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Umbilical cord serum elementomics of 52 trace elements and early childhood neurodevelopment: Evidence from a prospective birth cohort in rural Bangladesh

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Abstract

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Author Contributions.

Y.W., F.C., and D.C.C. were responsible for the study's conception; Y. W., L.W., X.C., H.H., and X.W. designed the study, conducted the data analyses, and wrote the manuscript; D.C.C., Q.Q., M.R. and L.W. collected the samples, processed the samples for analysis, and helped evaluate the element levels; R.Z., W.D., Z.H., H.S., and M.G.M. contributed to the discussion and revised the manuscript. All authors approved the final version of the manuscript. All authors had full access to all the data in the study and accepted responsibility to submit for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data sharing

Additional data available from the corresponding author at ywei@njmu.edu.cn upon reasonable request. The lead authors affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107370.

Background: Prenatal exposures to neurotoxic metals and trace elements are associated with early childhood neurodevelopmental outcomes. However, consequences of simultaneous exposure to mixtures of elements remain unclear.

Objective: To examine individual and joint effects of prenatal trace element exposure on early childhood neurodevelopment.

Methods: Using a well-established Bangladesh prospective birth cohort (2008–2011), we measured concentrations of 52 trace elements in umbilical cord serum of 569 mother–infant pairs using inductively coupled plasma mass spectrometry. Neurodevelopment was evaluated at 20–40 months of age using Bayley Scales of Infant and Toddler Development, Third Edition. Stability elastic net (ENET) was used to screen elements individually associated with the outcome; candidate exposures were combined by weighted linear combination to form a risk score representing their mixture effect on early childhood neurodevelopment.

Results: Stability ENET identified 15 trace elements associated with cognitive composite score and 14 associated with motor composite score, which were linearly combined to form the element risk score (ERS). Children with higher $ERS_{cognitive}$ had lower probability of cognitive developmental delay ($OR_{highest\ vs\ lowest}$: 0.21; 95 %CI: 0.10, 0.40; $P < 0.001$; $P_{trend} < 0.001$). Children with ERS_{motor} in the top quintile had a significantly lower risk of motor developmental delay (OR: 0.16; 95 %CI: 0.09, 0.31; $P < 0.001$; $P_{trend} < 0.001$) versus the lowest quintile. In Bayesian kernel machine regression analyses, lithium [conditional posterior inclusion probability (cPIP) = 0.68], aluminum (cPIP = 0.83) and iron (cPIP = 1.00) contributed most to the lower cognitive composite score; zinc (cPIP = 1.00), silver (cPIP = 0.81), and antimony (cPIP = 0.65) mainly contributed to the change of motor composite score.

Conclusion: Co-exposure to lithium/aluminum/iron or zinc/silver/antimony appears to impact children's neurodevelopment. ERS score reflecting maternal exposure could indicate children's risk of neurodevelopmental delay, warranting further studies to explore the underlying mechanism.

Keywords

Umbilical cord serum; Elementomics; Early childhood neurodevelopment; Bangladesh

1. Introduction

Mounting evidence suggests that neurodevelopment during fetal and early life, a time of intense brain maturation, forms the basis of academic achievement and economic productivity (Forrest et al., 2018; McCormick et al., 2020; Walker et al., 2011). Bangladesh has a large number of children at risk of poor development, ranking as one of the top ten countries of 34 low-income and middle-income countries in 2010 based on measures of children who were either stunted or lived in extreme poverty (Lu et al., 2016). In Bangladesh, poverty was associated with children's cognition as early as 7 months and the association continues until 5 years of age (Hamadani et al., 2014). Heavy metals in drinking water in Bangladesh largely exceed the levels in developed countries in Europe and North America and the safety threshold set by the World Health Organization. During pregnancy, toxic metals such as lead (Pb) and mercury (Hg) are transferred from the mother to the fetus via placenta (Grandjean and Landrigan 2006). Fetal brain development is extraordinarily sensitive to chemicals such as Hg, Pb and aluminum (Al), which can interfere with brain developmental processes and may lead to neurodevelopmental deficits in early childhood (Grandjean et al., 1998; Karwowski et al., 2018; Thomason et al., 2019; Yorifuji et al., 2011), which was supported by animal studies as well (Aschner and Clarkson 1988; Hao et al., 2021; Heyer and Meredith 2017; Schell et al., 2003). However, one previous study showed contradictory result that low level of Pb exposure has no or limited association with early childhood neurodevelopment (Taylor et al., 2017). In addition, a recent systematic review indicated that dietary Hg (i.e., methylmercury) exposure during pregnancy is unlikely to be associated with low neurodevelopmental functioning in early childhood (Dack et al., 2022). Identifying children who are susceptible to neurodevelopmental delay is necessary for prevention and intervention to improve children's health outcomes.

Prenatal exposure to arsenic (As), Hg, magnesium (Mn), and cadmium (Cd), and early-childhood exposure to Al and Pb were associated with childhood neurodevelopment (Barbone et al., 2019; Kao et al., 2021; Lee et al., 2018a; Levin-Schwartz et al., 2021; Ma et al., 2021a; Ma et al. 2021b). Recent studies indicate that elements such as zinc (Zn), iron (Fe), copper (Cu), strontium (Sr), and selenium (Se) may be associated with childhood neurodevelopmental outcomes (Amorós et al., 2019; Li et al., 2020; Močenić et al., 2019; Quezada-Pinedo et al., 2021; Yang et al., 2013). Most studies have focused on revealing the health effects of single elements or a mixture of several candidates. However, people in real life are exposed simultaneously to a broader list of elements beyond these candidates (Li et al., 2020; Sarigiannis et al., 2021). In addition, potential nonlinear exposure–response relationships and potential interactions (synergistic or antagonistic relationship) between elements also pose statistical challenges for their discovery. Further, consequences of simultaneous exposure to mixtures of elements remain unclear. Assessing the mixed effects of multiple elements is necessary. Therefore, it is necessary to investigate a broader panel of element exposures (Braun et al., 2016). Inductively coupled plasma mass spectrometry (ICP-MS) techniques enable accurate measurement of more than 70 elements simultaneously (Zhang et al., 2018), providing an opportunity to decipher exposures associated with adverse neurodevelopmental outcomes.

We took advantage of a well-established birth cohort in rural Bangladesh to measure the concentrations of 52 trace elements in umbilical cord serum samples collected at delivery using ICP-MS technology and further determined the elemental exposures that associated with early childhood neurodevelopment.

2. Methods

2.1 Study population

The study population was derived from a Bangladesh birth cohort established in 2008–2011, which has been detailed previously (Huang et al., 2021). Briefly, this prospective cohort recruited pregnant women who met the following inclusion criteria: maternal age ≥ 18 years at current pregnancy, ultrasound-confirmed pregnancy < 16 weeks of gestation, primary drinking water source was groundwater from a tube well, and residence that remained unchanged for the duration of the pregnancy. All participants provided informed consent before enrollment and were informed of the purpose of the study.

Demographic characteristics such as mother's height and weight, mother's exposure to second-hand smoke, parents' education levels, household income level, and the number of past pregnancies were collected at enrollment with a self-report questionnaire. Monthly household income level was assessed directly by asking husbands their monthly income. Gestational age at birth was based on clinical assessment using data from the last menstrual period, the first accurate ultrasound examination during the first trimester, and clinical examination. Health care workers who were trained following a standard protocol recorded gestational age and measured birthweight. Birthweight rounded to the nearest 10 g was measured within 120 min after delivery using a pediatric scale that was calibrated before each use. Research staff recorded information on newborn sex, delivery location, delivery type, and birthweight at delivery using a standardized reporting form.

Each participant received prenatal care and multivitamins from clinics operated by Dhaka Community Hospital in the Sirajdikhan and Pabna Sadar Upazilas, which were among the few health care providers in the catchment areas. A total of 1613 women in early pregnancy participated in the reproductive health study. Of these, 99 were lost to follow-up to birth, 121 withdrew from study activities, 132 pregnancies resulted in miscarriage, 5 were multiple pregnancies, and 72 pregnancies resulted in stillbirth, which resulted in 1184 singleton livebirths. After birth, an additional 24 were lost to follow-up and 4 withdrew from study activities. A total of 1156 women and their children participated in the follow-up studies, including 815 children who participated in neurodevelopmental assessments at age 20–40 months. This study included 569 mother–infant pairs who provided umbilical cord serum samples, had complete data on all of the essential covariates, and had been assessed on the Bayley Scales of Infants and Toddler Development, Third Edition (BSID-III) (Appendix Fig. 2).

2.2 Element measurements

Umbilical cord serum samples were collected at delivery. Detailed methods for analysis of cord serum element concentrations have been described previously (Huang et al., 2021). Briefly, element concentrations were analyzed by ICP-MS following acid digestion. The trained obstetrician double-clamped the umbilical cord after delivery of the child. A vacutainer needle was used to puncture the umbilical vein to collect the umbilical blood sample into a trace element-free vacutainer tube (BD Vacutainer Royal Blue Cap, Becton Dickinson) with K_2EDTA. When the umbilical cord blood began to clot, the blood was manually massaged out of the umbilical cord vein into the vacutainer tube. Cord blood samples were immediately shipped on dry ice to the Trace Metals Laboratory at the Harvard T.H. Chan School of Public Health. Serum was isolated by centrifugation and frozen at −20 ℃ immediately after processing. And samples underwent one freeze–thaw cycle in order to create aliquots prior to storage at −80 ℃. Samples were treated with 30% hydrogen peroxide (1 mL per 1 g of blood) overnight and diluted to 10 mL with deionized water. All operations were performed at 20 °C and 40% humidity in P2 laboratory.

Fifty-six elements were analyzed in the umbilical cord serum using the iCAP Qc ICP-MS system (Agilent 7700x ICP-MS) at Shanghai Biotree Biotech Co., Ltd. The system generates a high-fore vacuum (9.5×10^{-3} mbar); analyzer pressure was kept at 1.09×10^{-7} mbar; RF power was 1450 W; spray chamber temperature was 2 °C; nebulizer gas flow was 0.90 L/min; dilution gas flow was 0.32 L/min; auxiliary gas flow was 0.8 L/min; cool gas flow was 15 L/min; and cell gas flow was 4 mL/min. Four macroelemets – Na, Mg, K, and Ca are not trace elements as they occur in large quantities in the body, therefore they were not included in the following analyses. Among the included 52 elements, Cr, Cu, Zn, Se, and Mo were essential elements; Mn, Ni, B, and V were probably essential elements; Pb, Cd, Hg, Al, As, Sn, and Li were potentially toxic elements (Mehri 2020). The calibration (standard) curves for 52 elements showed that Pearson correlation coefficient R values between instrument responses and known concentrations of the analytes were all > 0.99 except for Fe, which was 0.98 (Appendix Table 1). Analysis of certified reference material Seronorm Trace Elements Serum L-2 (ref. 203113; Sero, Billingstad, Norway) were regularly performed, within each batch analysis, to validate the accuracy of the analytical procedure. Quality control samples were analyzed in parallel with the study samples (every 10 study samples with one standard quality control sample). The precisions (relative standard deviation, RSD) of quality control samples were shown in Appendix Table 2. The average of three replicate readings for each individual sample was reported as the final sample concentration used in the results. The quality control also included a procedure blank in this method, and the procedure blank was used for the calculation of LOD. The limit of detection (LOD) was calculated as 3 times the average of 10 consecutive measurements of the blank diluent (0.1% [v/v] Triton X-100, 1% [v/v] HNO3 plus 10 μg/L internal standards including 6Li [No gas], 103Rh [No gas], 103Rh [He], 115In [No gas], 115In [He], 187Re [No gas], and 187 [He]).

2.3 Neurodevelopmental outcomes

Child neurodevelopment status was assessed at 20–40 months of age using a translated and culturally adapted version of the BSID-III (Bayley 2006; Gleason et al., 2020; Lee et al., 2018a; Rodrigues et al., 2016). The objectively measured assessment includes five subscales: cognition, receptive language, expressive language, fine motor, and gross motor. The BSID-III assessments was administered by trained study personnel blinded to participants' environmental exposure measures. The quality control included review of all BSID-III videotapes by site leaders (Ibne Hasan and Halder), frequent observed assessments (Mazumdar), and review of randomly selected 5% of the videotaped administrations of the BSID-III by a senior neuropsychologist (Bellinger). Raw scores were calculated by summing the number of items successfully completed for each sub-domain. Lower scores indicate higher risk of developmental delay. Scaled scores were calculated using the US population-based norms, standardized to composite scores with a mean of 100 and standard deviation (SD) of 15 (Robertson 2010). Composite scores on the cognitive, language and motor scales were categorized as "delay" (i.e., a standard score of < 85, which was −1 standard deviation below the population mean of 100) or "normal" (i.e., a standard score of ≥ 85) on the BSID-III.

2.4. Covariates

Covariates were chosen through review of the literature based on their relationship with exposures and/or outcomes, including clinic, age of mother, sex of child, gestational age, Home Observation for Measurement of the Environment (HOME) score, mother's exposure to second-hand smoke, parents' education levels (no formal education, primary education, and secondary or higher education), and household income level (Lee et al., 2018a; Rodrigues et al., 2016). Mother's age, parents' education levels, mother's exposure to second-hand smoke and household income level were assessed at the time of enrollment via questionnaire. At birth, information regarding child's sex was collected. At the 20–40-month follow-up visit, trained study stuff administered a questionnaire to collect child's age and the HOME Inventory to assess stimulation/support available to the child in the home environment.

2.5. Statistical analyses

Distribution of characteristics related to children, mothers, and families was described as median [interquartile range (IQR)] or frequency and proportion [n(%)]. Concentrations of the elements were $\log_e$-transformed to better approximate a normal distribution. Pearson's correlation (for normal distribution) and Spearman's rank correlation (for skewed distribution) were used to evaluate correlations among $\log_e$-transformed element concentrations.

Linear regression was performed to assess the association of single elements with early childhood neurodevelopmental outcomes, with adjustment for age of mother, sex of child, gestational age, HOME score, exposure to second-hand smoke, maternal education level, household income level, and clinic. Element levels were categorized by quartiles to investigate the dose–response relationship using the lowest quartile as the reference. False discovery rate (FDR) was used to control for multiple comparisons, and FDR q-value ≤ 0.05 was considered statistically significant.

Ordinary least squares-based variable selection methods are commonly used but are prone to over-fitting. In addition, although association analyses for single element are informative, these results are likely confounded by co-exposed elements, which cannot be solved by simultaneously adding all elements in one regression model, particularly in the presence of multicollinearity among element exposures. Thus, we used elastic net (ENET) penalized linear regression with R package glmnetUtils, a flexible multi-chemicals model for variable selection, to assess the associations of umbilical cord serum element levels in a multi-chemicals condition. In addition, we applied the "stability selection" procedure upon ENET to prevent over-fitting and avoid the impact of potential outliers. Stability selection provides superior variable selection performance, keeping low false positives and identifying more truly significant variables than other variable selection procedures. Stability ENET penalized linear regression can be understood as a bootstrap aggregation procedure in which variable selection is repeatedly performed on bootstrap resampling, and all repeated results are aggregated. Stability selection was done by bootstrap resampling with a 90% sample size by repeating 500 times to assess the stability of selected variables. An ENET model including all elements [main effect ENET (mENET)] was performed to retain elements with main effects; an ENET model including all two-element pairs with adjustment for all elements was performed to detect potential interaction effects [interaction effect ENET (iENET)].

Further, to assess potential nonlinear exposure–response and interaction relationships, Bayesian kernel machine regression (BKMR) analysis was performed using R package bkmr. A hierarchical variable selection algorithm was adopted to identify dominant elements for the observed associations of element mixtures while controlling for multiple comparison. We fitted BKMR by grouping candidate elements into three groups based on hierarchical cluster analyses. The posterior inclusion probability (PIP) obtained from BKMR quantifies the relative importance of each group (group-PIP) and each element [conditional-PIP (cPIP)]. Empirically, elements that cPIP ranked the first within the corresponding group were regarded as important to the specific association. The relative importance of a specific chemical within a group depends more on the ranking of cPIPs in that group (Bobb et al., 2015; Domingo-Relloso et al., 2019). Estimates from BKMR were generated after 100,000 iterations of the Markov chain Monte Carlo. PIPs for elements pairwise interactions on BSID-III composite scores were evaluated by using R package NLinteraction (Joseph et al., 2020).

An element risk score (ERS) was constructed for candidate elements identified above. The underlying idea behind ERS is to build a risk score as a weighted sum of the element concentrations with weights estimated by stability ENET linear regression. We categorized ERS into quintiles and calculated the percentage of participants with neurodevelopmental delay (cognitive delay, motor delay). Next, we fit the logistic model to compare the risk of neurodevelopmental delay between ERS quintiles. Regression models were adjusted for age of mother, sex of child, gestational age, HOME score, mother's exposure to second-hand smoke, mother's education level, household income level, and clinic.

All statistical analyses were conducted with R software version 3.6.1 (The R Foundation for Statistical Computing).

3. Results

3.1. Characteristics of the study population

Table 1 presents demographics, baseline, maternal, and child characteristics of the 569 mother–infant pairs. Among 569 mothers, the median age was 22 (IQR: 19, 25) years, 15.1% of mothers and 27.2% of their spouses had no formal education, and 43.1% had been exposed to second-hand smoke. The median age of 569 children was 27.76 (IQR: 25.92, 30.32) months at time of assessment, and 49.0% were males and 51.0% were females. The median (IQR) BSID-III composite scores in cognitive, language, and motor domains were 85 (IQR: 80, 90), 89 (IQR: 83, 92), and 85 (IQR: 82, 91), respectively. In total, 146/569 (25.7%) children had cognitive delay, 187/569 (32.9%) had language delay, and 190/569 (33.4%) had motor delay. Median (IQR) BSID-III scaled scores for 569 children were: cognitive, 7 (IQR: 6, 8); receptive language, 8 (IQR: 7, 9); expressive language, 8 (IQR: 7, 9); fine motor, 8 (IQR: 7, 9); and gross motor, 8 (IQR: 7, 8).

We quantified 52 trace elements by iCAP ICP-MS (Fig. 1A). These elements were modestly correlated with each other (Appendix Fig. 1); pairwise correlation coefficients ranged from – 0.51 (Li and Ag) to 0.94 (Ba and Ce). Distributions of element levels are in Appendix Table 2. In > 50% of test samples, Cd (56.38%), Lu (70.47%), and Tl (69.66%) concentrations were lower than the limit of detection and thus were classified as "detectable" or "undetectable" for subsequent analyses.

3.2. Effects of individual elements in multiple linear and stability ENET regressions

Linear regression models of trace elements and BSID-III composite scores indicated that Ga, Ag, Sb, Ba, Ce, Eu, and Gd had a significant association with motor composite score (FDR-$q < 0.05$) (Appendix Table 3). Compared with the lowest quartile, the highest quartile of Ga (coefficient [b] = 2.20; 95% CI: 0.46, 3.95; FDR-q for trend test [FDR-q_{trend}] = 0.003), Ag (b = 1.56; 95% CI: 0.07, 3.04; FDR-q_{trend} = 0.011), Ba (b = 2.32; 95% CI: 0.58, 4.06; FDR-q_{trend} = 0.007), Ce (b = 2.41; 95% CI: 0.69, 4.13; FDR-q_{trend} = 0.001), Eu (b = 2.18; 95% CI: 0.43, 3.93; FDR-q_{trend} 0.017), and Gd (b = 2.65; 95% CI: 0.90, 4.39; FDR-q_{trend} = 0.003) were associated with higher motor composite score. Conversely, lower level of Sb ($b_{\text{quartile 4 vs. quartile 1}}$ = −3.29; 95% CI: −5.02, −1.57; FDR-q_{trend} = 0.004) was associated with higher motor composite score.

Fig. 1 and Appendix Table 4 show the results of variable selection for stability mENET and iENET models on neurodevelopmental outcomes. We identified 15 trace elements with main effects (Li, Al, Ti, V, Mn, Fe, Y, Nb, Ag, Sb, Cs, Ba, Pr, W, Tl) and two pairs of elements with interactive effects (Cd × Pr, Be × Cs) on cognitive composite score (Fig. 1B). In addition, an inverted U-shaped association between Zn, Sb, Ag and Ce with motor composite score were identified (Fig. 2B). Similarly, we identified 14 trace elements with main effects (Li, Be, Sc, Zn, Ga, Y, Zr, Nb, Ag, Sb, Cs, Ce, Hg, Tl) and five pairs of elements with interaction effects (Be × Cs, Cd × Cs, Sb × Ba, Mn × Pr, Ti × Cs) on motor composite score (Fig. 1C).

3.3. Exposure–response and interactive relationship by BKMR

BKMR analyses incorporating the trace elements identified by ENET models explored exposure–response relationships between each trace element and each composite score when setting all other trace elements to their corresponding median levels (Fig. 2). A linear inverse association was estimated between some trace elements (Li, Al, Mn, Fe, Y, Sb, and Ba) and cognitive composite score, while several other trace elements (Ti, V, Nb, Cs, Pr, W, and Tl) were positively associated with cognitive composite score. Visually, a weak relationship was estimated between Ag and cognitive composite score (Fig. 2A). BKMR also identified inverse associations between both Y and Zr with motor composite score, and weak positive associations for Ga and Tl. The associations of Li, Be, Sc, Nb, Cs, and Hg with motor composite score appeared null, and a weak inverted U-shaped relationship was visually estimated between motor composite score and Ag, as well as Ce. A strong positive relationship was estimated between Zn and motor composite score, except for the estimated potentially nonlinear effect at high Zn concentrations. In addition, inverted U-shaped associations between Zn, Sb, Ag and Ce and motor composite score were identified (Fig. 2B).

We additionally estimated PIPs for pairwise interactions between element pairs selected above. The inverted U-shaped relationship between Sb and motor composite score varied by levels of Ba (PIP for interaction: 0.92), while PIPs of other interaction pairs were < 0.5 (Fig. 3, Appendix Fig. 3).

3.4. Element mixtures and early childhood neurodevelopmental outcomes

The percentage of participants with neurodevelopmental delay and the distributions of sociodemographic factors across ERS quintiles are shown in Fig. 4. All ERSs were significantly associated with early childhood neurodevelopmental outcomes. Higher ERS was associated with lower probability of cognitive developmental delay ($OR_{quintile\ 5\ vs\ quintile\ 1}$ of ERS: 0.21; 95% CI: 0.10, 0.40; P < 0.001) (Fig. 4A–C). Children with ERS at the 80th percentile or higher showed 0.16-times risk of motor developmental delay (95% CI: 0.09, 0.31; P < 0.001) versus children with ERS at the 20th percentile or below (Fig. 4D–F). P-values for trend of cognitive developmental delay and motor developmental delay were both < 0.001.

We further used BKMR analysis to assess the contributions of individual candidate elements to the overall mixture effect on early childhood neurodevelopmental outcomes. As displayed in Table 2, in hierarchical variable selection analyses the group-PIPs for three element groups with cognitive composite score were 0.53, 0.13, and 0.03. Analyses identified Li (cPIP = 0.68), Al (cPIP = 0.83) and Fe (cPIP = 1.00) that presented stronger univariate exposure response relationships as important contributors to the overall association with cognitive composite score. Zn (group-PIP = 0.95; cPIP = 1.00), Ag (group-PIP = 0.65; cPIP = 0.81), and Sb (group-PIP = 0.99; cPIP = 0.65) were the most important contributors to the overall association with motor composite score.

4. Discussion

This study measured the most comprehensive element exposure profiles in umbilical cord serum to link these exposures with early childhood neurodevelopmental outcomes (Claus Henn et al., 2017; Lee et al., 2018a). Individual trace elements and several pairs of interactive elements appear to be associated with early childhood cognitive or motor development by using linear regression models and stability ENET. And the results in this study showed that each 1-unit increase in $\log_e$-transformed levels of an element or elements mixture was associated with how BSID-III composite score would change. Among these, the association between Mn and cognitive development has been consistently reported in candidate element studies in the Bangladesh cohort (Claus Henn et al., 2017; Lee et al., 2018a). Higher Mn concentration in umbilical cord blood was associated with lower cognitive score. In addition, an IQR increase in maternal blood Mn was associated with lower mental (MDI) and psychomotor (PDI) development indices. Because exposures in humans never occurs in isolation, individual effects may not necessarily capture actual complete relationships, and the association between a specific element and the outcome could be modified by other elements. Therefore, it is important to investigate multiple chemicals simultaneously to clarify their relationships with outcomes. Our analyses demonstrated that children with lower ERS—the proposed score representing the mixture effect of multiple element exposures—have higher risk of neurodevelopmental delay. Regarding the cognitive delay ERS, Li and Al are potentially toxic elements, while V and Mn are probably essential elements. Among the elements included in the motor delay ERS construction, Li and Hg are potentially toxic elements, while Zn is an essential element. ERS is a useful tool for characterizing cumulative risk from chemical mixtures, accounting for statistical challenges such as high degrees of correlation and chemical–chemical interactions. Prediction models using ERS could be used to assess risk stratification and discrimination power for predicting specific health endpoints.

Trace element mixture analyses identified Li, Al and Fe as the strongest contributors to the lower cognitive composite score based on hierarchical variable selection coupled to BKMR models. Results from preclinical studies in rats and zebrafish show long-term adverse neurodevelopmental consequences of intrauterine exposure to either Li or antipsychotics (Nery et al., 2014; Sechzer et al., 1986), which has not been found in clinical investigations. More research is needed to provide an estimation of the risk of Li exposure for the developing child. Al is a well-established neurotoxicant and is linked to cognitive function impairment and Alzheimer’s disease (Bishop et al., 1997; Bondy 2016). In utero, Al can transport across and accumulate in the placenta and may influence fetal development, and Al exposure in childhood is a potential risk factor for autism spectrum disorder (Kruger et al., 2010). One previous study also examined the neurological effects of Al exposure in healthy infants and found that hair Al levels are associated with poor motor function but not with language or cognitive development (Karwowski et al., 2018). Serum Fe is the Fe circulating in the blood that binds to plasma transferrin. One study reported a positive association between Fe exposure and cognitive outcomes, which is inconsistent with our findings (Hanieh et al., 2015). Notably, other studies have indicated an association between mothers with higher ferritin levels in pregnancy and lower cognitive scores at 6-month follow-up, which appears to be consistent with our findings and indicates a complicated relationship between maternal Fe exposure and children’s cognitive development (Hanieh et al., 2015; Kulik-Rechberger et al., 2016). Also, serum Fe levels vary at different times of the day, and are probably not suitable as a diagnostic indicator alone. In addition, breastfeeding and food are also the main sources of children’s Fe intake, which may be associated with children’s cognitive development. For the known trace elements, essentiality and toxicity are unrelated and toxicity is a matter of dose or exposure.

Zn, Ag, and Sb were the most important contributors to the overall association with motor composite score. Adequate Zn nutrition is important to ensure growth and neurodevelopment of children, healthy immune function, and normal pregnancy outcomes (Hanieh et al., 2015; Lind et al., 2004). Fetal Zn accumulation is a function of maternal Zn status, and thus newborn Zn deficiency is likely among women with inadequate dietary Zn intake during pregnancy. The association between Ag and cognitive function is rarely reported, except for two zebrafish and rat experimental studies that indicate adverse neurodevelopmental effects of Ag exposure (Powers et al., 2010; Sharma and Sharma 2012). The role Ag plays in the determination of motor function remains uncertain, and more research is needed to verify the mechanism behind the relationship. Humans can be exposed to Sb by breathing air, drinking water, and eating foods that contain the element, as well as by skin contact with substances that contain Sb. However, the effects of Sb on human health are not yet fully understood. Sb levels are positively correlated with inattention and hyperactivity/impulsivity scores rated by teachers (Lee et al., 2018b). Further, other evidence suggests that Sb may cause cerebellar ataxia (Khalil et al., 2006). Several potential interaction effects of two-element pairs were detected by the iENET model, and PIPs for pairwise interactions between element pairs selected above were further estimated. Notably, our study found an inverted U-shaped relationship between Sb and motor composite score that varied by levels of Ba. In addition, a previous study indicates that high concentration of Ba in whole blood is a protective factor for cognitive function in the elderly (Gu et al., 2021). However, the association between Ba and motor function is rarely reported, especially regarding the interaction effect between Ba and Sb on neurodevelopmental outcomes (Cusick et al., 2018). Due to the extremely low content of Ba in the human body and the influence of environmental factors, further studies are needed to fully characterize these relationships.

We did not identify any element associated with language composite score in this study. Both biological and environmental factors influence language development, but causal relationships remain unclear. Commonly documented risk factors of language development include a family history of language disorders or dyslexia, being male, being a younger sibling in a large family, and fewer years of parental education (Rudolph 2017). Conversely, several studies indicated that prenatal/perinatal factors do not seem to be important for language disorders (Tomblin et al., 1997; Whitehouse et al., 2014). However, higher prenatal Bisphenol A level in urine may be significantly and positively associated with a higher risk of poorer language skills among toddler boys (Jensen et al., 2019). Prenatal exposure to Pb and Hg has been associated with childhood language development (Calamandrei et al., 2020; Lin et al., 2013). It is possible that the levels of these elements in this study were not at a level that would reveal such associations or interactions among trace elements, which attenuated the associations. In addition, many other factors, such as nutrition, socioeconomic status, and home stimulation, also may determine children's early language development.

The major strength of our study is the implementation of two complementary statistical methods, stability ENET and BKMR, to evaluate the contribution of element mixtures on early childhood neurodevelopmental outcomes among prospective participants as well as nonlinear relationships and synergistic and antagonistic interaction relationships. While stability ENET handles multi-collinearity well through penalties, it provides superior variable selection performance by keeping the number of false positives low and provides the opportunity to select a group of non-zero collinear variables, identifying variables that are more important. Stability ENET also has the advantage of working well under sparsity and large-p/small-n situations. By contrast, BKMR allows for potential complex nonlinear and nonadditive relationships for the examined association by using a flexible kernel function. The detected nonlinear relationships of some trace elements and overall significant associations with early childhood neurodevelopmental outcomes in BKMR further highlights the need to apply stability ENET along with BKMR-like methods to comprehensively reveal complicated exposure–outcome relationships. It is worth noting that the stability ENET penalized regression results were partly consistent with that of multiple linear regression, indicating that the main components among simultaneous multiple elements associated with motor composite score were Sb and Ag, which also were identified in hierarchical variable selection coupled to BKMR models. The consistent results from three methods greatly reduces the chance of false discovery.

However, this study has some limitations. First, each element has a unique distribution within the organs and circulatory system, although this study only measured the element concentrations in umbilical cord blood serum that may be unable to completely reflect the element exposures level and distribution in the body. Second, BSID-III composite scores were standardized scores based on a population of children of the same age from the United States because no Bangladeshi norms have been published to date, which may not provide an accurate indication of the impact of these element exposure levels on early childhood neurodevelopmental outcomes in Bangladeshi population, highlighting the importance to develop Bangladeshi norms and innovate reliable approaches to assess neurodevelopment of children in resource-poor countries. Third, we used cross-validation and stability procedures to minimize the false positives of the ENET penalized models, yet our findings were not externally validated. Element exposures in cord serum could have tight relationship with the geological and environmental conditions. Notably, mother–infant pairs in low-income Bangladesh are exposed to higher levels of metals than the general population, hindering appropriate validation resources. However, the findings could be examined by future well-designed experimental studies to uncover underlying biological mechanisms. In addition, the proposed ERS reflecting element mixture exposures may capture partial risk of delayed neurodevelopment, which could be further complemented by multiple potential biological pathways affected by other parental and offspring environmental, nutritional, socioeconomic, or home stimulation factors.

5 . Conclusions

This is the most extensive prospective cohort study of element exposures among mother–infant pairs in Bangladesh. Our results suggest that co-exposure to a broad list of trace elements—particularly Li/Al/Fe or Zn/Ag/Sb—appears to impact children's early cognitive and motor function development. Children with higher ERS were less likely to have neurodevelopmental delay, highlighting the importance of assessing the effects of element mixtures on early childhood neurodevelopmental outcomes. These results provide further support for future studies that deepen mechanistic understanding of these exposure–outcome associations and validate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BMI	Body mass index
FDR	False discovery rate

ICP-MS	Inductively coupled plasma mass spectrometry
OR	Odds ratio
7Li	Lithium
9Be	Beryllium
11B	Boron
23Na	Sodium
24Mg	Magnesium
27Al	Aluminum
39K	Potassium
44Ca	Calcium
45Sc	Scandium
47Ti	Titanium
51V	Vanadium
52Cr	Chromium
55Mn	Manganese
56Fe	Iron
59Co	Cobalt
60Ni	Nickel
63Cu	Copper
66Zn	Zinc
71Ga	Gallium
75As	Arsenic
78Se	Selenium
85Rb	Rubidium
88Sr	Strontium
89Y	Yttrium
90Zr	Zirconium
93Nb	Niobium
95Mo	Molybdenum

107Ag	Silver
111Cd	Cadmium
118Sn	Tin
121Sb	Antimony
133Cs	Cesium
137Ba	Barium
139La	Lanthanum
140Ce	Cerium
141Pr	Praseodymium
146Nd	Neodymium
147Sm	Samarium
151Eu	Europiumxxx
157Gd	Europium
159Tb	Terbium
163Dy	Dysprosium
165Ho	Holmium
166Er	Erbium
169Tm	Thulium
172Yb	Ytterbium
175Lu	Lutetium
178Hf	Hafnium
181Ta	Tantalum
182W	Wolfram
202Hg	Hydrargyrum
205Tl	Thallium
208Pb	Lead
209Bi	Bismuth
232Th	Thorium
238U	Uranium

95% CI	95% Confidence interval

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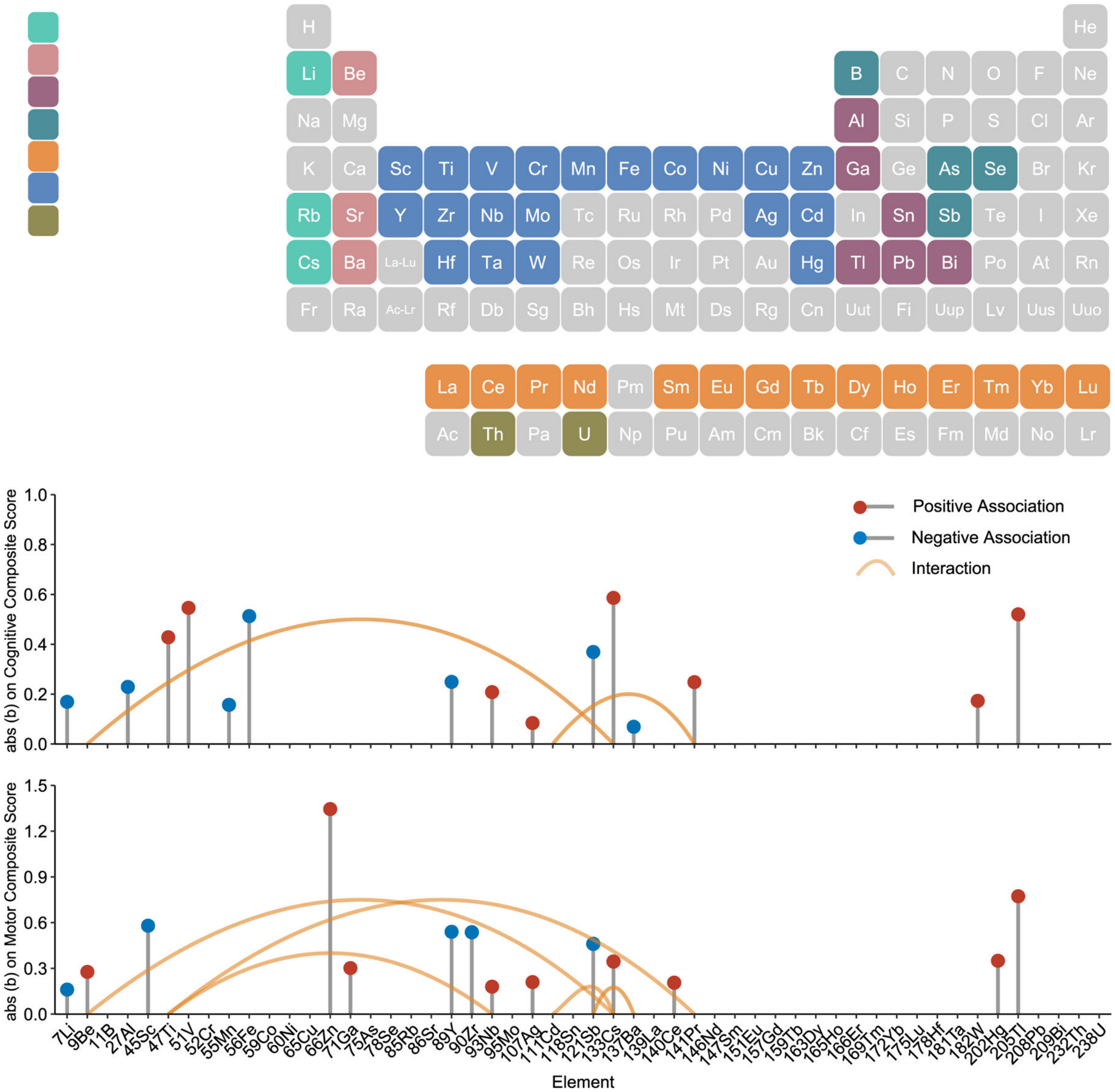

Fig. 1. Periodic table of the elements and elements identified in stability ENET regression.
(A) Periodic table of the elements, with detected elements marked with indicated colors. (B) Elements identified in stability ENET regression. Red dot indicates the element is positively associated with BSID-III composite score; blue dot indicates the element is positively associated with BSID-III composite score. Orange curve indicates an interaction relationship between two elements on BSID-III composite score. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

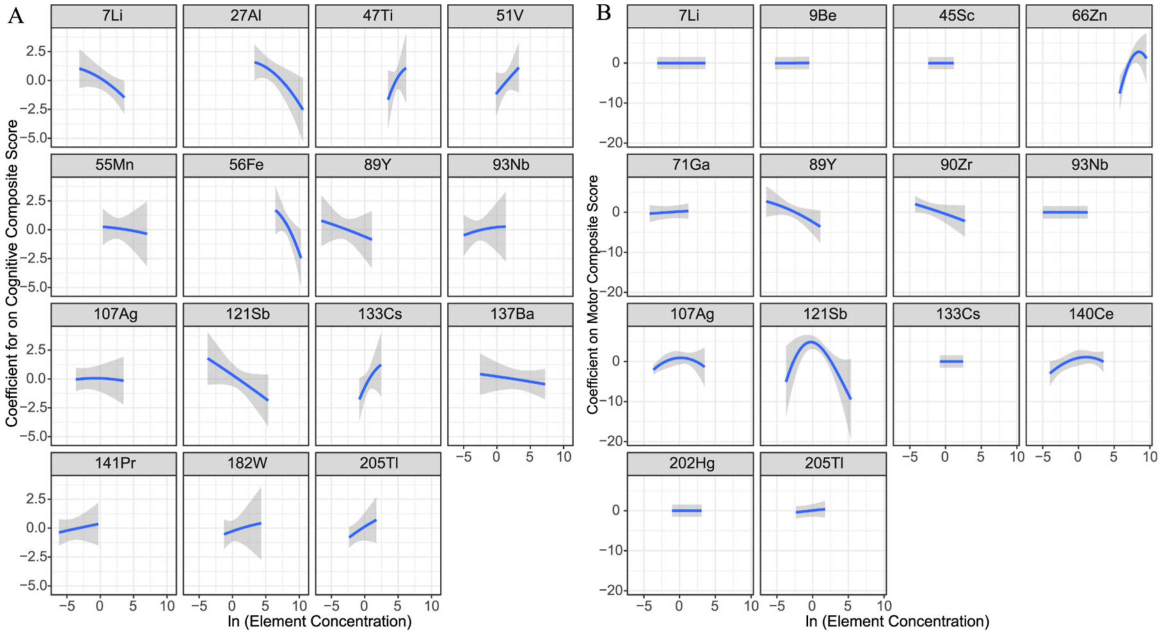

Fig. 2. Univariate dose–response relationships of significant elements.

Univariate dose–response association (estimates and credible intervals) of umbilical cord serum element levels on early childhood BSID-III composite scores, holding all other elements at their median concentrations among 569 mother–infant pairs in the Bangladesh prospective birth cohort study, 2008–2011. Models were adjusted for age of mother, sex of child, gestational age, Home Observation for Measurement of the Environment (HOME) score, exposure to second-hand smoke, maternal education level, household income level, and clinic. Blue solid line and gray bands represent the modified coefficient and its 95% credible intervals, respectively. Elements were $\log_e$-transformed. (A) Univariate dose–response association (estimates and credible intervals) of umbilical cord serum element levels on cognitive composite score. (B) Univariate dose–response association (estimates and credible intervals) of umbilical cord serum element levels on motor composite score. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

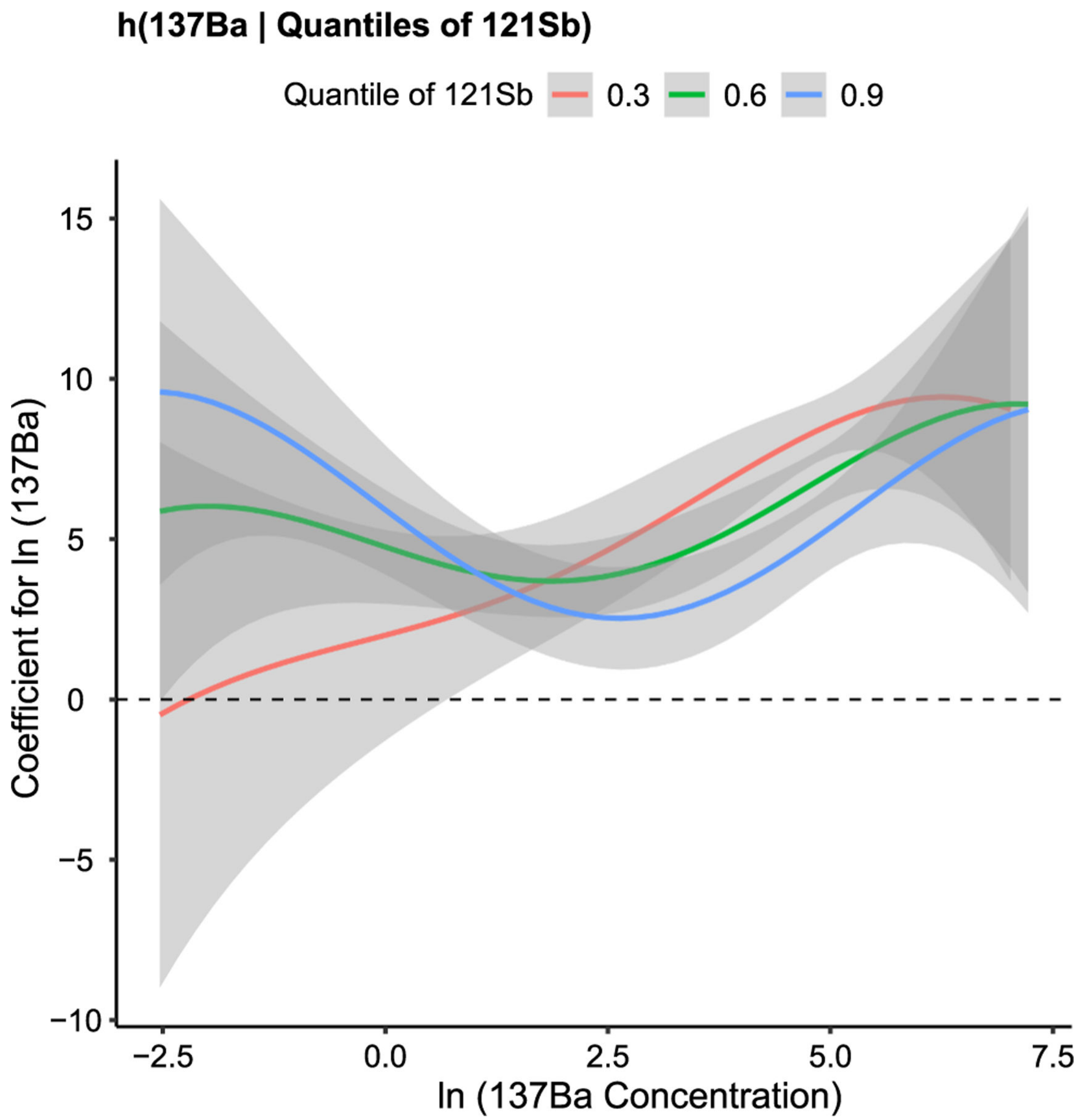

Fig. 3. Interaction relationship between Sb and Ba on motor composite score.

Bivariate exposure–response functions for Sb when Ba is fixed at 30th, 60th, or 90th percentile. Colored solid line and gray bands represent the modified coefficient and its 95% credible intervals, respectively. Nonlinear interaction effect between Sb and Ba on motor composite score was estimated by models adjusted for covariates including age of mother, sex of child, gestational age, Home Observation for Measurement of the Environment (HOME) score, exposure to second-hand smoke, maternal education level, household income level, and clinic.

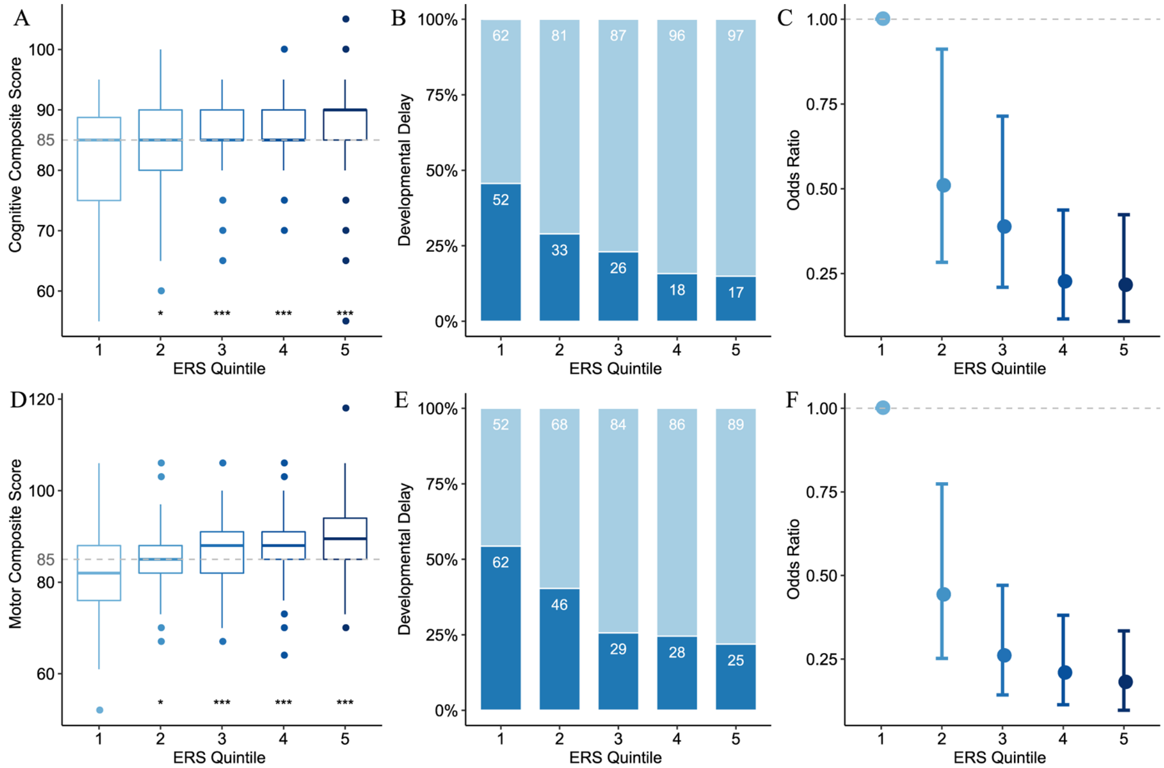

Fig. 4. Associations between element mixtures and early childhood neurodevelopmental outcomes.

(A) Boxplot comparing cognitive composite scores across element risk score (ERS) quintiles. Children with higher ERS had higher cognitive composite scores. One star indicates P < 0·05, two stars indicates P < 0·01, and three stars indicates P < 0·001. (B) Percent stacked bar plot displaying evolution of the proportion of normal (light blue) and developmental delay (dark blue) subgroups (sum equals 100%) based on cognitive composite score. (C) Odds ratio across ERS quintiles based on cognitive composite score. X-axis indicates the ERS quintiles. The first quintile was used as reference. Dots indicate mean odds ratio; bars indicate 95% confidence interval. (D) Boxplot comparing motor composite scores across ERS quintiles. Children with higher ERS had higher motor composite scores. (E) Percent stacked bar plot displaying evolution of the proportion of normal (light blue) and developmental delay (dark blue) subgroups (sum equals 100%) based on motor composite score. (F) Odds ratio across ERS quintiles based on motor composite score. X-axis indicates ERS quintiles. The first quintile was used as reference. Dots indicate mean odds ratio (OR); bars indicate 95% confidence interval (95% CI). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Characteristics of 569 mother–infant pairs.

Characteristics	**Summary statistics (*N* = 569)**
Maternal characteristics	***Median (IQR) or n (%)***
Age at enrollment (years)	22 (19, 25)
Marriage age (years)	18 (17, 18)
BMI level (kg/m ^{2}) (%)	
<18.5	171 (30.1)
18.5–25.0	347 (61.0)
≥25.0	51 (9.0)
Education level (%)	
No formal education	86 (15.1)
Primary education	180 (31.6)
Secondary or higher	303 (53.3)
Spouse's education level (%)	
No formal education	155 (27.2)
Primary education	168 (29.5)
Secondary or higher	246 (43.2)
Monthly household income level (Taka) (%)	
≤4000	273 (48.0)
4001–6000	232 (40.8)
>6000	64 (11.2)
Second-hand smoke (%)	
No	324 (56.9)
Yes	245(43.1)
Clinic (%)	
SCC	319 (56.1)
BCC	250 (43.9)
Child characteristics	***Median (IQR) or n (%)***
Birth gestational age (weeks)	38 (37, 40)
Child's age at time of assessment (months)	27.76 (25.92, 30.32)
HOME score	43.00 (41.00, 45.00)
Sex (%)	
Male	279 (49.0)
Female	290 (51.0)
Breastfeed (%)	
Yes	561 (98.6)
No	8 (1.4)
Birth parameters	
Weight (kg)	2.86 (2.65, 3.10)
Length (cm)	46.00 (46.00, 48.00)
Head circumference (cm)	33.00 (32.00, 33.00)

Characteristics	**Summary statistics (*N* = 569)**
Growth parameters at 20–40 months visit	
Weight (kg)	11.00 (10.00, 12.00)
Length (cm)	82.00 (80.00, 85.00)
Head circumference (cm)	45.00 (44.00, 46.00)
BSID-III at 20–40 months visit	
Cognitive scaled score	7 (6, 8)
Receptive scaled score	8 (7, 9)
Expressive scaled score	8 (7, 9)
Gross motor scaled score	8 (7, 9)
Fine motor scaled score	8 (7, 8)
Cognitive composite score	85 (80, 90)
Language composite score	89 (83, 92)
Motor composite score	85 (82, 91)

BCC: Birhampur Community Clinic; SCC: Sirajdikhan Community Clinic; BMI: Body mass index; HOME: Home Observation for Measurement of the Environment.

Table 2

Contributions of individual candidate elements to the overall mixture effect on early childhood neurodevelopmental outcomes by BKMR analysis.

mENET Model	Group †	Group-specific PIP	Component-wise PIP
Cognitive composite score			
7Li *	1	**0.53**	**0.68**
27Al *	**2**	**0.13**	**0.83**
47Ti	2	0.13	0.07
51 V	1	0.53	< 0.01
55Mn	2	0.13	0.03
56Fe *	**3**	**0.03**	**1.00**
89Y	1	0.53	< 0.01
93Nb	1	0.53	0.02
107Ag	1	0.53	0.22
121Sb	1	0.53	< 0.01
133Cs	1	0.53	0.02
137Ba	2	0.13	0.06
141Pr	1	0.53	< 0.01
182 W	1	0.53	0.02
205Tl	1	0.53	0.03
Motor composite score			
7Li	1	0.99	< 0.01
9Be	2	0.65	< 0.01
45Sc	1	0.99	< 0.01
66Zn *	**3**	**0.95**	**1.00**
71 Ga	2	0.65	0.01
89Y	2	0.65	0.15
90Zr	2	0.65	0.02
93Nb	2	0.65	< 0.01
107Ag *	**2**	**0.65**	**0.81**
121Sb *	**1**	**0.99**	**0.65**
133Cs	1	0.99	< 0.01
140Ce	1	0.99	0.35
202Hg	1	0.99	< 0.01
205Tl	2	0.65	0.01

* Ranking the first within the corresponding group.

† Groups for candidate elements were based on hierarchical cluster analyses as detailed in Methods.