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Tung, Pei Wen; Burt, Amber; Karagas, Margaret; Jackson, Brian P.; Punshon, Tracy; Lester, Barry; and Marsit, Carmen J., "Prenatal exposure to metal mixtures and newborn neurobehavior in the Rhode Island Child Health Study" (2022). *Dartmouth Scholarship*. 4286.
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Original Research Article

ENVIRONMENTAL EPIDEMIOLOGY

OPEN

Prenatal exposure to metal mixtures and newborn neurobehavior in the Rhode Island Child Health Study

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Background: Prenatal exposure to metals can affect the developing fetus and negatively impact neurobehavior. The associations between individual metals and neurodevelopment have been examined, but little work has explored the potentially detrimental neurodevelopmental outcomes associated with the combined impact of coexisting metals. The objective of this study is to evaluate prenatal metal exposure mixtures in the placenta to elucidate the link between their combined effects on newborn neurobehavior.

Method: This study included 192 infants with available placental metal and NICU Network Neurobehavioral Scale data at 24 hours–72 hours age. Eight essential and nonessential metals (cadmium, cobalt, copper, iron, manganese, molybdenum, selenium, zinc) detected in more than 80% of samples were tested for associations with atypical neurobehavior indicated by NICU Network Neurobehavioral Scale using logistic regression and in a quantile g-computation analysis to evaluate the joint association between placental metal mixture and neurobehavioral profiles.

Results: Individually, a doubling of placental cadmium concentrations was associated with an increased likelihood of being in the atypical neurobehavioral profile (OR = 2.39; 95% CI = 1.05 to 5.71). In the mixture analysis, joint effects of a quartile increase in exposure to all metals was associated with 3-fold increased odds of newborns being assigned to the atypical profile (OR = 3.23; 95% CI = 0.92 to 11.36), with cadmium having the largest weight in the mixture effect.

Conclusions: Prenatal exposure to relatively low levels of a mixture of placental metals was associated with adverse newborn neurobehavior. Examining prenatal metal exposures as a mixture is important for understanding the harmful effects of concomitant exposures in the vulnerable populations.

Keywords: Metals; Mixtures; Cadmium; Placenta; NNNS; Neurodevelopment

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The authors declare that they have no conflicts of interest with regard to the content of this report.

This work was supported by the National Institutes of Health [NIH-NIEHS R24 ES028507, NIH-NIEHS P30 ES019776, NIH-NIGMS P20 GM104416, and NIH-NIEHS P01 ES022832] and the US Environmental Protection Agency [US EPA grant RD83544201]. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the US EPA. Further, the US EPA does not endorse the purchase of any commercial products or services mentioned in the presentation.

Data used in this analysis may be available in deidentified format through the RICHS dataverse.

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Environmental Epidemiology (2022) 6:e194

Received: 30 September 2021; Accepted 5 January 2022

Published online 28 January 2022

DOI: 10.1097/EE9.0000000000000194

Introduction

Understanding the health impacts of metal exposure during the sensitive developmental periods *in utero* and early in life is imperative; neurodevelopmental processes are underway, and both fetuses and newborns are sensitive to even very subtle exposures to potentially toxic metals. The placenta is a crucial organ throughout pregnancy considering its roles of transporting water, gases and nutrients between mother and fetus, regulating the progression of pregnancy, metabolism of endogenous and exogenous factors.[1] Some studies have also documented that the placenta plays an important role in neurodevelopment, as the variety of neurotransmitters produced by the placenta throughout pregnancy stimulate normal fetal brain development.[2,3] Therefore, the placenta's part in the associations between prenatal exposure to metals and subsequent developmental outcomes in newborns has garnered attention.

Prevalent exposure to nonessential metals such as cadmium (Cd) and lead (Pb) is concerning as the population is commonly

What this study adds

In this study, we utilize placenta as a biomarker for prenatal metal exposure and evaluate whether metal mixture is linked to newborn neurobehavioral performance assessed using the NNNS. This approach and topic make this manuscript of interest to the audience of Environmental Epidemiology, and this manuscript represents the first to link an examination of a mixture of metal exposure and early life measures of neurobehavioral development. Findings from this study may shed insight on developing useful policies to mitigate and prevent exposures as well as identify infants at risk to allow for appropriate and early intervention.

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exposed to them through sources such as dietary intake or smoking.[4–6] Cd exposure has been frequently linked to kidney functions, and emerging evidence has shown that Cd toxicity leads to cognitive deficits in children.[4,7,8] Lead (Pb) is a well-established neurodevelopmental toxicant that can result in neurodevelopmental deficits even at very low levels.[9,10] Arsenic (As) and mercury (Hg) are also neurotoxicants known for their negative effects on early development.[11–15] On the other hand, exposure to essential trace elements or nutrients such as manganese (Mn) and copper (Cu) can also raise concerns. Essential trace nutrients are involved in numerous biological and developmental processes and the human body requires them to function properly, yet abnormal levels of such elements have been linked to adverse health outcomes in children. For instance, studies have established respiratory and neurological effects upon Mn exposure, and Mn toxicity has been linked to impaired neurodevelopment in children.[5,7,8,16,17] Lower Cu levels were found in children with attention deficit/hyperactivity disorder when compared with children in the control group.[18]

An abundance of evidence on the impacts of exposure to individual metal on neurodevelopment have provided valuable insights on the importance of regulating metal exposures and protecting vulnerable populations from life-long developmental consequences. With the variety, ubiquity, and persistence of metals in our environment, it is plausible that multiple metals act concurrently, and pose threats to normal development. Metals could share common pathways to disrupt development, such as the generation of reactive oxygen species that lead to oxidative stress, effects on enzyme activities, or impacts to immunological functions, thus even at very low levels, simultaneous exposure to multiple metals can be especially detrimental.[16,19–21] However, the exact mechanisms of metals' joint effect on neurodevelopment is unclear and the potential additive or protective effects of metals have yet to be thoroughly examined.[22]

Previous epidemiologic studies usually address metal exposure and neurodevelopment using single-metal models or, at most, binary combination of metals, although these traditional approaches can be biased by the limited number of metals evaluated in an analysis. More importantly, as the population may never actually be exposed to only one metal at any given time, it is necessary to investigate multiple metals in one setting to better grasp the magnitude of neurodevelopmental effects upon exposure to coexisting metals. Our study objective is to examine placental metals as a mixture using a recently developed method, quantile g-computation, and evaluate the potential impact of the metal mixture on newborn neurobehavior performance indicated through NICU Network Neurobehavioral Scale (NNNS) latent profiles in the Rhode Island Child Health Study (RICHS) population. We hypothesize that a mixture of both essential and nonessential placental metals can impact neurobehavioral performance of an infant with nonessential elements most strongly contributing to poor performance.

Methods

Study population

Mother-infant pairs in the RICHS study population were recruited from the Women and Infants Hospital of Rhode Island (N = 840). Briefly, the objective and design of the RICHS cohort was to understand aberrant fetal growth, thus the study population was oversampled for term infants born large for gestational age (≥90th birth weight percentile) and small for gestational age (≤10th birth weight percentile) based on the Fenton growth chart.[23] Adequate for gestational age infants were matched to large for gestational age and small for gestational age infants on sex, maternal age (±2 years), and gestational age (±3 days). The study included mothers who were at least 18 years of age and did not have life-threatening medical complications. Eligible infants were born free of life-threatening medical complications or congenital or chromosomal abnormalities. Obstetric medical information was obtained from a structured medical chart review, and demographic, lifestyle, and exposure histories were collected from interviewer-based questionnaires. All participants provided written informed consent approved by the Institutional Review Boards at the Women and Infants Hospital and Emory University. For this study, a series of 192 consecutive participants recruited in 2010–2011 with samples collected specifically for placental metals assessment were included.

Metals assessment

Placenta parenchyma tissue was biopsied approximately 2 cm from the cord insertion site and free of maternal decidua within 2 hours of delivery. Samples were snap frozen in liquid nitrogen and stored at -80°C until processed. Laboratory methods of assessing placental metal concentrations have been described previously.[24] Placental levels of 24 trace elements (aluminum [Al], arsenic [As], calcium [Ca], cadmium [Cd], cobalt [Co], chromium [Cr], copper [Cu], iron [Fe], mercury [Hg], potassium [K], magnesium [Mg], manganese [Mn], molybdenum [Mo], sodium [Na], nickel [Ni], phosphorus [P], lead [Pb], sulfur [S], antimony [Sb], selenium [Se], tin [Sn], uranium [U], vanadium [V], zinc [Zn]) were quantified in 192 samples using standardized ICP-MS protocols at the Dartmouth Trace Elements Analysis laboratory. Of this panel of metals, 14 were detectable in more than 80% of the samples (Table S1; http://links.lww.com/EE/A175). The current study focused on the prenatal exposure to a mixture of potentially toxic metals and micronutrients, and eight metals were included in the following mixture analyses (Cd, Co, Cu, Fe, Mn, Mo, Se, Zn). A small number of samples showed values below the limit of detection (LOD) for three metals, Cd (9.4%), Co (12.5%), and Mo (2.6%), and these values were substituted with LOD/·. The LOD for placental Cd, Co, and Mo were 2.12 ng/g, 2.12 ng/g, and 4.24 ng/g, respectively.

Neurobehavior assessment

NNNS is an assessment initially designed to examine a variety of neurobehavioral performances in drug-exposed and high-risk infants.[25,26] A standardized and comprehensive examination of both behavioral and neurologic functioning, NNNS was commonly used for at risk and preterm infants, and later extended its application to low-risk and term infants in the general population.[27,28] Researchers have also established the predictive characteristics of NNNS for developmental outcomes later in life. In a generally healthy, low-risk population like ours, Sucharew et al found that NNNS profiles were associated with early neurodevelopmental outcomes indicated through lower motor performance.[28] In the maternal lifestyle study, infants from the profile with the least optimal NNNS scores showed several adverse developmental and behavioral outcomes, including lower MDI scores at ages 1 and 2, more behavioral problems assessed through the Child Behavior Checklist at age 3, and lower IQ at 4.5 years of age.[29]

In the RICHS study population, NNNS was administered by certified psychometrists in 625 newborns (74%) after 24 hours of birth and before discharge.[30] We utilized latent profile analysis (LPA) to further categorize the study population into mutually exclusive neurobehavior profiles.[29,31] Based on the NNNS score patterns, profiles produced through the LPA method had minimized heterogeneity within a profile and maximized heterogeneity across different profiles. There are 13 summary scales in NNNS, but the habituation construct was not assessed for 54.9% of the newborns in RICHS as they would need to be in the required sleep state for assessment, so the LPA method included the remaining 12 summary scores from NNNS and generated five discrete profiles for the RICHS newborns. Figure

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S1 (http://links.lww.com/EE/A175) shows the NNNS summary score patterns and descriptions for the five profiles in the RICHS study population. Newborns categorized into profile 5 demonstrated the most atypical neurobehavior, as the summary scale patterns showed more extreme neurobehavioral performances, such as highest excitability, arousal, hypertonicity, and stress signs, along with exhibiting lowest regulation and quality of movement compared with the other profiles.[31]

Covariates

Based on previous RICHS studies and literature review, covariates considered for analyses include infant sex, maternal age, maternal race, prepregnancy body mass index (BMI), educational status, and smoking status during pregnancy. Infant sex was obtained from medical records. Maternal age and prepregnancy BMI were considered as continuous variables. Self-reported maternal race information was dichotomized into *White* or *other,* given the small number of individuals in any of the non-White race/ethnicity groups. Highest educational attainment was also self-reported and was recoded into two groups, *more than high school* or *high school or less*. We also conducted sensitivity analysis to test whether regression results were robust when smoking status during pregnancy was included. Women reported their smoking status during pregnancy and were defined as *smoked at any point during pregnancy* or *no smoking during pregnancy*.

Statistical analysis

Descriptive information on demographic and gestational characteristics were compared between the subcohort with available placental metal data (N = 192) and those without (N = 433) using chi-square tests and t-tests. For the eight metals included in the mixture, the mean, standard deviation, minimum and maximum values, and quartile ranges were assessed.

As described in our previous work, and similar to other research groups, we utilized LPA to generate NNNS profiles with model fit criteria used to determine the ultimate number of profiles.[29,32] Based on these criteria, the 5-profile model showed the best fit.

The association between individual metals and NNNS profiles were assessed with multivariable logistic regression models, controlled for covariates previously mentioned. Based on descriptive analyses and histograms, metals were $\log_2$-transformed for normal distribution in the single-metal models. NNNS profiles were further dichotomized into two groups, profile 5 versus all the other profiles. Based on the score patterns (Figure S1; http://links.lww.com/EE/A175), profile 5 showed most of the extreme NNNS summary scores, thus newborns categorized in this profile were considered as having the most atypical neurobehavioral performance in the RICHS study population.[31]

As concurrent exposure to metals is likely the norm in the study population, we aimed to further evaluate potential impact of metals as a mixture on newborn neurobehavior. Therefore, the quantile g-computation approach was used to understand the joint association between metal mixture and NNNS profile assignment (atypical profile 5 vs. all other profiles) in this study. Previously described in detail in Keil et al,[33] quantile g-computation is based on the concept of generalized linear model to estimate the impact on the outcome when simultaneously increasing all exposures in the mixture by one quantile. Adapted from weighted quantile sum regression (WQS), one of the main differences is the assumption of directional homogeneity.[34] Unlike WQS, quantile g-computation does not require directional homogeneity, and different exposures within the mixture may contribute oppositely (positively or negatively) to the mixture's impact on the outcome.[33] With this approach, exposures will first be categorized into quartiles, and then fitted into regression models. Each exposure will be given a positive or negative weight, and weights from all components of the mixture will sum to 1. In the event that directional homogeneity does not apply, both positive and negative weights will sum to 1, and an individual exposure's weight can then be interpreted as the proportion of the positive (or negative) partial effect on the outcome due to the specific component of the mixture.[35] For this study, we report the conditional odds ratio, and also estimate the joint effect of metal mixture on neurobehavior profile assignment with the inclusion of previously mentioned covariates through adjusted quantile g-computation models.

LPA analysis for NNNS profile membership was performed with Mplus version 8.4. All other statistical analyses were conducted using R version 3.5.1.

Results

Demographic and gestational characteristics are shown in Table 1. Among 192 newborns with available NNNS assessment scores and placental metal data in the RICHS cohort, 91 (47.4%) were female and 73.4% of the mothers were White, with 75.5% reported obtaining some posthigh school education. Average gestational age in the subcohort was 39.31 weeks, and the average maternal age was 29.79 years old. Demographic characteristics were similar between the included participants and those without available placental metal information (N = 433), although newborns included in this study were heavier averaging 3,644 grams.

Box plots for the eight metals (Cd, Co, Cu, Fe, Mn, Mo, Se, Zn) across five NNNS profiles are presented in Figure 1. Correlations between the metals are shown in Table S2 (http://links.lww.com/EE/A175), and the strongest correlation was between Mn and Se (r = 0.45).

Apart from Cu and Se, in single-metal models, unadjusted logistic regression showed increased odds of belonging to the atypical profile 5 as placental metal level increases, although the confidence intervals included the null (Figure 2). In adjusted models, every doubling of placental Cd was associated with increased odds of the newborn belonging to the atypical profile 5 (OR = 2.39; 95% CI = 1.05 to 5.71). On the other hand, with every doubling of placental Cu, we observed decreased odds of newborns belonging to the atypical profile 5 (OR = 0.42; 95% CI = 0.05 to 2.23).

Quartile ranges of each metal are presented to provide additional information on the distribution of the metals included in the mixture used in the following quantile g-computation analyses (Table 2). In the g-computation mixtures analyses (Table 3), as all metals in the mixture increase by one quartile, we observed increased odds of newborns belonging to the atypical profile 5 (OR = 3.23; 95% CI = 0.92 to 11.36). This approach also demonstrated Cd as the driving factor for the overall positive association between increased levels of all metals as a mixture and atypical neurobehavior, as this metal was assigned the largest positive weight, with Zn, Mn, and Fe following with smaller positive weights. Alternatively, Cu showed the largest negative weight among the metals (Figure 3). The RICHS study population had a low percentage of women who smoked during pregnancy, resulting in a very limited number of smokers in each profile (among infants categorized into the atypical profile 5, only one mother reported smoking during pregnancy). Accordingly, smoking status during pregnancy was included in a sensitivity analysis, and results showed attenuated effects of the overall mixture effect on neurobehavior (OR = 2.14; 95% CI = 0.71 to 7.61).

Discussion

Exposure to combinations of metals in our environment is inevitable, hence understanding the joint effects of coexisting metals is

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TABLE 1.

Demographic and Gestational Characteristics

Characteristic	Newborns With NNNS and Placental Metal Data (N = 192)	Newborns With NNNS and Without Placental Metal Data (N = 433)
	n (%)	
Infant gender		
Female	91 (47.4%)	230 (53.1%)
Male	101 (52.6%)	203 (46.9%)
Birth weight category		
SGA	29 (15.1%)	94 (21.7%)
AGA	93 (48.4%)	263 (60.7%)
LGA	70 (36.5%)	76 (17.6%)
Maternal race		
White	141 (73.4%)	300 (69.3%)
Other	47 (24.5%)	120 (27.7%)
Unknown	4 (2.1%)	13 (3.0%)
Infant race		
White	123 (64.1%)	285 (65.8%)
Other	64 (33.3%)	137 (31.6%)
Unknown	5 (2.6%)	11 (2.5%)
Maternal education status		
No more than high school	47 (24.5%)	119 (27.5%)
Some posthigh school*	145 (75.5%)	314 (72.5%)
Maternal smoking status		
Yes	26 (13.5%)	63 (14.5%)
No	165 (85.9%)	365 (84.3%)
Unknown	1 (0.5%)	5 (1.2%)
	Mean ± SD	
Birth weight (g)	3,644 ± 680.37	3,404 ± 644.89
Gestational age (weeks)	39.31 ± 0.95	39.35 ± 0.97
Maternal age (years)	29.79 ± 5.63	29.34 ± 5.43
Maternal BMI (kg/m^2)	27.48 ± 7.14	26.37 ± 6.87

*Posthigh school education included junior college, college or any post graduate schooling education.

critical. Although studies have since elucidated metal-associated health effects in the general population, the potential impacts of multiple placental metals on newborn neurobehavioral performance remain unclear. Additionally, possible protective effects of some essential metals are even less studied. In this study, we observed simultaneously increased levels of eight

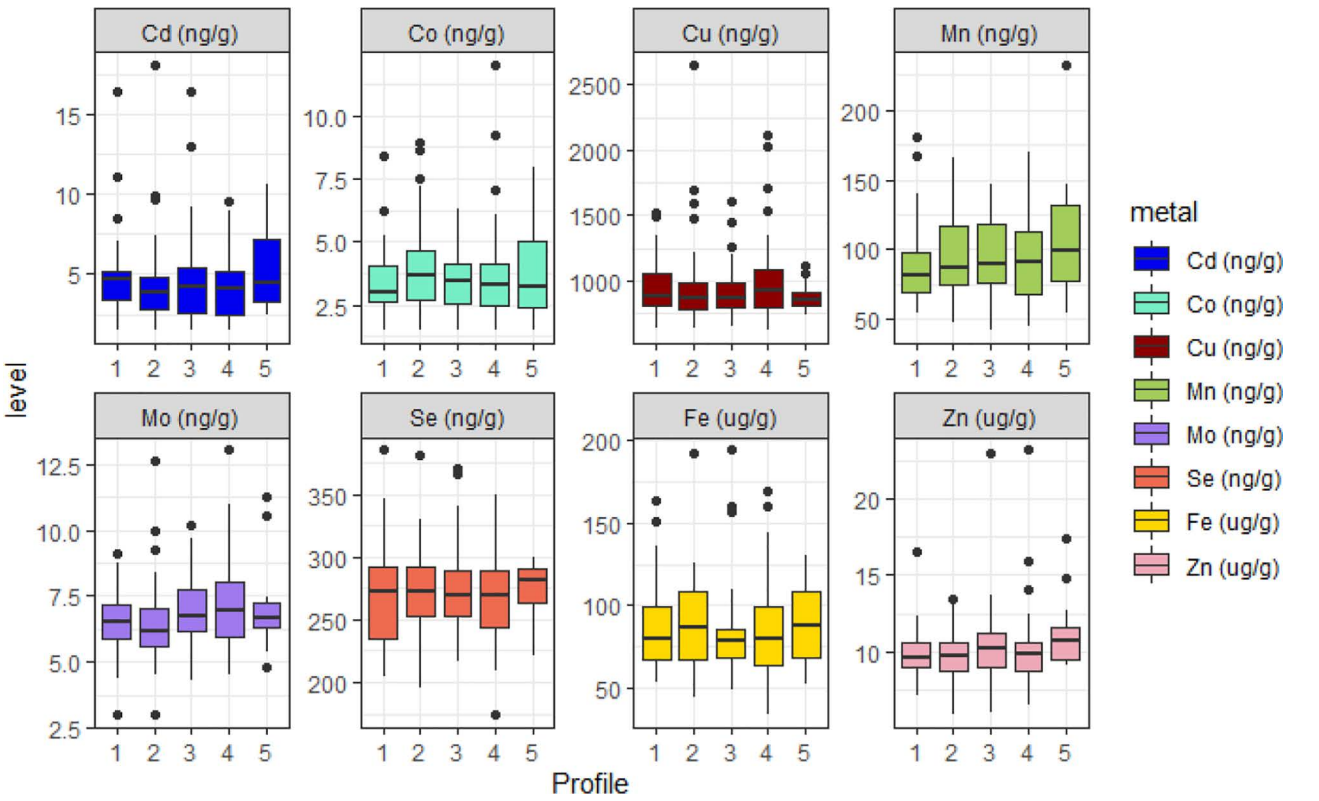

Figure 1. Metal distribution by NNNS profiles. Levels of the eight placental metals (*y* axis) included in the mixture analysis are shown across NNNS profiles 1–5 (*x* axis) in the RICHS study population.

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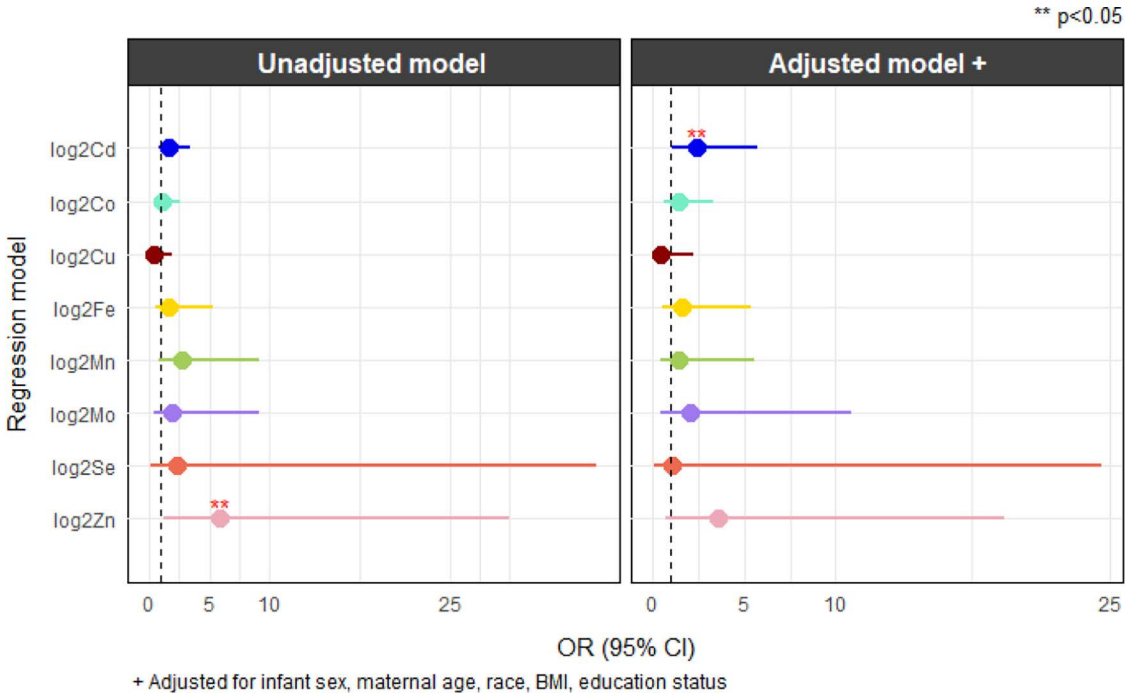

Figure 2. Associations between individual metals and neurobehavioral performance indicated through NNNS profiles. Log_2-transformed levels of eight metals were individually assessed in logistic regression models. Odds ratio and 95% CI indicated the odds of newborns belonging to the atypical profile 5 with every doubling of placental metal concentration.

placental metals as a mixture were associated with increased odds of impaired neurobehavior, which was indicated via. membership in an atypical NNNS profile. The "atypical" profile was characterized by infants showing the most signs of arousal, stress/abstinence, excitability, and hypertonicity, along with poorer regulation and quality of movement. With the quantile g-computational method, we further identified Cd as the primary metal associated with the "atypical" neurobehavior profile.

Placental levels of the eight metals included in our mixture analyses were generally comparable to what was reported in other study populations. In particular, Cd levels in RICHS would be amongst some of the lowest reported of ranges around the world (average: 4 ng/g), although it should be noted that the studies reported were from 1977 to 2011, and exhibit a decreasing trend over time.[36] While the mean (4.56 ng/g) and interquartile range (IQR = 2.80–5.38 ng/g) of placental Cd measured in our study was relatively similar to that measured in the INMA Project (mean: 4.45 ng/g; IQR = 2.79–6.49 ng/g), RICHS Mn levels demonstrated a higher mean of 95.37 ng/g (SD = ±30.08) and wider range of levels (IQR = 73.66–115.91 ng/g) compared with the INMA Project (mean: 70 ng/g; IQR = 52.50–82.24 ng/g).[37,38] RICHS placental Cu levels (mean: 0.97 μg/g) were comparable to another Spanish study (mean: 0.97 μg/g), although slightly higher than that measured in the New Hampshire Birth Cohort Study (mean: 0.88 μg/g).[39,40]

Common sources of Cd in the American population are dietary intake and tobacco smoke exposure.[4,6] Our study population had a relatively low percentage of self-reported smoking during pregnancy, and we did not obtain dietary information throughout pregnancy from the participants, which represents a limitation of the study. Despite the relatively low concentrations of Cd and low-to-moderate correlations between Cd and other metals included in the mixture, we interpret our results with caution and note that Cd exposure, individually and concurrently with other metals, during the sensitive developmental period *in utero* may lead to adverse effects on neurobehavior.

In agreement with our single-metal findings, several previously published studies also found prenatal Cd exposure to be associated with adverse impacts on neurodevelopment later in life. A Japanese birth cohort study found that maternal blood Cd concentrations negatively affect the postural-motor area of neurodevelopment in 2-year-old boys.[41] Cd measured in maternal urine was found to be inversely associated with cognitive score in the Spanish INMA Project and children's IQ (verbal, performance, and full scale) measured in 5-year olds in rural Bangladesh.[42,43] Cord blood Cd also negatively associate with performance IQ in children at 5 years of age in a South Korean study.[44] However, there remains some inconsistencies in the association between prenatal Cd exposure and developmental or behavioral outcomes in newborns or young children across different study

TABLE 2.

Mean Levels and Quartile Ranges for Metals Included in the Mixture (ng/g)

Metal	Mean	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Cd	4.56	1.06–2.80	>2.80–4.19	>4.19–5.38	>5.38–17.99
Co	3.65	1.18–2.56	>2.56–3.36	>3.36–4.32	>4.32–11.95
Cu	971.72	623.10–799.98	>799.98–878.90	>878.90–1,042.90	>1,042.90–2,643.50
Fe (μg/g)	84.93	33.67–65.49	>65.49 –81.05	>81.05–99.30	>99.30–194.03
Mn	95.40	42.34–73.66	>73.66–89.12	>89.12–115.91	>115.91–231.49
Mo	6.76	3.57–5.85	>5.85–6.58	>6.58–7.42	>7.42–13.04
Se	270.68	174.44–247.17	>247.17–271.79	>271.79–291.22	>291.22–384.91
Zn (μg/g)	10.11	5.94–8.98	>8.98–9.96	>9.96–10.92	>10.92–23.13

TABLE 3.

Quantile g-Computation Estimates (Odds Ratio and 95% CI) for Being Placed in the Atypical Profile 5 for a Quartile Increase in All Metals

	Unadjusted	Adjusted*
Mixture†	2.47 (0.82, 7.40)	3.23 (0.92, 11.36)

*Adjusted for infant sex, maternal age, race, BMI, education status
†8 metals: Cd, Co, Cu, Fe, Mn, Mo, Se, Zn.

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populations. The INMA Project did not find a significant association between placental Cd and general cognitive score in preschool children, and no association was observed between maternal blood Cd and behavioral outcomes in children between the ages of 1–8 years old in the HOME study.[38,45] Aside from Cd, we observed potential adverse effects of Mn on neurobehavior, which is consistent with some prior studies.[7,46] However, we also note the limitation in our assumption of linearity in the mixture analysis, which does not address a U-shaped association between Mn and behavioral outcomes reported in some prior work.[47,48] Similarly, the potential adverse effect of iron on neurobehavior observed in our study is consistent with several reports, although the exact impact of iron dysregulation on behavioral outcomes warrants further investigation.[49–51]

Difference seen across these studies could be attributable in part to difference in sample type used in exposure assessment. Some metals that readily pass the placental tissue (i.e., Pb and Mn) and would more likely be detected in the fetuses' blood or other tissues, would not be well represented and thus there impact on neurobehavior will be missed in this analysis, while others, such as Cd may be more likely retained in the placenta where they can exert toxic effects.[52–54] Elements like Zn, Cu, Fe, and Se that are essential for fetal growth and development are tightly regulated but their trans-placental efficiency is less well understood. Researchers have identified metal-transporters that transport them across the placenta into the fetal system, and found the processes may be affected by competition of toxic metals like Cd.[55–59] Using the placenta as a biomarker has its challenges, yet the reflected exposure window and its ability of regulating passage of these elements throughout the gestational period could provide valuable insight on prenatal exposure characteristics.

With the myriad of metals present in the environment, it is challenging to capture all combinations of metals. Researchers have since focused on several trace metals, both toxic and nontoxic, that represent common exposures in the general population and/or are more well-known for their effects on human health. Kim et al suggested an interaction between blood Cd and Pb with findings of an inverse association with Pb for both mental development index (MDI) and psychomotor development index (PDI) scores among 6-month-old infants with above-median Cd levels.[60] In the MOCEH study, researchers also explored the association between combined metal exposure (Cd, Hg, and Pb) and children's neurodevelopment using the Bayley Scales of Infant Development. In a Bayesian kernel machine regression analysis, they found a joint effect from late pregnancy Pb and Hg exposures on MDI and PDI scores at 6 months.[61] Using the same Bayesian kernel machine regression approach for mixtures, Valeri et al observed a negative effect on cognitive scores from joint exposure to As, Mn, and Pb.[62] Alternatively, Kordas et al did not observe an association between metals (blood Pb, hair Cd, Mn, and As) and MDI or PDI scores in young children.[63]

Our observed overall mixture effect also highlighted Cu and Se to be inversely related to the risk of atypical neurodevelopment. Cu and Se are considered as essential for proper organ functioning and metabolic processes, although abnormal levels can be detrimental to health. Adequate Cu intake is crucial in forming red blood cells and maintaining normal immune functions.[64] The main source of Cu in the general population is through diet, including vegetables, fruits, cereal, and nuts.[65] Although very uncommon, Cu-associated toxicity can impair numerous biological processes. Studies over the decades have largely focused on Cu deficiency and the associated effects on brain activities, such as Menkes disease, of which infants are subject to developmental disability.[64,66] It is also suggested that Cu-deficient infants are at risk to psychomotor impacts and hypotonia, among many other vessel, bone, and skin abnormalities.[64,67] In relation to neuropsychological outcomes, however, there remain discrepancies on the epidemiologic association between prenatal Cu concentrations and neurodevelopment or behavior early in life. For instance, a study demonstrated maternal Cu level adversely impacting the Bayley Scales of Infant Development mental scale accessed at 12 months, although in a Polish study, no association was found between prenatal Cu exposure and psychomotor development at 1–2 years of age.[68,69] In our single-metal analysis, Cu was the only metal with an OR < 1, indicating higher placental Cu level may lower odds of newborns belonging to the atypical NNNS profile. This association was likewise observed in our mixture analysis.

Se is generally regarded as a protective trace element to human health, with studies documenting positive effects on cognitive function and the cardiovascular and immune systems.[70–72] Se is also a crucial component of selenoproteins which oversee antioxidant defense mechanisms and protect the neuronal system.[73,74] One of the proposed mechanisms of toxic metals eliciting adverse health impacts is by generating oxidative stress and

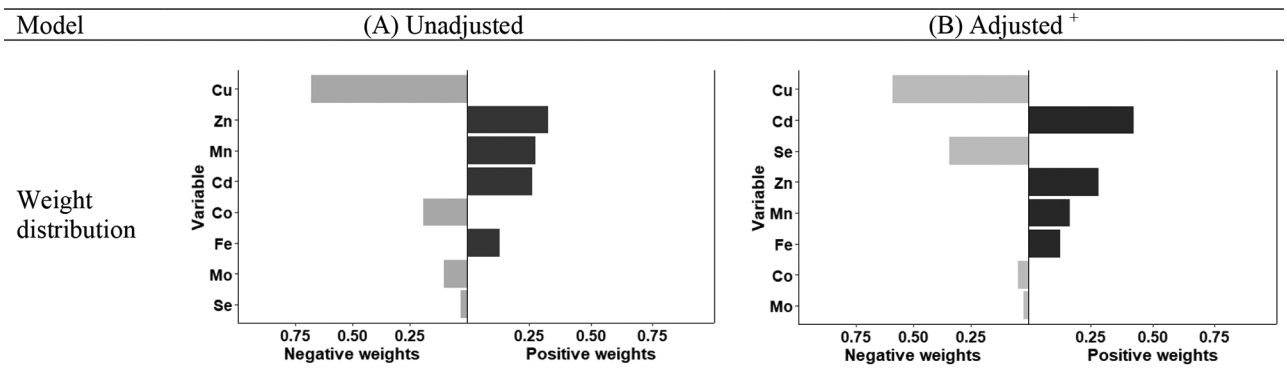

Figure 3. Weights for each metal in the quantile g-computation model. Weights represent the proportion of the positive or negative partial effect for each component (metal) in the mixture on newborn neurobehavior. Shadings of the bars correspond to the overall effect size—the darker colored bars are shown in the positive direction as the overall mixture effect is positive.

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targeting normal dopamine pathway functions.[75,76] It is possible that as part of the metal mixture, Se could display antagonistic and antioxidant properties, which in turn mitigated neurodevelopmental defects resulted from coexposure to neurotoxic metals such as Cd and Mn.[74,77] A Chinese study investigating two-way metal interactions found that higher Se levels may be protective toward Mn-induced toxic effects on neurodevelopment.[78] However, further studies of placental metal mixtures that include Se as a component are needed.

By considering the real-life circumstances of concurrent exposures to multiple metals, evaluating metals as a mixture in the sensitive prenatal period indicated potential adverse impacts on newborn neurobehavior. A reasonable motivation in investigating prenatal metal exposure to metal mixtures was to reflect the reality of exposure patterns in the study population. The application of quantile g-computation enabled us to assess exposure mixture-response association, and this method also helps to identify the "bad actor" among the variety of metals present in the environment. With this information, future interventions can be designed to first target and eliminate exposure to the "bad actor" to effectively decrease negative impacts to newborn neurodevelopment. In addition, newborns that are most affected by exposures are identified early and appropriate medical follow-ups and interventions can be implemented to mitigate long term adverse developmental outcomes.

Quantiling of exposure levels when generating quantile g-computation results makes this method insensitive to exposure outliers and in turn reduces outliers' influences on model coefficients. Another advantage of this analysis method was that we were able to assess both directions of associations of metal exposures and neurobehavior. Metals like Cd are toxic and nonessential, while others such as Cu and Se, are essential to normal biological functions, and so the quantile g-computation served as an informative approach to addressing this question. A limitation, although, is the assumption of linearity. Larger studies which can assess a broader range of exposures would be needed to better examine nonlinear associations between metals and these outcomes.

Among the 24 metals accessed in the RICHS placenta samples, only 14 metals were detectable in more than 80% of the samples. Several simultaneously occurring toxic trace elements that were well-known to impact neurodevelopment, such as As, Pb, and Hg, were excluded due to high percentages of <LOD. Another factor that may have affected the evaluation of placental metal mixture-neurobehavior association in our study is the relatively low to modest level for all eight metals in the RICHS study population. Although the subcohort with available placental metal data is representative to the full RICHS cohort, the small sample size may also affect the precision of model estimates. Coupled with our generally healthy, thus smaller proportion of atypical neurobehavior newborns, it is likely that we lack sufficient power to robustly detect an association between metal mixture and NNNS profiles. Therefore, it is also important for future research to include larger sample sizes, especially if the inherent metal exposure levels are low in the targeted population, to establish any potential neurodevelopmental impacts upon concurrent metal exposures.

Conclusions

In summary, we observed a significant association between placental Cd levels and atypical neurobehavior. As multiple placental metals were jointly investigated as a mixture, we also found the overall mixture effect to demonstrate an increased odds of newborns being assigned to the atypical NNNS profile, with Cd regarded as the driving factor of the mixture's adverse effect on neurobehavioral performance. Investigating prenatal metal exposure as a mixture provided additional insight on the adverse neurobehavior effects elicited from combined metal exposure. Future analyses are warranted to provide and verify more robust associations between concomitant metal exposures and newborn neurobehavioral outcomes that may have persistent effects later in life.

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