Environment International 120 (2018) 373–381

Contents lists available at ScienceDirect

Environment International

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

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

Maya A. Deyssenroth[a], Chris Gennings[a], Shelley H. Liu[b], Shouneng Peng[c], Ke Hao[c], Luca Lambertini[a,d], Brian P. Jackson[e], Margaret R. Karagas[f], Carmen J. Marsit[g], Jia Chen[a,h,i,j,*]

[a] Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[b] Center for Biostatistics, Department of Population Health Science and Policy, Icahn School of Medicine at Mount Sinai, New York, NY 20019, USA
[c] Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[d] Department of Obstetrics, Gynecology and Reproductive Science, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[e] Department of Earth Sciences, Dartmouth College, Hanover, NH 03755, USA
[f] Department of Epidemiology, Geisel School of Medicine at Dartmouth, USA
[g] Department of Environmental Health, Emory University, Atlanta, GA 30322, USA
[h] Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[i] Department of Medicine, Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[j] Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

ARTICLE INFO

Handling Editor: Olga-Ioanna Kalan

Keywords:
Multi-metal exposure
Placenta
Gene coexpression network
Birth weight

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" ($\beta = -0.02$ [−0.04, −0.01]) and "metabolic hormone secretion" ($\beta = 0.02$ [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

* Corresponding author at: One Gustave Levy Place, Box 1057, New York, NY 10029, USA.
E-mail address: jia.chen@mssm.edu (J. Chen).

https://doi.org/10.1016/j.envint.2018.08.010
Received 14 May 2018; Received in revised form 1 August 2018; Accepted 3 August 2018

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; Lesseur 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. Interviewer-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.