Published in final edited form as:

Environ Int. 2017 November ; 108: 204–211. doi:10.1016/j.envint.2017.08.022.

Maternal Residential Air Pollution and Placental Imprinted Gene Expression

Samantha L. Kingsley^{a,*}, Maya A. Deyssenroth^b, Karl T. Kelsey^{a,c}, Yara Abu Awad^d, Itai Kloog^e, Joel D. Schwartz^d, Luca Lambertini^{b,f}, Jia Chen^{b,g,h,i}, Carmen J. Marsit^j, and Gregory A. Wellenius^a

^aDepartment of Epidemiology, Brown University School of Public Health, Providence, RI, USA

^bDepartment of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY USA

^cDepartment of Pathology and Laboratory Medicine, Brown University, Providence, RI, USA

^dDepartment of Environmental Health, Harvard T. H. Chan School of Public Health, Boston, MA, USA

^eDepartment of Geography and Environmental Development, Ben-Gurion University of the Negev, Beer Sheva, Israel

^fDepartment of Obstetrics, Gynecology and Reproductive Science, Icahn School of Medicine at Mount Sinai, New York; NY USA

^gDepartment of Pediatrics, Icahn School of Medicine at Mount Sinai, New York; NY USA

^hDepartment of Medicine, Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York; NY USA

ⁱDepartment of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York; NY USA

^jDepartment of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Abstract

Background—Maternal exposure to air pollution is associated with reduced fetal growth, but its relationship with expression of placental imprinted genes (important regulators of fetal growth) has not yet been studied.

* **Corresponding author:** Samantha L. Kingsley, PhD, Post-Doctoral Research Associate, Brown University School of Public Health, Box G-S121-2, Providence, RI 02912, Samantha_Kingsley@brown.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Competing financial interests.

The authors declare no competing financial interests.

Objectives—To examine relationships between maternal residential air pollution and expression of placental imprinted genes in the Rhode Island Child Health Study (RICHS).

Methods—Women-infant pairs were enrolled following delivery between 2009 and 2013. We geocoded maternal residential addresses at delivery, estimated daily levels of fine particulate matter (PM_{2.5}; n=355) and black carbon (BC; n=336) using spatial-temporal models, and estimated residential distance to nearest major roadway (n=355). Using linear regression models we investigated the associations between each exposure metric and expression of nine candidate genes previously associated with infant birthweight in RICHS, with secondary analyses of a panel of 108 imprinted genes expressed in the placenta. We also explored effect measure modification by infant sex.

Results—PM_{2.5} and BC were associated with altered expression for seven and one candidate genes, respectively, previously linked with birthweight in this cohort. Adjusting for multiple comparisons, we found that PM_{2.5} and BC were associated with changes in expression of 41 and 12 of 108 placental imprinted genes, respectively. Infant sex modified the association between PM_{2.5} and expression of *CHD7* and between proximity to major roadways and expression of *ZDBF2*.

Conclusions—We found that maternal exposure to residential PM_{2.5} and BC was associated with changes in placental imprinted gene expression, which suggests a plausible line of investigation of how air pollution affects fetal growth and development.

Keywords

air pollution; genomic imprinting; placenta

1. Introduction

The placenta plays a vital role in fetal development as it regulates the exchange of nutrients, gas, and waste between mother and fetus, and optimizes the maternal-fetal environment for proper development (Sandovici et al. 2012). An important group of genes responsible for these functions, and for that adaptability, are those genes whose expression is controlled through genomic imprinting. Genomic imprinting is a form of epigenetic gene regulation in which one parental allele is expressed and the other is silenced (Jirtle and Skinner 2007). The highly controlled pattern of expression of these genes is critical to normal development and plays a particularly important role in the placenta functioning (Fowden et al. 2006; Miozzo and Simoni 2002; Reik and Walter 2001). For example, loss of genomic imprinting (LOI) early in development can lead to placental and fetal growth restriction (Lambertini et al. 2012a), and recent studies have shown that even relatively modest differences in imprinted gene expression are associated with fetal growth/birth weight (Kappil et al. 2015a; Lambertini et al. 2012b). Moreover, in both human and animal studies imprinted gene expression has been associated with exposure to a number of environmental exposures, including, maternal nutrition, alcohol use, tobacco use, and BPA exposure, and imprinted gene expression has been highlighted as a potentially useful environmental sensor (Kappil et al. 2015b).

Maternal exposure to ambient air pollution during fetal development has been associated with reduced fetal growth, even at exposure levels lower than National Ambient Air Quality Standards (Dadvand et al. 2013; Lamichhane et al. 2015; Sapkota et al. 2010; Stieb et al. 2012; Sun et al. 2016; Zhu et al. 2015). However, the underlying molecular mechanisms remain unknown. Maternal residential proximity to major roadways has been associated with changes in placental DNA methylation and maternal exposure to ambient fine particulate matter (PM_{2.5}) has been associated with placental gene expression (Kingsley et al. 2016; Saenen et al. 2015). However, to our knowledge, no study has examined the association between maternal exposure to air pollution and placental imprinted gene expression as a potential mechanism for altering fetal growth.

Accordingly, we examined the relationship between maternal residential levels of PM_{2.5} and placental expression of imprinted genes in a cohort of 410 women-infant pairs from the Rhode Island Child Health Study (RICHS). We hypothesized that mothers exposed to higher levels of PM_{2.5} have altered imprinting profiles compared to mothers exposed to lower levels of PM_{2.5}. We also examined the associations between placental imprinted gene expression and residential exposure to black carbon (BC) and proximity to nearest major roadway as markers of traffic-related pollution.

2. Materials and Methods

2.1 Study Population

The Rhode Island Child Health Study (RICHS) enrolled women-infant pairs from March 2009 to May 2013 following delivery at Women and Infants Hospital of Rhode Island (Marsit et al. 2012). Eligibility criteria included singleton, viable, full term births to mothers 18 years or older without a life-threatening complication for the mother or a congenital or chromosomal abnormality of the infant. Infants born small for gestational age (SGA) and large for gestational age (LGA) were selected and matched to infants born appropriate for gestational age (AGA) based on gender, gestational age (± 3 days), and maternal age (± 2 years). Exposure histories, residential address, and lifestyle and demographic information were collected from structured medical chart reviews and in person interviewer-administered questionnaires. Information on residential addresses was available for 410 participants. The study protocol was approved by the Institutional Review Boards of Brown University and Women and Infants Hospital of Rhode Island.

2.2 Imprinted Gene Expression in Placenta

Placenta samples were taken within two hours of delivery from four quadrants of the maternal side of the placenta, 2 cm from the umbilical cord-insertion site and free of maternal decidua. The samples were immediately placed in RNALater (Life Technologies, #AM7024) and stored at 4°C for at least 72 hours, then blotted dry, snap-frozen in liquid nitrogen, homogenized and stored at -80 °C until analysis. DNA was extracted using QIAmp DNA mini kit (Qiagen, #51306) and RNA was extracted using RNeasy mini kit (Qiagen, #74106).

We examined expression of 108 established imprinted genes, the selection of which was informed by two databases (Jirtle 2012; Morison et al. 2001) encompassing empirically determined imprinted genes as well as those computationally predicted, and limited to those genes that have been found to be consistently expressed in a majority (>50%) of the sample, as previously described (Kappil et al. 2015a). The Nanostring nCounter system was used to perform a direct count of the presence of imprinted gene mRNA expressed in the placenta. Briefly, samples of RNA were incubated overnight with reporter and capture probes. After removal of unbound probes, the samples were aligned and immobilized on imaging cartridges. To obtain code counts, the cartridges were sealed and scanned in an nCounter Digital Analyzer (Kappil et al. 2015a). The nanoString Norm package was used to normalize nCounter data (Waggott 2014). The raw code count data was normalized against the geometric mean of spike-in positive control probes and against the geometric mean of the housekeeping genes, *GAPDH*, *RPL19*, and *RPLP0*. The limit of detection for each sample was set at two standard deviations above the mean of the included negative control probes.

2.3 Exposure Assessment

Using ArcMap 10.1 (ESRI; Redlands, CA) we geocoded participant addresses at time of delivery. We estimated daily $PM_{2.5}$ levels at each maternal residential address using a hybrid spatial-temporal model that uses land-use regression methodologies and satellite measurements of aerosol optical depth (AOD) at a 1 km resolution, as previously described (Kloog et al. 2014). Briefly, land use regression and satellite-based AOD are used to fit a daily calibration regression using ground-level $PM_{2.5}$ measurements with a high prediction accuracy ($R^2 = 0.88$) (Kloog et al. 2014). To estimate exposure on a finer scale ($200\text{ m} \times 200\text{ m}$), the differences in AOD and measured $PM_{2.5}$ are regressed against local land use features such as distance to source emission points, traffic density, and visibility (Kloog et al. 2014). $PM_{2.5}$ estimates were available for the entire study period. We estimated daily BC levels at each maternal residential address using an extended version of a validated spatial-temporal land-use regression model that includes daily average BC estimates from five Rhode Island BC monitors, meteorological data from nearly two dozen local weather stations, state land use data, latitude and longitude, daily meteorological factors and other characteristics (eg: day of week, day of season), and interaction terms between land-use measures and daily meteorological factors (Gryparis et al. 2007). The model performed well in both cold (November–April) and warm seasons (May–October) with 10-fold cross validated R^2 of 0.73 and 0.75, respectively. BC estimates were available from the start of the study period to 2011 (excludes 22 participants). BC and $PM_{2.5}$ estimates were averaged over the length of pregnancy and used as exposure.

We calculated the Euclidean distance to nearest major roadway and defined major roadways as those with US Census feature class codes A1 (primary highway with limited access), A2 (primary road without limited access), or A3 (secondary and connecting roads). We considered participants living 150m of an A1 or A2 roadway or 50m of an A3 roadway as “exposed” and unexposed otherwise (Kingsley et al. 2016).

2.4 Covariates

We considered several maternal and infant characteristics collected from in-person interviewer-administered questionnaires as potential confounders. We categorized parity (1, 2, 3), maternal race (Caucasian, other), maternal education (less than high school, some college, college or more), annual household income (<\$25,000, \$25,000–49,999, \$50,000–79,999, \$80,000–99,999, \$100,000), health insurance (none/self-pay, private, public/other), and prenatal care during pregnancy (yes, no). Maternal alcohol, tobacco, and prenatal vitamin use during pregnancy (defined as regularly taking prenatal vitamins) were each dichotomized as yes or no. Marital status was defined as married or not married (single, separated, or divorced). Maternal body mass index (BMI) before pregnancy was calculated from self-reported height and weight. Maternal age was measured in years. Covariate data for the infant included sex and gestational age at birth (weeks).

To address potential confounding by neighborhood socioeconomic status (SES), we calculated z-scores for each of six census tract-level variables (median household income; percent of households with interests, dividends, or rent income; percent of residents with high school diploma; percent with college degree; percent with professional occupation; and median value of owner-occupied housing units), and summed the z-scores creating a z-sum score (Diez Roux et al. 2001).

2.5 Statistical Analyses

We used two approaches to examine the associations between maternal residential PM_{2.5} and expression of imprinted genes: a candidate gene approach and an agnostic approach. First, we used linear regression models to investigate the associations between PM_{2.5} (n=355) and the expression of nine candidate genes that were previously found to be associated with infant birthweight in this cohort: *BLCAP*, *DLK1*, *H19*, *IGF2*, *MEG3*, *MEST*, *NDN*, *NNAT*, and *PLAGL1* (Kappil et al. 2015a). We used causal diagrams to select potential confounders for inclusion in regression models (Hernán et al. 2002). All models were adjusted for maternal age (centered at the mean and modeled as a quadratic term), maternal education, tobacco use during pregnancy, prenatal vitamin use, maternal BMI, parity, annual household income, maternal health insurance, marital status, maternal race, infant sex, and neighborhood SES z-sum score. We repeated these analyses to examine associations with BC (n=336) and residential proximity to nearest major roadway (n=355) as markers of traffic-related pollution. In sensitivity analyses we additionally adjusted analyses for conception date as a sinusoidal term in models for PM_{2.5} and BC to address potential confounding by temporal trends in exposure.

Second, to identify placental imprinted genes whose expression is associated with air pollution exposures, but whose expression has not been previously linked to birth weight in this cohort, we examined the associations between PM_{2.5} and the expression of each of the 108 imprinted genes using linear regression models. For this approach we calculated q-values to control the false discovery rate (FDR<0.05) using the Benjamini-Hochberg method (Benjamini and Hochberg 1995). A q-value of 0.05 indicates that on average 5% of tests with q-values<0.05 are false positives. As a more conservative approach, we also used Bonferroni correction to control the family-wise error rate of 0.05, meaning that the

probability of one false positive in this family of tests that is due to chance is 0.05 (Rosenthal and Rubin 1984). We calculated a critical value of $0.05/108=0.00046$, which indicates that all p-values less than this critical value are considered statistically significant. We repeated these analyses to examine associations with BC and proximity to major roadways. All models were adjusted for the same covariates as the candidate gene approach.

To account for the oversampling of SGA and LGA infants in this cohort, we used inverse-probability-of-selection weights in all regression models. We used data from Women and Infants Hospital from March 2009–May 2011 to calculate the probability of selection into this cohort and the probability of selection given infant growth status (SGA, AGA, or LGA). These probabilities were used to calculate stabilized weights for SGA, AGA, and LGA infants.

$$sw_i = \frac{P(S=1)}{P(S=1|X=x)}$$

Where $P(S=1)$ denotes the marginal probability of being selected into the study and $P(S=1|X=x)$ denotes the probability of being selected into the study conditional on infant growth status (X : 1=SGA, 2=AGA, and 3=LGA).

We explored potential effect measure modification by infant sex by including an interaction term for each exposure metric and infant sex in regression models. We conducted all analyses using R (v3.2.0).

3. Results

The majority of participants were married (64%), Caucasian (77%), received prenatal care (99%), used prenatal vitamins (94%), and reported not using tobacco (95%) or alcohol (99%) during pregnancy (Table 1). The average maternal age was 30 years (SD=5.6) and average gestational age was 39 weeks (SD=0.9). Maternal and child characteristics did not differ by tertiles of maternal residential PM_{2.5} exposure. The mean pregnancy-average PM_{2.5} level was 7.96 µg/m (SD=0.65). This is below the current National Ambient Air Quality Standard for annual PM_{2.5} of 12 µg/m³ (EPA 2013) and is lower than 2010 annual PM_{2.5} levels in Los Angeles, California (12.6 µg/m³), New York, New York (11.5 µg/m), and Boston, Massachusetts (10 µg/m³) (EPA 2010).

In adjusted models, PM_{2.5} was associated with altered gene expression for seven of the nine candidate genes previously found to be associated with infant birth weight in this cohort (Kappil et al. 2015a). Specifically, PM_{2.5} was associated with increased expression of *BLCAP*, *H19*, *IGF2*, *MEG3*, *MEST*, and *PLAGL1* and lower gene expression for *NNAT* (Figure 1). The magnitude (absolute value) of the change in gene expression ranged from 4.2% (*BLCAP*) to 15.5% (*MEG3*) per interquartile range (IQR) shift in PM_{2.5}. Results were not materially different in sensitivity analyses with additional adjustment for seasonal time trends, although 95% confidence intervals were typically wider indicating decreased precision (Supplemental Table S1).

Next, we examined associations between PM_{2.5} and all 108 imprinted genes (Supplemental Table S2). After adjusting for multiple comparisons to preserve the false discovery rate, PM_{2.5} was associated with expression of 41 imprinted genes (Figure 2). Changes in gene expression ranged from – 13.6% (*FAM50B*) to 27.3% (*VTRNA2.1*) per IQR shift in PM_{2.5}. Eighteen of the 41 associations also survived the more conservative Bonferroni correction.

We repeated these analyses for BC and residential proximity to major roadways as markers of traffic related pollution. Applying the candidate gene approach, an IQR (0.14 µg/m³) shift in BC was associated with a 6.3% (95% CI: –10.1%, –2.3%) lower *BLCAP* gene expression (Figure 1). In analyses examining all 108 imprinted genes and adjusted for multiple comparisons, BC was associated with expression of 12 of the 108 imprinted genes (Figure 3). Only two of these associations (*NLRP2* and *ABCA1*) also survived Bonferroni correction. We did not find any associations between placental imprinted gene expression and maternal residential proximity to major roadways (Supplemental Material Table S2).

The association between PM_{2.5} and *CHD7* varied by infant sex (heterogeneity p-value=0.0001 and q-value=0.012) with associations in opposite directions for males and females (Table 2). We found a similar dimorphic pattern for the association between residential proximity to major roadway and *ZDBF2* expression (heterogeneity p-value=0.0003 and q-value=0.03). We did not find evidence of heterogeneity in gene expression by infant sex for BC.

4. Discussion

Environmental exposures have been associated with numerous types of epigenetic changes, including imprinted gene regulation (Kappil et al. 2015b; Marsit 2015). Genomic imprinting arises early in fetal development and it has been suggested that these epigenetic patterns may function as a biosensor for the impact of *in utero* environmental exposures (Hoyo et al. 2009; Jirtle and Skinner 2007). In addition, imprinting plays an important role in fetal development and growth, a process that has been found to be disrupted by exposure to air pollutants (Dadvand et al. 2013; Lamichhane et al. 2015; Sapkota et al. 2010; Stieb et al. 2012; Sun et al. 2016; Zhu et al. 2015).

Kappil et al. (2015a) previously examined the associations between placental imprinted gene expression and birthweight in the RICHs cohort and found that higher expression of *BLCAP*, *DLK1*, *H19*, *IGF2*, *MEG3*, *MEST*, *NDN*, *NNAT*, and *PLAGL1* was associated with higher odds of an infant being born LGA while higher expression of *NNAT* was associated with higher odds of being born SGA. Given the well-documented association between PM_{2.5} and fetal growth, in a first analysis we found that expression of seven of these genes was associated with PM_{2.5}. We next examined the association between PM_{2.5} and expression of all 108 placental imprinted genes, and found that PM_{2.5} was associated with changes in expression of 41 of these imprinted genes, after controlling the false discovery rate. These results suggest, for the first time, that expression of placental imprinted genes is a plausible mediator of the association between PM_{2.5} and reduced fetal growth.

Several studies have examined the link between imprinted gene expression in placenta and markers of fetal growth, particularly intrauterine growth restriction (IUGR) (Diplas et al. 2014; McMinn et al. 2006). Our results suggest the possibility that PM_{2.5} exposures could induce changes in placental expression of imprinted genes that in turn reduce fetal growth. Consistent with this hypothesis, we found that PM_{2.5} was associated with increased expression of *IGF2* (Insulin Like Growth Factor 2) and *H19*, both of which are regulated by the same imprinting control region and have been associated with growth disorders including IUGR (Abu-Amro et al. 2008; Bourque et al. 2010; Cordeiro et al. 2014; Weksberg et al. 2010). However, not all of our findings were concordant with our hypothesis. For example, IUGR placentas have lower expression of *PLAGL1* (PLAG1 Like Zinc Finger 1), *MEG3* (maternally expressed 3), and *MEST* (Mesoderm Specific Transcript), but we unexpectedly found that PM_{2.5} was associated with higher, rather than lower, expression of these genes (Diplas et al. 2014; McMinn et al. 2006). These findings underscore that the regulation of fetal growth by placental imprinted genes is multifactorial without a straightforward one-to-one correspondence between environmental exposures, gene expression and birth weight.

In “agnostic” analyses of all 108 placental imprinted genes, we found that PM_{2.5} was associated (FDR<0.05) with expression of 41 genes, 18 of which survived the more conservative Bonferroni correction (*CD44*, *CDKAL1*, *CDKN1C*, *COPG2*, *FAM50B*, *GAA*, *IGF1R*, *NNAT*, *NPAP1*, *PEG10*, *PCNA*, *PHLDA2*, *PSIMCT.1*, *SHANK2*, *SLC22A18AS*, *UBE3A*, *ZNF264*, and *ZNF703*). These imprinted genes are associated with a number of distinct cellular functions, and in prior studies, their alteration has been linked to various health outcomes, raising the possibility that modulation of expression of these genes may mediate other documented impacts of air pollution on fetal and child health. For example, PM_{2.5} was associated with higher expression of *PHLDA2* (Pleckstrin Homology Like Domain Family A Member 2). Previous studies have found that IUGR placentas have higher expression of *PHLDA2* (Cordeiro et al. 2014; Diplas et al. 2014; McMinn et al. 2006), which is maternally expressed. Therefore higher expression of this gene supports the parental conflict theory of maternal restriction during fetal development favoring IUGR. Many of the 18 imprinted genes found to be associated with PM_{2.5} are involved in promoting (*IGF1R* and *PEG10*) or restricting (*CDKN1C* and *PHLDA2*) cell growth (Piedrahita 2011). Some of these genes (*CDKN1C*, *COPG2*, *PHLDA2*, and *SLC22A18AS*) are also associated with growth disorders, such as Beckwith-Wiedemann syndrome and Silver-Russell syndrome (MacIsaac et al. 2012; Zollino et al. 2010). These findings provide initial plausibility for the hypothesis that the effects of PM_{2.5} on fetal growth may be mediated through placental epigenetic regulation.

Maternal PM_{2.5} exposures have also been linked to other adverse outcomes, including alterations in child neurodevelopment (Chiu et al. 2016; Suades-Gonzalez et al. 2015). Many of the placental imprinted genes associated with PM_{2.5} have important functions in the brain and in neurodevelopment such as *CDKN1C*, *FAM50B*, *NPAP1*, *PHLDA2*, *SLC22A18AS*, *SHANK2*, and *UBE3A*. Expression of three of these genes (*PHLDA2*, *NPAP1*, *FAM50B*) has been previously linked with child neurological function in this same cohort (Green et al. 2015). Additionally, *NPAP1* and *UBE3A* are found in a segment on chromosome 15 that plays a critical role in three neurogenetic disorders (Chamberlain 2013). Additional studies are needed to investigate the potential mediating role of these genes on the association

between maternal PM_{2.5} exposure and newborn neurobehavioral profiles or other child health outcomes.

PM_{2.5} was most strongly associated with expression of *VTRNA2.1*. This gene has been previously identified as a metastable epiallele in the human population, meaning its pattern of expression can be influenced by the experienced environment during the earliest points of development and that this pattern of expression can be maintained into childhood and beyond (Silver et al. 2015). Our data suggests that subtle environmental stressors such as ambient air pollution can affect the expression pattern of this gene in placenta, and would further suggest this gene to be an exquisitely sensitive environmental sensor worthy of further examination of not only in terms of its environmental susceptibility but also its functional impact in human placenta.

With respect to BC, only expression of *BLCAP* (Bladder Cancer Associated Protein) was associated with exposure in our candidate gene analysis. *BLCAP* is involved in cell growth and is maternally expressed, suggesting that higher *BLCAP* expression could be associated with reduced fetal growth. However, previously in this cohort, higher *BLCAP* expression was associated with higher odds of LGA, suggesting that the relationship between BC, *BLCAP* expression and birth weight remains incompletely understood. In our agnostic approach, BC was associated with variation in the expression of 12 other placental imprinted genes, two of which survived Bonferroni correction: *ABCA1* and *NLRP2*. *ABCA1* (ATP Binding Cassette Subfamily A Member 1) encodes a membrane protein that transports cholesterol (Bhattacharjee et al. 2010) and its association with BC suggests that *in utero* exposure to traffic pollution may alter the maternal-fetal cholesterol transport mechanism. *NLRP2* (NLR Family Pyrin Domain Containing 2) encodes a cytoplasmic protein that is associated with inflammation (Minkiewicz et al. 2013), although its role in the function of the placenta is unknown.

We found differences in the association between PM_{2.5} and expression of *CHD7* (Chromodomain Helicase DNA Binding Protein 7) by infant sex. Mutations of *CHD7* have been previously associated with CHARGE syndrome, which is characterized by Coloboma, Heart malformations, Atresia choanae, Retarded growth and development, Genital hypoplasia, and Ear anomalies (Pagon et al. 1981). CHARGE patients may also have hypogonadotropic hypogonadism and delayed puberty due to changes in the hypothalamic-pituitary-gonadal axis resulting in hormone deficiencies (Jongmans et al. 2006). Urogenital abnormalities are more common in male patients than female patients, but both sexes show deficient levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Jongmans et al. 2006; Zentner et al. 2010). Our findings warrant further investigation into the sexual dimorphism of *CHD7* and suggest one potential mechanism underlying sex-specific susceptibility for environmental exposures such as air pollution.

We also found infant sex differences in the association between residential distance to nearest major roadway and *ZDBF2* (Zinc Finger DNA binding factor type containing 2). *ZDBF2* is a unique imprinted gene in that it contains both maternal and paternal marks (Duffie et al. 2014). During the pre-implantation period and in the placenta after implantation, the maternal allele is silenced. The paternal allele is silenced in the embryo

after implantation and is maintained in somatic tissues. This transient tissue-specific imprinting supports the hypothesis that alterations during fetal programming can have lasting effects into adulthood (Marsit 2015).

This study had important strengths and limitations. First, the placenta is a heterogeneous organ comprised of many cell types. Previous studies have shown associations between air pollution and alterations in markers of inflammation in adults (Hajat et al. 2015). It is hypothesized, but not yet convincingly demonstrated, that maternal air pollution exposure may induce an inflammatory response in the placenta and fetal membranes (Vadillo-Ortega et al. 2014). We cannot differentiate whether our results reflect purely pollutant-induced changes in placental gene expression, pollutant-induced changes in placental infiltration by inflammatory cells, or some combination of the two. Second, as in any observational study, we cannot exclude the possibility of residual confounding by unknown variables, despite adjusting for a number of established potential confounders including maternal demographic and lifestyle characteristics, infant characteristics, and neighborhood SES. Third, there is potential for exposure misclassification due to unavailable information about residential history, amount of time spent at home, indoor air pollution sources, and housing characteristics. However, we used sophisticated spatial-temporal models to estimate PM_{2.5} and BC exposure at each residential address which reduces the likelihood of exposure misclassification in comparison to the commonly-used approach of basing exposure estimates solely on data from a network of sparse regulatory monitors. Fourth, we performed a large number of statistical hypothesis tests and some of the findings could reflect false positives. However, to mitigate this possibility we used a two-stage approach in which we first examined specific *a priori* hypotheses based on a limited set of candidate genes to reduce the number of statistical tests. The second stage was an agnostic approach in which we controlled for multiple comparisons using the Benjamini-Hochberg method and additionally using the more conservative Bonferroni correction. Lastly, this study focused on expression of imprinted genes, and not on the underlying control of their expression through DNA methylation in imprinting control regions, as we make the assumption that the alterations observed given these exposures and in the context of a normative cohort are unlikely to result in overt changes to imprinting status which would be facilitated by altered ICR methylation. Future studies could more completely interrogate the epigenetic underpinnings of these observed variations.

On the other hand, unique strengths of our study include a large, well-characterized study population with detailed measures of placental gene expression, a novel mechanistic hypothesis, and a combination of hypothesis-driven and agnostic analytic approaches. Indeed, to our knowledge, this is the first study to investigate the association between maternal air pollution exposure and placental expression of imprinted genes.

5. Conclusions

We found that maternal exposure to residential PM_{2.5} and BC was associated with changes in expression of imprinted genes in placenta. These findings suggest a plausible line of investigation of how air pollution affects growth and development. As this is the first study

to examine the association between maternal air pollution exposures and placental imprinted gene expression, additional studies are clearly needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute of Mental Health [R01MH094609], the National Institute of Environmental Health Sciences [R01ES022223, P01 ES022832], the National Institutes of Health [R21-ES023073] and a graduate student fellowship from the Institute at Brown for Environment and Society [to S.L.K.]. The contents of this report are solely the responsibility of the authors and do not necessarily represent the official views of the sponsoring organizations.

References

- Abu-Amro S, Monk D, Frost J, Preece M, Stanier P, Moore GE. The genetic aetiology of Silver-Russell syndrome. *Journal of medical genetics*. 2008; 45:193–199. [PubMed: 18156438]
- Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society*. 1995; 57:289–300.
- Bhattacharjee J, Jetta F, Giacomello E, Bechi N, Romagnoli R, Fava A, Paulesu L. Expression and localization of ATP binding cassette transporter A1 (ABCA1) in first trimester and term human placenta. *Placenta*. 2010; 31:423–430. [PubMed: 20338636]
- Bourque DK, Avila L, Penaherrera M, von Dadelszen P, Robinson WP. Decreased placental methylation at the H19/IGF2 imprinting control region is associated with normotensive intrauterine growth restriction but not preeclampsia. *Placenta*. 2010; 31:197–202. [PubMed: 20060582]
- Chamberlain SJ. RNAs of the human chromosome 15q11-q13 imprinted region. *Wiley interdisciplinary reviews RNA*. 2013; 4:155–166. [PubMed: 23208756]
- Chiu YH, Hsu HH, Coull BA, Bellinger DC, Kloog I, Schwartz J, Wright RO, Wright RJ. Prenatal particulate air pollution and neurodevelopment in urban children: Examining sensitive windows and sex-specific associations. *Environment international*. 2016; 87:56–65. [PubMed: 26641520]
- Cordeiro A, Neto AP, Carvalho F, Ramalho C, Doria S. Relevance of genomic imprinting in intrauterine human growth expression of CDKN1C, H19, IGF2, KCNQ1 and PHLDA2 imprinted genes. *Journal of assisted reproduction and genetics*. 2014; 31:1361–1368. [PubMed: 24986528]
- Dadvand P, Parker J, Bell ML, Bonzini M, Brauer M, Darrow LA, Gehring U, Glinianaia SV, Gouveia N, Ha EH, Leem JH, van den Hooven EH, Jalaludin B, Jesdale BM, Lepeule J, Morello-Frosch R, Morgan GG, Pesatori AC, Pierik FH, Pless-Mulloli T, Rich DQ, Sathyanarayana S, Seo J, Slama R, Strickland M, Tamburic L, Wartenberg D, Nieuwenhuijsen MJ, Woodruff TJ. Maternal exposure to particulate air pollution and term birth weight: a multi-country evaluation of effect and heterogeneity. *Environmental health perspectives*. 2013; 121:267–373. [PubMed: 23384584]
- Diez Roux AV, Merkin SS, Arnett D, Chambless L, Massing M, Nieto FJ, Sorlie P, Szklo M, Tyroler HA, Watson RL. Neighborhood of residence and incidence of coronary heart disease. *The New England journal of medicine*. 2001; 345:99–106. [PubMed: 11450679]
- Diplas AI, Lambertini L, Lee M-J, Sperling R, Lee YL, Wetmur JG, Chen J. Differential expression of imprinted genes in normal and IUGR human placentas. *Epigenetics*. 2014; 4:235–240.
- Duffie R, Ajjan S, Greenberg MV, Zamudio N, Escamilla del Arenal M, Iranzo J, Okamoto I, Barbaux S, Fauque P, Bourc'his D. The Gpr1/Zdbf2 locus provides new paradigms for transient and dynamic genomic imprinting in mammals. *Genes & development*. 2014; 28:463–478. [PubMed: 24589776]
- EPA. Air Quality Statistics Report. 2010.
- EPA. , editor. EPA. National Ambient Air Quality Standards for Particulate Matter. 2013.
- Fowden AL, Sibley C, Reik W, Constancia M. Imprinted genes, placental development and fetal growth. *Hormone Research*. 2006; 65:50–58. [PubMed: 16612114]

- Green BB, Kappil M, Lambertini L, Armstrong DA, Guerin DJ, Sharp AJ, Lester BM, Chen J, Marsit CJ. Expression of imprinted genes in placenta is associated with infant neurobehavioral development. *Epigenetics*. 2015; 10:834–841. [PubMed: 26198301]
- Gryparis A, Coull BA, Schwartz J, Suh HH. Semiparametric latent variable regression models for spatiotemporal modelling of mobile source particles in the greater Boston area. *J Roy Stat Soc C-App*. 2007; 56:183–209.
- Hajat A, Allison M, Diez-Roux AV, Jenny NS, Jorgensen NW, Szpiro AA, Vedal S, Kaufman JD. Long-term exposure to air pollution and markers of inflammation, coagulation, and endothelial activation: a repeat-measures analysis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Epidemiology*. 2015; 26:310–320. [PubMed: 25710246]
- Hernán MA, Hernández-Díaz S, Werler MM, Mitchell AA. Causal Knowledge as a Prerequisite for Confounding Evaluation: An Application to Birth Defects Epidemiology. *American journal of epidemiology*. 2002; 155:176–184. [PubMed: 11790682]
- Hoyo C, Murphy SK, Jirtle RL. Imprint regulatory elements as epigenetic biosensors of exposure in epidemiological studies. *Journal of epidemiology and community health*. 2009; 63:683–684. [PubMed: 19679714]
- Jirtle RL. Imprinted Genes: by Species. *Imprinted Gene Databases*. 2012
- Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nature reviews Genetics*. 2007; 8:253–262.
- Jongmans MC, Admiraal RJ, van der Donk KP, Vissers LE, Baas AF, Kapusta L, van Hagen JM, Donnai D, de Ravel TJ, Veltman JA, Geurts van Kessel A, De Vries BB, Brunner HG, Hoefsloot LH, van Ravenswaaij CM. CHARGE syndrome: the phenotypic spectrum of mutations in the CHD7 gene. *Journal of medical genetics*. 2006; 43:306–314. [PubMed: 16155193]
- Kappil M, Green B, Armstrong D, Sharp A, Lambertini L, Marsit C, Chen J. Placental expression profile of imprinted genes impacts birth weight. *Epigenetics*. 2015a; 10:842–849. [PubMed: 26186239]
- Kappil M, Lambertini L, Chen J. Environmental Influences on Genomic Imprinting. *Current environmental health reports*. 2015b; 2:155–162. [PubMed: 26029493]
- Kingsley SL, Eliot MN, Whitsel EA, Huang YT, Kelsey KT, Marsit CJ, Wellenius GA. Maternal residential proximity to major roadways, birth weight, and placental DNA methylation. *Environment international*. 2016; 92–93:43–49.
- Kloog I, Chudnovsky AA, Just AC, Nordio F, Koutrakis P, Coull BA, Lyapustin A, Wang Y, Schwartz J. A new hybrid spatio-temporal model for estimating daily multi-year PM2.5 concentrations across northeastern USA using high resolution aerosol optical depth data. *Atmospheric Environment*. 2014; 95:581–590.
- Lambertini L, Lee MJ, Marsit CJ, Chen J. Genomic Imprinting in Human Placenta. In: Zheng, DJ., editor. *Recent Advances in Research on the Human Placenta*: InTech. 2012a.
- Lambertini L, Marsit C, Sharma P, Maccani M, Ma Y, Hu J, Chen J. Imprinted gene expression in fetal growth and development. *Placenta*. 2012b; 33:480–486. [PubMed: 22465419]
- Lamichhane DK, Leem JH, Lee JY, Kim HC. A meta-analysis of exposure to particulate matter and adverse birth outcomes. *Environmental health and toxicology*. 2015; 30:e2015011. [PubMed: 26796890]
- MacIsaac JL, Bogutz AB, Morrissy AS, Lefebvre L. Tissue-specific alternative polyadenylation at the imprinted gene Mest regulates allelic usage at Copg2. *Nucleic acids research*. 2012; 40:1523–1535. [PubMed: 22053079]
- Marsit CJ. Influence of environmental exposure on human epigenetic regulation. *The Journal of experimental biology*. 2015; 218:71–79. [PubMed: 25568453]
- Marsit CJ, Maccani MA, Padbury JF, Lester BM. Placental 11-Beta Hydroxysteroid Dehydrogenase Methylation Is Associated with Newborn Growth and a Measure of Neurobehavioral Outcome. *PLoS One*. 2012; 7:e33794. [PubMed: 22432047]
- McMinn J, Wei M, Schupf N, Cusmai J, Johnson EB, Smith AC, Weksberg R, Thaker HM, Tycko B. Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. *Placenta*. 2006; 27:540–549. [PubMed: 16125225]

- Minkiewicz J, de Rivero Vaccari JP, Keane RW. Human astrocytes express a novel NLRP2 inflammasome. *Glia*. 2013; 61:1113–1121. [PubMed: 23625868]
- Miozzo M, Simoni G. The role of imprinted genes in fetal growth. *Biol Neonate*. 2002; 81:217–228. [PubMed: 12011565]
- Morison I, Paton C, Cleverley S. The imprinted gene and parent-of-origin effect database. *Nucleic acids research*. 2001
- Pagon R, G J Jr, Zonana J, Yong S. Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: CHARGE association. *The Journal of pediatrics*. 1981; 99:223–227. [PubMed: 6166737]
- Piedrahita JA. The role of imprinted genes in fetal growth abnormalities. *Birth defects research Part A, Clinical and molecular teratology*. 2011; 91:682–692. [PubMed: 21648055]
- Reik W, Walter J. Genomic Imprinting: Parental Influence on the Genome. *Nature Reviews Genetics*. 2001; 2:21–32.
- Rosenthal R, Rubin DB. Multiple Contrasts and Ordered Bonferroni Procedures. *Journal of Educational Psychology*. 1984; 76:1028–1034.
- Saenen ND, Plusquin M, Bijlens E, Janssen BG, Gyselaers W, Cox B, Fierens F, Molenberghs G, Penders J, Vrijens K, De Boever P, Nawrot TS. In Utero Fine Particle Air Pollution and Placental Expression of Genes in the Brain-Derived Neurotrophic Factor Signaling Pathway: An ENVIRONAGE Birth Cohort Study. *Environmental health perspectives*. 2015; 123:834–840. [PubMed: 25816123]
- Sandovici I, Hoelle K, Angiolini E, Constancia M. Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming. *Reproductive biomedicine online*. 2012; 25:68–89. [PubMed: 22560117]
- Sapkota A, Chelikowsky AP, Nachman KE, Cohen AJ, Ritz B. Exposure to particulate matter and adverse birth outcomes: a comprehensive review and meta-analysis. *Air Quality, Atmosphere & Health*. 2010; 5:369–381.
- Silver MJ, Kessler NJ, Hennig BJ, Dominguez-Salas P, Laritsky E, Baker MS, Coarfa C, Hernandez-Vargas H, Castellino JM, Routledge MN, Gong YY, Herceg Z, Lee YS, Lee K, Moore SE, Fulford AJ, Prentice AM, Waterland RA. Independent genomewide screens identify the tumor suppressor VTRNA2-1 as a human epiallele responsive to periconceptional environment. *Genome biology*. 2015; 16:118. [PubMed: 26062908]
- Stieb DM, Chen L, Eshoul M, Judek S. Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. *Environmental research*. 2012; 117:100–111. [PubMed: 22726801]
- Suades-Gonzalez E, Gascon M, Guxens M, Sunyer J. Air Pollution and Neuropsychological Development: A Review of the Latest Evidence. *Endocrinology*. 2015; 156:3473–3482. [PubMed: 26241071]
- Sun X, Luo X, Zhao C, Zhang B, Tao J, Yang Z, Ma W, Liu T. The associations between birth weight and exposure to fine particulate matter (PM_{2.5}) and its chemical constituents during pregnancy: A meta-analysis. *Environmental pollution*. 2016; 211:38–47. [PubMed: 26736054]
- Vadillo-Ortega F, Osornio-Vargas A, Buxton MA, Sanchez BN, Rojas-Bracho L, Viveros-Alcaraz M, Castillo-Castrejon M, Beltran-Montoya J, Brown DG, O'Neill MS. Air pollution, inflammation and preterm birth: a potential mechanistic link. *Medical hypotheses*. 2014; 82:219–224. [PubMed: 24382337]
- Waggott, DM. NanoStringNorm: Normalize Nanostring miRNA and mRNA data. R package version 1.1.17. 2014.
- Weksberg R, Shuman C, Beckwith JB. Beckwith-Wiedemann syndrome. *European journal of human genetics : EJHG*. 2010; 18:8–14. [PubMed: 19550435]
- Zentner GE, Layman WS, Martin DM, Scacheri PC. Molecular and phenotypic aspects of CHD7 mutation in CHARGE syndrome. *American journal of medical genetics Part A*. 2010; 152A:674–686. [PubMed: 20186815]
- Zhu X, Liu Y, Chen Y, Yao C, Che Z, Cao J. Maternal exposure to fine particulate matter (PM_{2.5}) and pregnancy outcomes: a meta-analysis. *Environmental science and pollution research international*. 2015; 22:3383–3396. [PubMed: 25163563]

Zollino M, Orteschi D, Marangi G, De Crescenzo A, Pecile V, Riccio A, Neri G. A case of Beckwith-Wiedemann syndrome caused by a cryptic 11p15 deletion encompassing the centromeric imprinted domain of the BWS locus. *Journal of medical genetics*. 2010; 47:429–432. [PubMed: 19843502]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- The mechanism by which air pollution exposure impacts fetal growth remains unknown.
- Placental imprinted genes are important regulators of fetal growth.
- Air pollution exposure and expression of placental imprinted genes was studied.
- Air pollution was associated with changes in placental imprinted gene expression.
- This suggests a potential mechanism of how air pollution may affect fetal growth.

Figure 1. Percent change in candidate gene expression (95% CI) per interquartile range (IQR)^a increase in PM_{2.5} and black carbon (BC) and for living near major roadways compared to far from major roadways.

^aIQR is 0.87 $\mu\text{g}/\text{m}^3$ for PM_{2.5} and 0.14 $\mu\text{g}/\text{m}^3$ for black carbon.

Figure 2. Percent change in gene expression (95% CI) per interquartile range (IQR)^a increase in PM_{2.5} that reached q-value < 0.05 (black circles) and survived Bonferroni correction (red triangles). Ordered by change in gene expression.
^aIQR is 0.87 µg/m³ for PM_{2.5}

Figure 3. Percent change in gene expression (95% CI) per interquartile range (IQR)^a increase in black carbon that reached q-value<0.05 (black circles) and survived Bonferroni correction (red triangles). Ordered by change in gene expression.
^aIQR is 0.14 $\mu\text{g}/\text{m}^3$ for black carbon

Table 1Characteristics of study population, by tertiles of average maternal residential PM_{2.5} over entire pregnancy

Variables	Mean ± SD or N (%)			
	Total (n=410) ^a	Tertile 1 7.6 µg/m ³ (n=137)	Tertile 2 (7.6, 8.22] µg/m ³ (n=136)	Tertile 3 >8.22 µg/m ³ (n=137)
Maternal Characteristics				
Age, years, mean + - SD	30.0 ± 5.6	29.8 ± 5.8	30.1 ± 5.5	30.2 ± 5.4
BMI before pregnancy, kg/m ² Parity	27.1 ± 7.0	26.4 ± 7.0	27.2 ± 6.5	27.7 ± 7.4
1	108 (26.3)	41 (29.9)	38 (27.9)	29 (21.2)
2	142 (34.6)	43 (31.4)	53 (39.0)	46 (33.6)
>3	159 (38.8)	53 (38.7)	44 (32.4)	62 (45.3)
Race, Caucasian	314 (76.6)	106 (77.4)	98 (72.1)	110 (80.3)
Education				
< High school	89 (21.7)	30 (21.9)	28 (20.6)	31 (22.6)
Some college	101 (24.6)	32 (23.4)	35 (25.7)	34 (24.8)
College or more	216 (52.7)	74 (54.0)	70 (51.5)	72 (52.6)
Income				
<\$25,000	85 (20.7)	26 (19.0)	27 (19.9)	32 (23.4)
\$25,000–49,999	64 (15.6)	18 (13.1)	24 (17.6)	22 (16.1)
\$50,000–79,999	73 (17.8)	23 (16.8)	25 (18.4)	25 (18.2)
\$80,000–99,999	52 (12.7)	14 (10.2)	21 (15.4)	17 (12.4)
\$100,000	98 (23.9)	40 (29.2)	24 (17.6)	34 (24.8)
Married	263 (64.1)	90 (65.7)	85 (62.5)	88 (64.2)
Health Insurance				
None/self-pay	7 (1.7)	2 (1.5)	1 (0.7)	4 (2.9)
Private	248 (60.5)	82 (59.9)	83 (61.0)	83 (60.6)
Public/other	149 (36.3)	53 (38.7)	50 (36.8)	46 (33.6)
No prenatal care during pregnancy	4 (1.0)	2 (1.5)	0 (0.0)	2 (1.5)
No prenatal vitamin use	24 (5.9)	3 (2.2)	11 (8.0)	10 (7.3)
Tobacco use during pregnancy	19 (4.6)	6 (4.4)	5 (3.6)	8 (5.8)
Alcohol use during pregnancy	4 (1.0)	1 (0.7)	2 (1.5)	1 (0.7)
Infant Characteristics				
Gestational age (weeks), mean ± SD	39.0 ± 0.9	39.0 ± 1.0	38.9 ± 0.9	39.1 ± 0.9
Infant sex, Male	213 (52.0)	61 (44.5)	71 (52.2)	81 (59.1)
Infant growth status				
SGA	68 (16.6)	22 (16.1)	28 (20.6)	18 (13.1)
AGA	226 (55.1)	81 (59.1)	71 (52.2)	74 (54.0)
LGA	116 (28.3)	34 (24.8)	37 (27.2)	45 (32.8)

Variables	Mean $\pm$ SD or N (%)			
	Total (n=410) ^a	Tertile 1 7.6 $\mu\text{g}/\text{m}^3$ (n=137)	Tertile 2 (7.6, 8.22] $\mu\text{g}/\text{m}^3$ (n=136)	Tertile 3 >8.22 $\mu\text{g}/\text{m}^3$ (n=137)
Neighborhood SES, sum of z-score ^b	-0.09 $\pm$ 5.4	0.45 $\pm$ 5.7	-0.57 $\pm$ 5.2	-0.17 $\pm$ 5.2
Residential air pollution				
PM _{2.5} ($\mu\text{g}/\text{m}^3$) [*]	7.96 $\pm$ 0.65	7.27 $\pm$ 0.27	7.93 $\pm$ 0.18	8.69 $\pm$ 0.38
BC ($\mu\text{g}/\text{m}^3$)	0.46 $\pm$ 0.09	0.46 $\pm$ 0.10	0.47 $\pm$ 0.09	0.45 $\pm$ 0.09
Near major roadway ^c	86 (21.0)	30 (21.9)	22 (16.2)	34 (24.8)

Abbreviations: SD is standard deviation, BMI is body mass index, PM_{2.5} is fine particulate matter, BC is black carbon, and SES is socioeconomic status.

^aNot all N values equal 410 because of missing values and not all percentages add to 100% due to rounding.

^bNeighborhood SES z-sum is the sum of the z-scores for median household income, percent of households with interests, dividends, or rent income, percent of residents with high school diploma, percent with college degree, percent with professional occupation, and median value of owner-occupied housing units.

^cNear major roadway defined as 150 m of A1/A2 or 50 m of A3.

^{*}p<0.05; p-values obtained from chi square tests for categorical variables and one-way ANOVA tests for continuous variables

Table 2

Percent change (95% confidence interval) in expression of *CHD7* per interquartile range (IQR)^a increase in PM_{2.5} and of *ZDBF2* for living near major roadways compared to far from major roadways, by infant sex

Imprinted Gene	Male	Female	p-value ^b	q-value ^b
<i>CHD7</i>	14.7 (4.8, 25.7)	-8.4 (-14.3, -2.2)	0.0001	0.012
<i>ZDBF2</i>	-16.5 (-28.4, -2.6)	18.1 (3.0, 35.5)	0.0003	0.03

Note: Models are adjusted for maternal age, maternal education, tobacco, prenatal vitamin use, maternal BMI, parity, annual household income, maternal insurance, marital status, maternal race, neighborhood SES.

^aIQR is 0.87 µg/m³

^bHeterogeneity p-value and q-value