Intrauterine multi-metal exposure is associated with reduced fetal growth through modulation of the placental gene network

Maya A Deyssenroth, Icahn School of Medicine at Mount Sinai
Chris Gennings, Icahn School of Medicine at Mount Sinai
Shelley H Liu, Icahn School of Medicine at Mount Sinai
Shouneng Peng, Icahn School of Medicine at Mount Sinai
Ke Hao, Icahn School of Medicine at Mount Sinai
Luca Lambertini, Icahn School of Medicine at Mount Sinai
Brian P Jackson, Dartmouth College
Margaret R Karagas, Geisel School of Medicine at Dartmouth
Carmen Marsit, Emory University
Jia Chen, Icahn School of Medicine at Mount Sinai

Journal Title: Environment International
Volume: Volume 120
Publisher: Pergamon-Elsevier Science LTD | 2018-11-01, Pages 373-381
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1016/j.envint.2018.08.010
Permanent URL: https://pid.emory.edu/ark:/25593/vh3dv

Final published version: http://dx.doi.org/10.1016/j.envint.2018.08.010

Copyright information:
© 2018 Elsevier Ltd

Accessed December 27, 2023 4:17 PM EST
Intrauterine multi-metal exposure is associated with reduced fetal growth through modulation of the placental gene network

Maya A. Deyssenroth, Chris Gennings, Shelley H. Liu, Shouneng Peng, Ke Hao, Luca Lambertini, Brian P. Jackson, Margaret R. Karagas, Carmen J. Marsit, Jia Chen

Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Center for Biostatistics, Department of Population Health Science and Policy, Icahn School of Medicine at Mount Sinai, New York, NY 20019, USA

Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Department of Obstetrics, Gynecology and Reproductive Science, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Department of Earth Sciences, Dartmouth College, Hanover, NH 03755, USA

Department of Epidemiology, Geisel School of Medicine at Dartmouth, USA

Department of Environmental Health, Emory University, Atlanta, GA 30322, USA

Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Department of Medicine, Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

ABSTRACT

Background: Intrauterine metal exposures and aberrations in placental processes are known contributors to being born small for gestational age (SGA). However, studies to date have largely focused on independent effects, failing to account for potential interdependence among these markers.

Objectives: We evaluated the inter-relationship between multi-metal indices and placental gene network modules related to SGA status to highlight potential molecular pathways through which in utero multi-metal exposure impacts fetal growth.

Methods: Weighted quantile sum (WQS) regression was performed using a panel of 16 trace metals measured in post-partum maternal toe nails collected from the Rhode Island Child Health Study (RICHS, n = 195), and confirmation of the derived SGA-related multi-metal index was conducted using Bayesian kernel machine regression (BKMR). We leveraged existing placental weighted gene coexpression network data to examine associations between the SGA multi-metal index and placental gene expression. Expression of select genes were assessed using RT-PCR in an independent birth cohort, the New Hampshire Birth Cohort Study (NHBCS, n = 237).

Results: We identified a multi-metal index, predominated by arsenic (As) and cadmium (Cd), that was positively associated with SGA status (Odds ratio = 2.73 [1.04, 7.18]). This index was also associated with the expression of placental gene modules involved in "gene expression" (β = −0.02 [−0.04, −0.01]) and "metabolic hormone secretion" (β = 0.02 [0.00, 0.05]). We validated the association between cadmium exposure and the expression of GRHL1 and INHBA, genes in the "metabolic hormone secretion" module, in NHBCS.

Conclusion: We present a novel approach that integrates the application of advanced bioinformatics and biostatistics methods to delineate potential placental pathways through which trace metal exposures impact fetal growth.

1. Introduction

Being born small for gestational age (SGA) is a major determinant of childhood and later life morbidity, including metabolic syndrome, neurodevelopmental deficits and coronary heart disease (Arcangeli et al., 2012; Jancevska et al., 2012). Established risk factors known to impact fetal growth include maternal age, parity and ethnicity (Jancevska et al., 2012). In addition to maternal characteristics, gestational exposure to environmental pollutants through maternal ingestion and inhalation are also known to play a role (Chou et al., 2011; Lauritzen et al., 2016; Peelen et al., 2016; Stillerman et al., 2008). Multiple studies to date have linked intrauterine trace metal levels to...
SGA status. These include exposure to elevated levels of toxic metals (i.e., arsenic (Claus Henn et al., 2016; Thomas et al., 2015), cadmium (Cheng et al., 2017; Johnston et al., 2014; Sun et al., 2014) and lead (Nishioka et al., 2014; Taylor et al., 2015), reduced levels of essential elements (i.e., copper, zinc and iron (Shen et al., 2015)) and several studies demonstrating curvilinear associations (i.e., manganese (Chen et al., 2014; Xia et al., 2016)). However, inconsistencies in the literature persist (Bermúdez et al., 2015; Loiacono et al., 1992; Osman et al., 2000; Thomas et al., 2015).

While heterogeneity in study designs likely plays an important role, the discrepancy may also reflect a focus on assessing the effects of individual metals. Such methods fail to account for potential mixture compositions in which the presence of toxic and essential co-pollutants at varying doses may alter the activity of the metal under consideration. While findings are beginning to emerge demonstrating modified effects within the context of two metals at a time (Al-Saleh et al., 2015; Everson et al., 2017), the role of the multi-metal environment on deviations of appropriate fetal growth is still underexplored.

The molecular pathways through which metals exert their effect on fetal growth are not clearly delineated. However, several studies point to the possibility that in utero exposure to metals at toxic levels may induce aberrations in processes mediated by the placenta, the organ overseeing appropriate fetal development (Gundacker and Hengstschläger, 2012). Alterations in the gene expression and DNA methylation profile of several placental loci, including genes involved in nutrient transport, endocrine signaling and imprinting, have been linked to fetal growth (Caviedes et al., 2016; Chen et al., 2015; Green et al., 2015; Kappil et al., 2015; Lesieur et al., 2013; Sabri et al., 2014). However, similar to studies linking trace metals to fetal growth, molecular biomarker studies thus far have focused on associations between individual genes and fetal growth. As biological processes are driven by interacting gene-sets, testing the independent association of individual genes likely results in information loss on the biologic context within which perturbations occur. In an effort to address the co-regulated organizational structure of genes, we recently delineated the human placental coexpression network and demonstrated deviations in specific network modules linked to aberrant fetal growth (Deyssenroth et al., 2017).

Similar to the bioinformatics methods developed to analyze genes within network contexts, novel statistical approaches that are able to model and delineate the independent and joint effects across multiple correlated exposures, are now available to address the gap in the literature regarding exposure response relationships (Liu et al., 2017; Stafoggia et al., 2017). These include weighted quantile sum (WQS) regression (Carrico et al., 2014) and Bayesian kernel machine regression (BKMR) (Bobb et al., 2015). While the exposure-response relationship modeled by the WQS-derived body burden index is constrained to linear, unidirectional associations, the machine learning based BKMR method allows more flexible modeling of the relationship between co-pollutants and the outcome. The former approach lends itself for enhanced interpretability of the findings while the latter approach allows for more in-depth evaluation of potentially complex, non-linear and non-additive exposure-response relationships.

In the current study, we integrate the application of novel biostatistics and bioinformatics approaches to identify an SGA-related multi-metal index and assess whether SGA-related placental gene networks are associated with this multi-metal index to highlight potential molecular pathways through which in utero trace metal exposure impacts fetal growth.

2. Materials and methods

2.1. Study participants

Mother-infant pairs were enrolled in the Rhode Island Child Health Study between 2009 and 2013, following delivery at Women and Infants Hospital (n = 899) (Kappil et al., 2015). Enrollment was restricted to mothers ≥18 years of age and infants without congenital or chromosomal abnormalities. Infants born small for gestational age (SGA, < 10% percentile) and large for gestational age (LGA, > 90% percentile), based on the sex-specific actual-age 2013 Fenton Growth Chart (Fenton and Kim, 2013), were matched on gender, gestational age and maternal age to infants born appropriate for gestational age (AGA). Anthropometric and clinical in-patient data were collected through structured reviews of medical records. Interview-based questionnaires were administered after delivery and prior to hospital discharge to collect demographic characteristics and exposure histories. Written informed consent was obtained from all enrolled participants, and the study was approved by the institutional review boards at Women and Infants Hospital and Emory University. The current study included SGA and AGA infants with complete molecular profile (placental RNA-Seq) and metal exposure data (n = 195).

Findings relating metal exposure to placental gene expression were validated in an independent cohort, the New Hampshire Birth Cohort Study (NHBCS), among SGA and AGA participants with available extracted placental RNA (n = 237). The NHBCS is a prospective birth cohort initially designed to assess the impact of intrauterine environmental exposures on child health and development. Participants were recruited from prenatal clinics in New Hampshire starting in 2009 (Emond et al., 2018). Similar to the RICHS cohort, available data in NHBCS includes Fenton growth curve measurements and metal levels assessed in maternal postpartum toenails. In contrast to RICHS, the NHBCS cohort was not oversampled for extreme birth weight groups and is therefore more reflective of growth distributions observed in the general population (Supplemental Table 1).

2.2. Metal assessment

First toenail clippings were requested from mothers and infants following discharge, and clippings were mailed back in provided envelopes. In RICHS, average time to collection was 2.8 months (range, 0.3–7.1 months) postpartum, while in NHBCS, all toenails were collected within 2–8 weeks postpartum. A panel of nineteen trace metals (silver (Ag), aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), uranium (U), vanadium (V), and zinc (Zn)) were analyzed at the Dartmouth Trace Element Analysis laboratory using standardized ICP-MS protocols. Briefly, visible dirt was manually removed from toenail samples and toenails were further cleaned with five washes in an ultrasonic bath using Triton X-100 and acetone followed by deionized water. Toenails were allowed to dry prior to low-pressure microwave digestion and ICP-MS analysis. Quality control measures included the use of certified reference materials (Japanese hair standard NIES #13), analytical duplicates and spikes, initial and continuing calibration verification and digestion of fortified blanks (Punshon et al., 2016; White et al., 2018). The current study focused on the measurements derived from maternal toenails. Three metals with >10% missing values (values below limit of detection (LOD) that fell below calibration blank), Ag, Co and Hg, were excluded from the analysis to maintain adequate sample size. Remaining measurement values falling below the LOD were replaced by the LOD/√2.

2.3. Placental gene network

A placental gene coexpression network consisting of 17 network modules was generated from available placental RNA-Seq data using the “WGCNA: weighted correlation network analysis” package in R as previously described (Deyssenroth et al., 2017). The first principal component of each module, the module eigengene, was derived as an average measure of module gene expression.
2.4. Quantitative real time PCR (qRT-PCR) validation

Genes from arsenic (C21orf91, KIAA1370, INO80D) and cadmium (GRHL1, INHBA, LEP) responsive placental network modules were selected for qRT-PCR verification in an independent cohort (NHBCS) based on connectivity within the module (kME > 0.8), association with the derived WQS index and availability of published PCR assays. In a preliminary study of 30 NHBCS participants (15 participants in the top quartile of exposure (As and Cd) and 15 participants in the lowest quartile of exposure (As and Cd), we did not observe differential expression among the As-responsive genes and As exposure while a trend towards significance was observed in the association between expression of the Cd-responsive genes and Cd exposure (data not shown). Based on these preliminary findings and due to limited RNA availability, the remainder of the NHBCS cohort was analyzed using the Cd-responsive genes.

Placental RNA samples were reverse-transcribed using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) following manufacturer’s protocol. Based on an evaluation of the RICHS RNAseq data for a panel of commonly utilized housekeeping genes (GAPDH, ACTB, SDHD, RPL19, RPL0 and UBOQ), RPL19 demonstrated the lowest coefficient of variation across the placental samples and was selected as the endogenous control gene in the current study. All samples were run in triplicate, alongside non-template controls and pooled internal controls for each assay (GRHL1, INHBA, LEP and RPL19) on each plate. Previously published primer sequences were utilized for INHBA (Katayama et al., 2017) and LEP (Haffejee et al., 2017), and all remaining assays were designed using the NCBI Primer-BLAST tool (Supplemental Table 2). The qRT-PCR reactions were conducted on a LightCycler 480 II Instrument (Roche Diagnostics Corporation, Indianapolis, IN) using the following thermocycling conditions: an initial denaturation step at 95 °C for 10 min, followed by 45 cycles at 95 °C for 30 s, 60 °C at 30 s and 72 °C at 30 s.

2.5. Statistical analysis

Differences in maternal-infant demographic and gestational characteristics across birth weight categories and study participants were assessed using a Mann-Whitney U Test for continuous variables and a chi-square test for categorical variables.

The association between exposure to individual metals (log2 transformed) and SGA status was assessed using logistic regression models. To account for the collinearity among metals, weighted quantile sum (WQS) regression analysis was conducted to derive metal mixture indices associated with SGA status using the “gWQS: generalized weighted quantile sum regression” package in R (Carrico et al., 2014; Czarnota et al., 2015; Renzetti et al., 2016). The weights assigned to the individual, tertile-scored metals within the composite index were simultaneously derived based on bootstrap sampling (n = 200), with the finalized weighted quantile score (WQS) estimated as the mean of the estimated estimates across the bootstrap samples. Two separate WQS indices were derived, an index positively associated with SGA status and an index negatively associated with SGA status, each of the associations between exposure in an independent cohort (NHBCS) and SGA status was additionally assessed using logistic regression models.

Generalized linear regression models were conducted to assess the association between SGA-associated metals (individual and WQS composite index) and SGA-associated network module eigengenes.

We also assessed gene-metal associations in an in silico analysis, interrogating the Comparative Toxicogenomics Database (Davis et al., 2017) for all gene associations reported for the 16 trace metals included in our analysis. The placental gene network was cross-referenced against this curated list of known gene-metal interactions to identify modules enriched for metal-responsive genes using a Fisher’s exact test (FDR < 0.25).

NHBCS qRT-PCR data were preprocessed and analyzed as follows. Cycle threshold (Cv) values ≥ 35 cycles were considered non-detectable. For each assay, samples with ≥ 2 non-detectable measurements were removed from the analysis, leaving a sample size of 262, 267 and 260 for the GRHL1, INHBA and LEP assays, respectively. Among these participants with placental expression data on GRHL1, INHBA and LEP, maternal toenail Cd levels were available for 180, 178, and 176 subjects, respectively. Triplicate Cv values for each sample/assay were averaged, and ΔCv values for each sample/assay were calculated using the following formula: ΔC = (C(TPL19) − C(Target)). Generalized linear models were evaluated assessing the association between maternal toenail Cd levels and expression of the three selected genes.

In the RICHS dataset, all regression models were adjusted for infant gender, maternal ethnicity, maternal BMI and maternal smoking status during pregnancy based on observed differences in these variables across birth weight categories (p < 0.20). Metal analyses were additionally adjusted for metal assay batch. For the qRT-PCR evaluation among the NHBCS samples, models were adjusted for PCR plate batch, infant gender, maternal BMI and maternal ever smoking status (> 95% of women reported white ethnicity).

All analyses were conducted using R version 3.3.1.

3. Results

The maternal-infant demographic and gestational characteristics among RICHS study participants are shown in Table 1. On average, birth weight was lower among SGA infants than AGA infants, as expected. A greater proportion of non-Caucasian women gave birth to SGA infants than Caucasian women. Additionally, a higher proportion of SGA infants were born among women who reported smoking during pregnancy than those who did not. No other clear differences in maternal-infant characteristics were observed across the two birth weight categories. The SGA/AGA subset of the RICHS cohort with available trace metal data differed from those without available trace metal data (Supplemental Table 3). Women who provided postpartum toenails were older, attained higher educational status and were less likely to smoke than women who did not provide postpartum toenails. The role of socioeconomic status in study retention observed in our study is in line with previous reports in longitudinal cohort studies (Launes et al., 2014).

The distribution of maternal metal levels assessed in RICHS study participants is shown in Table 2. Essential metals, including Zn, Fe, and Se, were detected at elevated levels compared to known toxicants, including Pb, As and Cd.

As shown in Fig. 1, logistic regression models of individual metals and fetal growth restriction indicated borderline increases in the odds of SGA status with increasing As levels (OR = 1.55, 95% CI [0.96, 2.50]) and Cd levels (OR = 1.37, 95% CI [0.96, 2.00]) and a decrease in
the odds of SGA status with increasing Ni levels (OR = 0.78, 95% CI (0.65, 0.94)) that reached statistical significance. In our WQS analysis, we observed a 2.7 increase in the odds of SGA status (OR = 2.73, 95% CI (1.97, 3.80)) for every unit increase in the derived metal mixture index (which represents tertiled metals, each weighted based on its contribution to the overall association with the outcome). The weights of As (44.4%) and Cd (17.9%) predominated in this metal mixture index, indicating their variable importance in driving the observed association. Similarly, a metal mixture index predominated by Ni (23.6%) and Al (22.3%) was significantly associated with decreased odds of SGA status (OR = 0.24, 95% CI [0.08, 0.76]) (Fig. 2). These exposure-outcome relationships were corroborated using BKM R. Here, each metal’s association to the outcome, with all other metals held at the median, also indicated that SGA status was positively associated with As and Cd and inversely associated with nickel (Supplemental Fig. 1). This profile also suggests that As, Cd and Ni are linearly related to SGA status. Further evaluation of bivariate relationships among the metals revealed no interactive relationships among the metals within the BKM R-derived multi-metal index (Supplemental Fig. 2).

We previously characterized the placental gene coexpression network from transcriptome-wide RNASeq data assessed in the same subset of RICHS study participants, and we reported 5 out of 17 derived gene network modules that demonstrated differential module activity across 3 birth weight categories (small, appropriate, and large for gestational age) based on a crude ANOVA analysis (Deyssenroth et al., 2017). Building off these reported findings, we performed an adjusted logistic regression analysis modeling the eigengenes of the 17 derived placental network modules, contrasting SGA and AGA infants. Two of the five previously reported modules maintained a significant association in this more focused analysis adjusted for covariates, with a significant inverse association between the “greenyellow” module and SGA status and a significant positive association between the “salmon” module and SGA status (Fig. 3). The “greenyellow” placental network module consists of 201 genes and is functionally enriched for biological processes related to gene expression based on Gene Ontology analysis, and the “salmon” placental network module consists of 162 genes and is functionally enriched for biological processes related to metabolic hormone secretion (Deyssenroth et al., 2017).

Among the SGA-related modules, a significant inverse association between the gene expression (greenyellow) module and As exposure ($\beta = -0.02 \, [-0.04, \, -0.01]$) as well as the SGA-associated metal mixture index ($\beta = -0.04 \, [-0.07, \, -0.01]$) was sustained using a generalized linear regression model. Similarly, borderline positive associations between the metabolic hormone secretion (salmon) module and Cd exposure ($\beta = 0.01 \, [0.0, \, 0.03]$) as well as the SGA-associated positive WQS index ($\beta = 0.02 \, [0.00, \, 0.05]$) was observed (Fig. 4).

A separate analysis based on mapping a curated list of known gene-metal interactions from the Comparative Toxicogenomics Database (CTD) (Davis et al., 2017) onto our placental gene network revealed an enrichment of metal-responsive genes in several network modules, including a moderate, significant fold enrichment of As-responsive genes in the gene expression (greenyellow) module (OR = 1.39 [0.99, 1.92], Supplemental Fig. 3). A connectivity map of the gene expression
In this study, we identified a multi-metal index predominated by As and Cd that is associated with SGA status. While previous studies have shown independent associations between fetal growth restriction and As as well as Cd exposure, this is the first study to demonstrate that the effect of these metals persist and predominate even after accounting for the presence of correlated co-pollutants. The identification of As and Cd as the predominant SGA-related bad actors among the evaluated panel of metals was substantiated with BKMR, an independent mixture modeling approach. Furthermore, this metal signature was also associated with SGA-related placental gene network modules that are enriched for biological processes related to gene expression and metabolic hormone secretion, implicating potential molecular pathways through which these environmental contaminants exert their intrauterine effect.

Of note, most trace metal levels detected in RICHS participants are on par with reported levels of these metals in other US populations (Slotnick et al., 2005). The observed levels of the predominant metals associated with SGA status in our study, As (mean = 0.049 µg/g, range = 0.012–0.445 µg/g) and Cd (mean = 0.016 µg/g, range = 0.001–0.170 µg/g), are, in fact, lower than what is reported in other populations (Slotnick et al., 2005). Notably, while guidelines for acceptable Cd levels measured in nails are not established, the Agency
for Toxic Substances and Disease Registry (ATSDR) lists As levels detected below 1 ppm in nails as within the normal range (ATSDR, n.d). In our study, all individuals fell well below this stipulated set-point, suggesting that adverse health effects are observable at what is currently accepted as safe human exposure levels. On a broader scale, our findings suggest that accounting for co-pollutant exposures may reveal lower ‘safe level’ thresholds than what is demonstrated by evaluating exposures in isolation.

We identified two SGA-associated placental network modules associated with the multi-metal index. The As/Cd responsiveness of several genes within these modules were previously described. For example, changes in the expression of loci mapped to the gene expression (greenyellow) module, including PAN3 (Yamagishi et al., 2014), SUZ12 (Kim et al., 2012) and a panel of ZNF genes (Severson et al., 2013), were reported in relation to As exposure. Similarly, loci mapped to the metabolic hormone secretion (salmon) module, including ARNT2 (Kluxen et al., 2012) and INHBA (Garrett et al., 2013), are known Cd-responsive genes. To identify metal-responsive genes in our placental gene network on a more comprehensive scale, we mapped studies indexed in the Toxicogenomics database that evaluated metals included in our study onto our placental gene network. Several of the observed enrichments aligned with the biological processes linked to the modules. For example, Fe is enriched in the “grey60” module, which predominates in heme-related genes involved in gas exchange. This analysis also indicated that the gene expression (greenyellow) module is enriched with As-responsive genes. In addition to corroborating previous reports of genes independently associated with As and Cd exposure, our findings on a gene-network level suggest that many of these genes participate in common pathways (i.e., gene expression and metabolic hormone secretion) that may be dysregulated upon exposure.

Both the metal and gene signatures were derived while accounting for the correlated and interactive components in each data-set, allowing us to derive the predominant actors within the respective metal and genetic contexts. Furthermore, our WQS-derived multi-metal index was independently corroborated using the machine learning method, BKMR. While WQS stipulates an additive relationship among the constituents in the mixture in relation to the outcomes, BKMR allows for complex interactions among components of the exposure mixture, and potentially non-linear and non-additive associations between co-pollutant exposures and the health endpoint. This added level of granularity enabled us to verify that As and Cd are linearly related to the outcome and rule out potential multiplicative interactions among the analyzed metals. Additionally, we were able to confirm our expression-related findings in a select group of genes both in an independent cohort and using a different technological platform. Given that the NHBCS differs in several key demographic characteristics from the RICHS, most notably in terms of birth weight distributions and maternal ethnicity, the confirmatory analysis suggests our findings are generalizable beyond our study.

Important caveats warrant caution in the interpretation of the findings reported herein. The co-exposure effect examined in the current study was limited to the available measurements of 16 trace metals. The maternal environment is likely influenced by a wider breadth of exogenous and endogenous factors, such as persistent organic pollutants and prenatal distress, which were not captured in the reported findings.

While gene expression levels were measured in the placenta, metal levels were measured in maternal toenails. This is a pertinent
consideration as agreement between placental and maternal toenail levels were previously reported for some but not all assessed metals (Punshon et al., 2016). The observed discrepancies between placental and toenail metal levels can arise due to several reasons. For example, despite extensive washing steps in the processing of the toenails prior to ICP-MS analysis, exogenous metal sources may contribute to toenail measurements.

In addition to biospecimen differences, assessments in maternal vs. fetal exposures can differ due to differences in bioavailability. For example, some metals not only readily cross the placental barrier but also accumulate in placental tissue while others (for example, cadmium) cross only partially or not at all. The bioavailability of metals in the placenta can also vary based on inter-individual differences in toxicokinetics, including genetically-determined placental metabolic and transport activity (Gundacker et al., 2016). Hence, maternal toenail metal levels may not accurately reflect exposure levels directly experienced by the placenta. Instead, changes in placental activity may capture the combination of metal-induced toxicity within the placenta as well as metal-induced changes in maternal physiology, resulting, for example, in altered uteroplacental blood flow and secretion of maternal hormones, which ultimately lead to altered fetoplacental development.

Metal exposure was assessed in toenails collected postpartum, following the outcome assessment of birth weight. Still, given the slow growth rate of toenails (1 mm/month), these postpartum measurements largely capture an integrated exposure measure spanning pregnancy (Garland et al., 1993; Yaemsiri et al., 2010). Hence, the detected changes in placental expression patterns are likely in response to constitutive rather than acute exposure. However, as both gene expression and metal exposure levels were measured at a single time point at term,
the temporal dynamics between metal-induced changes in gene expression levels to tease out potential windows of susceptibility could not be further evaluated. Furthermore, since expression and exposure levels were determined contemporaneously with the outcome, the directionality of the association between these metrics and the outcome could not be established.

While our findings point to a possible convergence between in utero metal exposures, alterations in placental processes and deviations in fetal growth, we did not formally test for mediation in the current study. Similarly, while we adjusted our models for potential confounding due to gender, we were underpowered to assess potential gender-specific effect modification in the associations between metal exposure and placental gene expression as well as SGA status. This is a particularly pertinent consideration given that As and Cd may act as endocrine disruptors (Iavicoli et al., 2009). Follow up studies with sufficient power to formally investigate whether changes in placental processes mediate the association between in utero metal exposures and SGA status and potential gender-specific effects are warranted to further evaluate the proposed pathway.

Finally, we were able to verify some, but not all, observed significant associations through technical replication in an independent cohort. The three cadmium-responsive genes selected for qRT-PCR validation across the NHBCS cohort were prioritized based on a preliminary study on a subset of the cohort. Further efforts will need to be undertaken to more comprehensively assess the generalizability of the reported findings, especially with respect to the As-related findings. Nevertheless, the findings reported in the current study provide an important advancement in the understanding of metal-induced changes in placental processes that disrupt appropriate fetal growth.

5. Conclusions

Leveraging placental transcriptomic and multi-metal exposure data, we delineate potential placental processes through which trace metal exposure impact fetal growth. The application of novel statistical and bioinformatics-based approaches that account for interrelationships within each dataset and facilitate integration across datasets informed the insight into pathways linking intrauterine trace metal exposures and placental processes relevant to perturbations in fetal growth reported in the current study. Implementation of such integrative approaches has the potential to advance mechanistic insight across the spectrum of exposure-disease paradigms. Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.08.010.

Acknowledgments

This work is supported by NIH-NIMH R01MH094609, NIH-NIEHS R01ES022223, NIH-NIEHS R01ES022223-03S, NIH-NIEHS P01ES022832, NIH-NIEHS P42ES007373, NIH-NIEHS P30ES023515, NIH-NIEHS R24ES028507, NIH-NIGMS P20GM104416, US-EPA RD83544201, and NIH-NIEHS K99ES029571-01.

Declarations of interest

None.

References
