > 0.05).