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RESEARCH ARTICLE

# Effects of preterm birth and postnatal exposure to metal mixtures on neurodevelopment in children at 24 months of age

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## Abstract

The effects of early-life metal exposure on neurodevelopment in very low birth weight preterm (VLBMP) children (with a birth weight of <1500 g and a gestational age of <37 weeks) have not been clearly established. We aimed to investigate associations of childhood exposure to multiple metals and preterm low birth weight with neurodevelopment among children at 24 months of corrected age. VLBWP children ($n = 65$) and normal birth weight term (NBWT) children ($n = 87$) were enrolled from Mackay Memorial Hospital in Taiwan between December 2011 and April 2015. Lead (Pb), cadmium (Cd), arsenic (As), methylmercury (MeHg), and selenium (Se) concentrations in the hair and fingernails were analyzed as biomarkers for metal exposure. The Bayley Scale of Infant and Toddler Development, Third Edition, was used to determine neurodevelopment levels. VLBWP children had significantly lower scores in all development domains compared to NBWT children. We also investigated preliminary exposure levels of VLBWP children to metals as reference values for future epidemiological and clinical survey. Fingernails are a useful biomarker for metal exposure to evaluate the effects on neurological development. A multivariable regression analysis revealed that fingernail Cd concentrations were significantly negatively associated with cognition ($\beta = -0.63$, 95% confidence interval (CI): −1.17 to −0.08) and receptive language function ($\beta = -0.43$, 95% CI: −0.82 to −0.04) among VLBWP children. VLBWP children with a 10-μg/g increase in the As concentration in their nails had a 8.67-point lower composite score in cognitive ability and a 1.82-point lower score in gross-motor functions. Effects of preterm birth and postnatal exposure to Cd and As were associated with poorer cognitive, receptive language, and gross-motor abilities. VLBWP children are at risk for neurodevelopmental impairments when exposed to metals. Further large-scale studies are needed assess to the risk of neurodevelopmental impairments when vulnerable children are exposed to metal mixtures.

**Keywords** Metal · Hair and fingernail · Neurodevelopment · Very low birth weight preterm children · Bayley Scale

## Abbreviations

As — arsenic
Bayley-III — the Bayley Scale of Infant and Toddler Development, Third Edition

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| | |
|---|---|
| Cd | cadmium |
| CV | coefficient of variation |
| LOD | limit of detection |
| MeHg | methylmercury |
| NBWT | normal birth weight term |
| Pb | lead |
| SD | standard deviation |
| Se | selenium |
| VLBWP | very low birth weight preterm |

## Background

The number of newborns in Taiwan has decreased in recent years, whereas the incidence of prematurity is increasing, and the rate was 10.4% in 2019 (HPA 2021). For the past 20 years, the Premature Baby Foundation of Taiwan has been longitudinally collecting neurodevelopmental evaluations at 6, 12, and 24 months of corrected age of very low birth weight preterm (VLBWP) infants (with a birth weight of <1500 g and a gestational age of <37 weeks). Compared to normal birth weight term (NBWT) children, VLBWT children are vulnerable and at risk of growth delays, chronic medical problems, cognitive deficits, motor coordination disorders, and academic disabilities (Caravale et al. 2005; McNicholas et al. 2013; Oliveira et al. 2011). The neurological prognosis of VLBWP children during early life will influence their neurodevelopment in childhood and adolescence.

Compared to adults, children may be at higher risk of exposure to environmental pollutants because of their behaviors (Tsou et al. 2015). Some metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are recognized as neurotoxicants that can interfere with neurodevelopmental functions in children. Pan et al. (2018) showed that postnatal Pb exposure was associated with a decreased intelligent quotient (IQ) at 9~11 years of age in China. A large-scale prospective cohort survey conducted in Bangladesh indicated that childhood Cd exposure was related to lower full-scale IQ levels and poorer prosocial behavior scores (Gustin et al. 2018). Total Hg concentrations were negatively correlated with the general cognitive, memory, and verbal scores among preschool children in Spain (Freire et al. 2010). An inverse relationship between As exposure and motor function was detected among 304 school-age children in Bangladesh (Parvez et al. 2011). However, those studies investigated the impacts of metal exposure on neurodevelopment only among children with a normal birth.

Compared to blood and urine samples, human hair and fingernails are used to reflect longer-term exposure, due to the growth rate of hair being approximately 1 cm/month (Jursa et al. 2018) and the rate of fingernails being 0.3 cm/month (Slotnick and Nriagu 2006). Also, collecting hair and fingernail samples is painless and more convenient for vulnerable VLBWP children. Davis et al. (2014) indicated that infant nails were a reliable biomarker for As exposure. Additionally, previous studies revealed good associations between metal concentrations in nails and other biomarkers (Levin-Schwartz et al. 2021; Parvez et al. 2011).

Evidence indicates that compared to NBWT children, VLBWP children exhibit impairments in several developmental domains. To our knowledge, few investigations of both preterm effects and childhood metal exposure of children have been conducted. The aim of this study was to assess correlations of childhood exposure to multiple metals and of preterm low birth weight with neurodevelopment among children at 24 months of corrected age. We also investigated biomonitoring data of VLBWP children's exposure to metals as reference values for future epidemiological and clinical survey in Taiwan.

## Methods

### Study population

VLBWP children (with a birth weight of <1500 g and a gestational age of <37 weeks) ($n = 65$) with neurodevelopment information at 24 months of corrected age, as well as postnatal exposure to heavy metals, were compared to NBWT children (with a birth weight of ≥2500 g and a gestational age of ≥37 weeks) at 24 months of age ($n = 87$). All subjects were recruited from Mackay Memorial Hospital (Taipei, Taiwan) between December 2011 and April 2015. Written informed consent was obtained from parents of participating children after receiving an explanation of the study. This study was approved by the institutional review board of Taipei Mackay Memorial Hospital (IRB no. MMH-I-S-596 and IRB no. 13MMHIS088). Both hair and fingernail samples were collected at 24 months of corrected age among VLBWP children and 24 months of age among NBWT children. A structured questionnaire was used to obtain demographic information of children and their parents at the same time.

### Exposure assessment

Pb, As, Cd, and Se concentrations in hair and fingernails were examined by inductively coupled plasma-mass spectrometry (ICP-MS; Thermo X-series II) according to USEPA method 200.8 (EPA USA 1994). Hair and fingernail samples of children were applied as non-invasive biomarkers to reflect internal exposure to environmental trace elements over long-term periods of time (Barbosa et al. 2005; Esplugas et al. 2019; Fiłon et al. 2020; Salcedo-Bellido et al. 2021). Hair samples from the occipital region of the scalp and fingernail samples were collected and rinsed three times with deionized water after sonication with a neutral detergent for 30 min. After being dried in an oven at 37 °C for 24 h, 0.1 g of hair sample was added to 5 mL of 69% nitric acid (J.T. Baker®) and wet-digested at 90 °C for 3 h. A fingernail sample (0.04 g) was placed in an acid-bath with 1.09 mL of 69% nitric acid and 0.2 mL of 30% hydrogen peroxide (Merck, Germany) overnight and wet-digested at 90 °C for 3 h. Certified reference material GBW09101b from the Shanghai Institute of Nuclear Research (Shanghai, China) was used to validate the quality of the measurements. The precision (coefficient of variation, CV) and accuracy were 0.06~6.79% and 92.1~110%, respectively. The limits of detection (LODs), calculated as three times the standard deviation (SD) of the blank concentration, for Pb, Cd, As, and Se were 0.012, 0.004, 0.009, and 0.071 μg/L, respectively.

Methylmercury (MeHg) concentrations in hair and fingernail samples were analyzed using the Brooks Rand BRL Method BR-0011 and the USEPA method 1630 with minor modifications (Hsi et al. 2016). Samples was rinsed three times with deionized water after sonication with a neutral detergent for 30 min and dried in an oven at 37 °C for 24 h. Samples (0.01 g) were heated with 2 ml of 25% KOH/methanol at 75 °C for 5.5 h. The cooled sample was added to 10 ml of CH2Cl2 and 2 ml of HCl before shaking for 30 min. Samples were filtered through a 0.45-μm filter, which was placed in a distillation vessel and purged for 1 h after deionized water was added. An aliquot in a closed purge vessel was supplemented with 300 μL of sodium acetate buffer and 40 μL of 1.0% sodium tetraethylborate. The ethyl analog of MeHg was separated from the solution, and MeHg concentrations were determined by an automated methylmercury analytic system (MERX, Brooks Rand, USA) using cold vapor atomic fluorescence spectrophotometry (CVAFS). Reference materials, IAEA-085 methylmercury, total mercury, and other trace elements in human hair of the International Atomic Energy Agency were applied to validate the quality of the measurements. The precision and accuracy were 10.28% ± 6.81% and 91.36% ± 8.71%, respectively, while the detection limit was 0.25 ng/g.

## Neurodevelopment assessment

The Bayley Scale of Infant and Toddler Development, Third Edition (Bayley-III) was applied to assess neurodevelopmental function of NBWT children at 24 months of age and VLBWP children at 24 months of corrected age. This scale is a useful tool for evaluating development of children between the ages of 1 and 42 months (Walder et al. 2009). Cognition, language, and motor developmental domains of children were assessed by psychologists. The cognitive domain assesses functions such as sensorimotor development, exploration and manipulation, object relatedness, concept formation, and memory. The language development domain is subdivided into receptive language and expressive language subscales, while the motor development domain is subdivided into fine-motor and gross-motor subscales. We present scaled scores of five subscales for cognition, receptive language, expressive language, fine-motor, and gross-motor development. The mean (SD) normative score of each subscale was 10 (3). Composite scores were derived from subscales for cognition, language, and motor developmental domains with a mean ± SD score of 100 ± 15. Higher scores indicated better neurodevelopmental function.

## Covariates

Information about children's demographic characteristics including gender, age, gestational age, weight, height, head circumference, Apgar scores at birth, parity, breastfeeding duration, and dietary intake were collected using trained interviewers with a structured questionnaire. Sociodemographic characteristics of the parents such as family income, maternal age at delivery, and maternal smoking were also obtained.

## Statistical analyses

Sociodemographic characteristics of the study population were described by the mean and SD for continuous variables and counts (%) for categorical variables. Metal concentrations in hair and fingernail samples were natural logarithm-transformed to normalize right-skewed data. Bivariate associations of exposure with neurodevelopment outcomes and covariates among VLBWP and NBET children were assessed with the Mann-Whitney *U*-test or the Kruskal-Wallis test and Spearman correlation coefficients. We also applied multivariable linear regression models to evaluate effects of metal mixtures exposure on neurodevelopmental outcomes among these two groups children. Variables with $p < 0.2$ in a univariate regression analysis were further included in the final model analysis. As covariates with a high correlation, only variables that induced the highest adjusted $R^2$ were included in the final multivariate regression model to eliminate collinearity. The variance inflation factor (VIF) was used to measure the severity of collinearity.

The interaction analysis of prematurity and postnatal metals exposure on children's neurodevelopment was assessed. According to the gestational age stratified by 37 weeks incorporate with metal concentrations in fingernails categorizing them into high and low based on a median value, children were categorized into four subgroups: having high metal exposure/with a gestational age of <37 weeks, having low metal exposure/with a gestational age of <37 weeks, having high metal exposure/with a gestational age of ≥37 weeks, and having low metal exposure/with a gestational age of ≥37 weeks. The neurodevelopmental scores between these four subgroups were assessed. The trend test was applied to evaluate the dose-response relationship. All statistical analysis were conducted using the SAS software (vers. 9.4, SAS Institute, Cary, NC, USA).

## Results

### Demographic characteristics

Sociodemographic characteristics of VLBEP compared to NBWT children are described in Table 1. The mean gestational age and birth weight of VLBWP children were 28.6 ± 3.2 weeks and 1077 ± 281 g, while those of NBWT children were 38.6 ± 1.3 weeks and 3084 ± 515 g, respectively. Birth outcomes such as gestational age, weight, height, head circumference, and Apgar score were lower among VLBWP children compared to NBWT children. The breastfeeding duration and rice intake levels in VLBWP children were significantly lower than those of NBWT children. In family characteristics, there was a significantly higher monthly family income, maternal age at delivery, and maternal smoking among the VLBWP group.

### Metal concentrations and neurodevelopmental scores

The different distributions of metal concentrations in hair and fingernails between these two group are presented in Fig. 1. Higher concentrations of MeHg in both biomarkers, Se in hair, and Cd in fingernails were observed among NBWT children. Se concentrations in fingernails of VLBWP children were higher than those of NBWT children. As and Pb levels in hair and fingernails between these two groups exhibited no significant differences. Table 2 shows developmental scores of Bayley-III of each group. There were significant differences in all subtests between groups. VLBWP children had lower scores (mean ± SD) in cognition (8.8 ± 2.6), receptive language (9.0 ± 2.2), expressive language (8.1 ± 2.7), fine-motor (8.4 ± 2.7), and gross-motor (7.1 ± 2.9) development than the normative score (10 ± 3).

### Associations between metal mixtures exposure and neurodevelopment

Negative correlations of metal levels in fingernails with developmental scores were obtained ($r = -0.34$~0.02), whereas no correlations between metal levels in hair and outcomes were observed ($r = -0.003$~0.14). Therefore, we applied metal concentrations in fingernails as an indicator of metal exposure for the following analysis. Covariates such as breastfeeding, parity, and maternal age with a $p$ value of >0.2 in a univariate regression analysis were further excluded in the final model analysis. The VIF in multivariable regression analysis is less than 2 indicating the rare presence of collinearity. Relationship between metal mixtures exposure and neurocognitive abilities from multivariable linear regression stratified by VLBWP and NBWT children are given in Table 3. The effects of As and Cd in fingernails on neurodevelopmental scores were significant. Hence, we only presented the results of As and Cd in Table 3. Linear regression analyses from VLBWP children revealed that fingernail Cd concentrations were significantly negatively associated with cognition ($\beta = -0.63$, 95% confidence interval (CI): −1.17 to −0.08) and receptive language ($\beta = -0.43$, 95% CI: −0.82 to −0.04).

**Table 1** Sociodemographic characteristics of the very low birth weight preterm (VLBWP) and normal birth weight term (NBWT) groups

| | VLBWP | NBWT | *p* |
|---|---|---|---|
| | Mean ± SD or *n* (%) | | |
| Children's characteristics | | | |
| *n* | 65 | 87 | |
| Male | 32 (49.2) | 40 (46.0) | 0.69 |
| Gestational age (weeks) | 28.6 ± 3.2 | 38.6 ± 1.3 | <0.0001 |
| Age (years) | 2.7 ± 0.5 | 2.9 ± 0.3 | 0.003 |
| 1-min Apgar score | 6.38 ± 1.7 | 9.7± 0.5 | <0.0001 |
| 5-min Apgar score | 8.1 ± 1.2 | 9.9 ± 0.4 | <0.0001 |
| Birth height (cm) | 35.8 ± 4.2 | 49.9 ± 2.1 | <0.0001 |
| Birth weight (g) | 1077 ± 281 | 3084 ± 515 | <0.0001 |
| Birth head circumference (cm) | 25.8 ± 2.7 | 33.1 ± 1.5 | <0.0001 |
| Parity | | | |
| 1 | 40 (64.5) | 62 (71.3) | 0.38 |
| ≥2 | 22 (35.5) | 25 (28.7) | |
| Breastfeeding (months) | 7.5 ±.6.4 | 10.7 ± 9.4 | 0.01 |
| Food consumption (servings/week) | | | |
| Rice | 19.2 ± 14.5 | 23.3 ± 12.3 | 0.02 |
| Meat products | 9.3 ± 6.7 | 8.3 ± 7.0 | 0.12 |
| Eggs | 6.2 ± 7.3 | 4.7 ± 3.8 | 0.28 |
| Dairy | 12.1 ± 17.5 | 12.9 ± 9.5 | 0.17 |
| Vegetables and fruits | 15.7 ± 11.7 | 18.6± 13.7 | 0.22 |
| Seafood | 8.6 ± 8.5 | 7.4 ± 7.4 | 0.49 |
| Freshwater fish | 4.3 ± 4.7 | 3.5 ± 3.7 | 0.35 |
| Saltwater fish | 3.7 ± 3.8 | 3.8 ± 4.0 | 0.75 |
| Shellfish | 2.5 ± 2.2 | 2.4 ± 2.6 | 0.51 |
| Sociodemographic characteristics | | | |
| Family income (US$/month) | | | |
| <2300 | 29 (47.5) | 68 (81.0) | <0.0001 |
| ≥2300 | 32 (52.5) | 16 (19.0) | |
| Maternal age at delivery (years) | 33.2 ± 4.4 | 31.6 ± 4.2 | 0.02 |
| Maternal smoking | 6 (14.3) | 1 (1.2) | 0.005 |

*SD* standard deviation

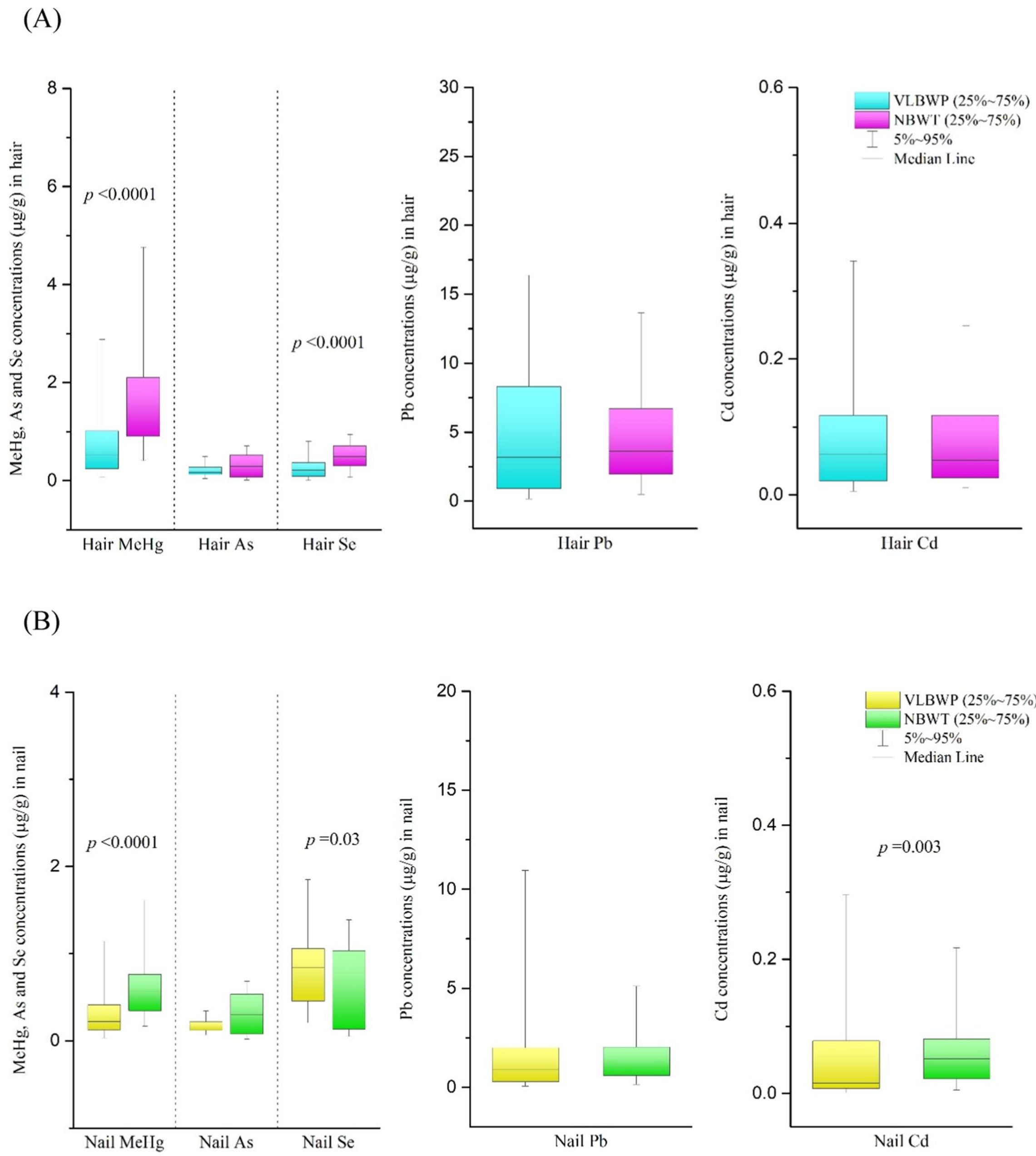


**Fig. 1** Metal concentrations in hair (**A**) and fingernails (**B**) among very low birth weight preterm (VLBWP) and normal birth weight term (NBWT) children

VLBWP children with a 10-μg/g increase in As concentrations in fingernails had a 8.67-point lower composite score in cognitive ability (95% CI: −16.44 to −0.91) and a 1.82-point lower score in gross motor (95% CI: −3.68 to 0.04). In NBWT children, there were no significant associations between Cd exposure and neurodevelopment outcomes. A significant negative correlation between As concentrations in fingernails with gross-motor scores ($\beta = -1.31$, 95% CI: −2.43 to −0.19).

## Effects of preterm birth and postnatal exposure to metal mixtures on neurodevelopment

We attempted to further evaluate the interaction that existed between prematurity and postnatal metals exposure. According to the gestational age stratified by 37 weeks and As and Cd concentrations in fingernails stratified by the median, children were categorized into four groups: having high metal exposure/with a gestational age of <37 weeks, having

**Table 2** Results of neurodevelopment evaluated by the Bayley Scale (Bayley-III) for very low birth weight preterm (VLBWP) children at 24 months of corrected age and normal birth weight term (NBWT) children at 24 months of age

| Score[a] | VLBWP Mean ± SD | NBWT mean ± SD | *p* |
|---|---|---|---|
| Cognitive | 8.8 ± 2.6 | 11.5 ± 2.6 | <0.0001 |
| Receptive language | 9.0 ± 2.2 | 11.2 ± 2.4 | <0.0001 |
| Expressive language | 8.1 ± 2.7 | 11.1 ± 2.5 | <0.0001 |
| Fine motor | 8.4 ± 2.7 | 11.5 ± 2.7 | <0.0001 |
| Gross motor | 7.1 ± 2.9 | 10.5 ± 1.7 | <0.0001 |

*SD* standard deviation

[a]Normative score: average 10, SD 3

low metal exposure/with a gestational age of <37 weeks, having high metal exposure/with a gestational age of ≥37 weeks, and having low metal exposure/with a gestational age of ≥37 weeks. Due to the small sample number of each subgroup, we could not analyze multivariate regression that included both dichotomized variables. Children with preterm birth (a gestational age of <37 weeks) and higher Cd exposure have the lowest cognitive, receptive language, and gross-motor scores (p for trend:<0.0001). The dose-response trends of As exposure and gestational age on neurodevelopment were observed (p for trend:<0.0001) (Table 4).

## Discussion

In our study, VLBWP children had significantly lower scores in all development domains than did NBWT children. We also investigated preliminary data of exposure to metals by VLBWP children as reference values for future epidemiological and clinical survey in Taiwan. Previous studies mostly investigated associations between metal exposure and development in full-term children. We examined effects of both preterm birth and metal mixtures exposure on neurodevelopment in the early life of children. Inverse correlations between metal concentrations in fingernails and

**Table 3** Linear regression analysis of cadmium (Cd) and arsenic (As) concentrations (μg/g, log-transformed) in fingernails with very low birth weight preterm (VLBWP) and normal birth weight term (NBWT) children's neurocognitive abilities

| | VLBWP | | | NBWT | | |
|---|---|---|---|---|---|---|
| | β | (95% CI) | *p* | β | (95% CI) | *p* |
| Cognitive score | | | | | | |
| Nail Cd | **−0.63** | **(−1.17, −0.08)** | **0.03** | 0.39 | (−0.53, 0.86) | 0.39 |
| Nail As | −1.49 | (−3.15, 0.18) | 0.07 | 0.01 | (−0.54, 0.57) | 0.95 |
| Receptive language score | | | | | | |
| Nail Cd | **−0.43** | **(−0.82, −0.04)** | **0.03** | −0.05 | (−0.80, 0.69) | 0.88 |
| Nail As | −0.15 | (−1.39, 1.09) | 0.80 | 0.02 | (−0.43, 0.48) | 0.92 |
| Expressive language score | | | | | | |
| Nail Cd | −0.35 | (−0.91, 0.22) | 0.22 | −0.11 | (−1.01, 0.79) | 0.81 |
| Nail As | −0.80 | (−2.65, 1.05) | 0.38 | −0.06 | (−0.61, 0.48) | 0.82 |
| Fine-motor score | | | | | | |
| Nail Cd | −0.26 | (−0.78, 0.26) | 0.32 | 0.33 | (−0.47, 1.13) | 0.40 |
| Nail As | −1.08 | (−2.76, 0.58) | 0.19 | −0.09 | (−0.57, 0.40) | 0.72 |
| Gross-motor score | | | | | | |
| Nail Cd | −0.30 | (−0.88, 0.27) | 0.30 | −0.02 | (−1.08, 1.06) | 0.97 |
| Nail As | **−1.82** | **(−3.68, 0.04)** | **0.05** | **−1.31** | **(−2.43, −0.19)** | **0.03** |
| Composite cognitive score | | | | | | |
| Nail Cd | **−2.96** | **(−5.51, −0.43)** | **0.02** | 2.02 | (−2.29, 6.34) | 0.35 |
| Nail As | **−8.67** | **(−16.4, −0.91)** | **0.03** | 0.31 | (−2.33, 2.96) | 0.81 |
| Composite language score | | | | | | |
| Nail Cd | **−2.74** | **(−5.40, −0.07)** | **0.04** | −0.14 | (−4.29, 4.00) | 0.94 |
| Nail As | −2.46 | (−10.68, 5.75) | 0.55 | −0.27 | (−2.81, 2.26) | 0.83 |
| Composite motor score | | | | | | |
| Nail Cd | −2.43 | (−5.46, 0.60) | 0.11 | −1.25 | (−12.8, 10.32) | 0.83 |
| Nail As | −8.73 | (−17.99, 0.53) | 0.06 | 1.84 | (−5.25, 8.92) | 0.60 |

The model was adjusted for children's gestational age, age, family income, and methylmercury, lead, and selenium concentrations in fingernails

The bold represented the statistical significance

*CI*, confidence interval

**Table 4** Combined effects of gestational age (GA) and low and high cadmium (Cd) and arsenic (As) exposure on neurodevelopmental scores of Bayley-III

| Metal exposure | | GA (weeks) | n | Cognitive score (mean ± SD) | Receptive language score (mean ± SD) | Gross-motor score (mean ± SD) |
|---|---|---|---|---|---|---|
| Cd levels in fingernails[a] | High | <37 | 19 | 7.57 ± 3.34 | 8.04 ± 2.65 | 5.76 ± 3.03 |
| | Low | <37 | 42 | 9.37 ± 1.93 | 9.47 ± 1.83 | 7.91 ± 2.51 |
| | High | ≥37 | 36 | 12.21 ± 1.98 | 12.21 ± 2.04 | 10.67 ± 1.33 |
| | Low | ≥37 | 25 | 11.16 ± 2.97 | 10.12 ± 2.35 | 10.33 ± 2.60 |
| *P* value* | | | | <0.0001 | <0.0001 | <0.0001 |
| *P* for trend[c] | | | | <0.0001 | <0.0001 | <0.0001 |
| As levels in fingernails[b] | High | <37 | 27 | 8.54 ± 2.12 | 8.92 ± 2.19 | 6.44 ± 3.19 |
| | Low | <37 | 34 | 8.95 ± 2.83 | 9.07 ± 2.26 | 7.70 ± 2.54 |
| | High | ≥37 | 34 | 12.29 ± 2.39 | 12.14 ± 1.93 | 10.43 ± 0.85 |
| | Low | ≥37 | 27 | 11.34 ± 2.59 | 10.63 ± 2.54 | 10.69 ± 2.50 |
| *P* value* | | | | <0.0001 | <0.0001 | <0.0001 |
| *P* for trend[c] | | | | <0.0001 | <0.0001 | <0.0001 |

*GA* gestational age, *SD* standard deviation

*Kruskal-Wallis test

[a]Cd levels in fingernails were categorized into high and low groups using the median concentration of 0.03 μg/g

[b]As levels in fingernails were categorized into high and low groups using the median concentration of 0.18 μg/g

[c]The trend analysis was conducted by a multiple linear regression analysis with controlling for children's age and family income

development outcomes were obtained. In the multivariable linear regression analysis, fingernail Cd concentrations were significantly negatively associated with cognition and receptive language among VLBWP children. Better cognitive and gross-motor scores were observed among VLBWP children with lower As exposure. The joint effects of preterm birth and Cd and As exposure on poor neurodevelopment revealed dose-response trends. Results indicated that VLBWP children were more vulnerable to metal exposure and at risk for neurodevelopmental impairments.

MeHg exposure levels in both biomarkers of NBWT children were significantly 2.5-times higher than those of VLBWP children in our results. We found a positive correlation between gestational age and MeHg exposure ($r$ = 0.36~0.43, $p < 0.0001$). A previous study indicated that 80% of Hg is approximately represented by MeHg, which can cross the blood-brain and placenta barriers and expose fetuses to MeHg (McDowell et al. 2004; Pérez et al. 2019). A shorter gestational age may reduce prenatal exposure to MeHg in VLBWP children. Lee et al. (2021) indicated that prenatal MeHg exposure was correlated with postnatal exposure ($r = 0.20$). Moreover, we observed that VLBWP children had higher fingernail Se concentrations that may reduce exposure to mercury owing to the high affinity of Se for Hg (Melgar et al. 2019).

The exposure parameters such as activity patterns, hormonal status (Barbosa et al. 2005), nutritional status (Kordas et al. 2016; Papadopoulou et al. 2019), and environmental factors (Hussein et al. 2008; Tippairote et al. 2019) may affect differences in biomarkers of metal concentrations among VLBWP and NBWT children. Hence, we observed inconsistent trends of Se concentrations in both biomarkers between groups. Fingernail Cd and As concentrations of NBWT children in our study were similar to those observed in Brazil (Carneiro et al. 2011), whereas higher Pb and Se levels in fingernails were shown in our study. It was noted that Pb, Cd, and As exposure levels of VLBWP children were comparable to those of NBWT children in our study. These findings provide metal exposure levels among VLBWP children as reference values for future epidemiological and clinical investigations.

Negative associations between metal levels in fingernails and developmental scores were obtained in our results, whereas rare correlations were observed with hair in our results. Previous studies applied fingernails as proxies for metal exposure in children (Carneiro et al. 2011; Hussein et al. 2008). Fingernails have been applied as a marker of long-term exposure owing to nails' growth rate of 0.3 cm/month (Barbosa et al. 2005; Slotnick and Nriagu 2006). Compared to full-term children, VLBWP children likely have a lower growth rate of nails which would still be suitable for reflecting long-term metal exposure. We can evaluate long-term associations between metal exposure and neurodevelopment using fingernails as biomarkers. Also, collecting fingernail samples is painless and more convenient than drawing blood and thus is suitable for vulnerable

VLBWP children. Furthermore, a previous study showed good associations between metal concentrations in nails and other biomarkers (Parvez et al. 2011). A study investigated Pb levels in multimedia biomarkers from blood, urine, hair, and fingernails and showed that nail Pb levels were significantly correlated with other biomarkers ($r$ = 0.15~0.26) (Levin-Schwartz et al. 2021). In our study, fingernail samples were a reliable biomarker of metal exposure for evaluating the effects on neurological development among VLBWP children.

The longitudinal survey, maternal and infant nutrition interventions in Bangladesh, investigated the Se status and cognitive function of children at 5 and 10 years of age, and they revealed negative associations between Se concentrations in hair above the spline knot at 0.66 μg/g and cognitive function scores (Skroder et al. 2017). In our results, 10.8% of VLBWP children ($n$ = 7) had hair Se levels higher than the value of 0.66 μg/g with a mean of 2.01 μg/g. We found that those children had lower scores of gross-motor function (mean ± SD: 6.43 ± 2.82). However, there were few participants with levels of Se in the hair above 0.66 μg/g in our analysis. Further study is needed to assess the neurotoxicity of excessive exposure to Se.

To our knowledge, the evidence of associations between early-life multiple metal exposure and neurodevelopment of VLBEP children is insufficient. Our findings showed that fingernail Cd concentrations were significantly negatively associated with cognition and receptive language functions. Cadmium was shown to indirectly affect the developing brain by disrupting endocrine hormone function (Gustin et al. 2018; Iijima et al. 2007). Prenatal and postnatal cadmium exposure may impair children's cognition development, learning abilities, and behavior (Ciesielski et al. 2012; Kippler et al. 2016). A 10-year longitudinal cohort study assessed effects of prenatal and childhood Cd exposure on neurodevelopment in Bangladeshi children. Their results revealed that childhood Cd exposure was negatively correlated with full IQ scores and most of the subtests. Also, children with the highest exposure tertile had prosocial behavior impairment (Gustin et al. 2018).

VLBWP children with lower As exposure had better cognitive and gross-motor abilities in our results. A previous study conducted in Taiwan demonstrated that preschool children exposed to higher As were significantly associated with a risk of developmental delays, which indicated that urinary total As levels were significantly correlated with developmental delays in the speech-language domain (OR = 3.57, 95% CI: 1.22 to 10.57) and in the global delays domain (OR = 2.00, 95% CI: 1.01 to 3.98) (Hsieh et al. 2014). A study in Bangladesh indicated that As exposure was negatively correlated with total motor composite scores ($\beta = -3.77$, 95% CI: −6.52 to −1.03) (Parvez et al. 2011). Although those studies collected biological samples from children with a normal birth, few studies have investigated effects of both very low birth weight preterm and metal exposure on neurodevelopment in the early life of children. Oliveira et al. (2011) revealed that VLBWP children were at risk of motor and cognitive impairments. They only assessed the effects of parents' level of education and the home environment on development of these children. Our results showed effects of preterm birth and postnatal exposure to metal mixtures on a risk of neurodevelopmental impairments.

The main strengths of this study are the demonstrated association of childhood multiple metal exposure and preterm low birth weight with neurodevelopment among children at 24 months of corrected age. We also included information on exposure and outcomes of full-term born children to validate that VLBWP children have risks of cognitive, language, and motor impairments. Fingernails were used as a biomarker of long-term metal exposure and revealed good correlations with neurodevelopmental scores. We adjusted potential neurotoxic contaminants, such as MeHg and Pb, that may cause neurodevelopmental problems in children (Freire et al. 2010; Hong et al. 2015). We investigated the joint effect of preterm birth and postnatal exposure to metal mixtures on poor neurodevelopmental scores. However, we enrolled only a small number of participants owing to a small population of VLBWP children. Additionally, we lacked an investigation of prenatal metal exposure, which is also associated with child neurodevelopmental outcomes (Guo et al. 2020).

## Conclusions

Our study reaffirmed that VLBWP children had significantly poorer neurodevelopment than NBWT children. We investigated preliminary exposure levels of VLBWP children to metals as reference values for future epidemiological and clinical studies. We revealed associations of childhood exposure to multiple metals and preterm low birth weight with neurodevelopment among children in early life. Metal exposure using fingernails as a biomarker can be used to evaluate the effects on neurological development among VLBWP children. Cd exposure was significantly negatively associated with cognitive and receptive language function, while VLBWP children with lower As exposure had better cognitive and gross-motor abilities. The joint effects of preterm birth and postnatal exposure to metal mixtures revealed dose-response trends. These results indicated that very low birth weight preterm children are at risk for neurodevelopmental impairments when exposed to metal mixtures. Further longitudinal large-scale surveys are needed to investigate associations between metal mixtures and development among these vulnerable children.

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**Availability of data and materials** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Author contribution** Chi-Sian Kao—conceptualization, formal analysis, writing—original draft; Yen-Tzu Fan—formal analysis, data validation; Ling-Chu Chien—formal analysis, methodology, funding acquisition; Kai-Wei Liao—formal analysis; investigation; Jui-Hsing Chang—resources, investigation; Chyong-Hsin Hsu—resources, investigation; Yi-Jhen Chen—formal analysis, methodology; Chuen-Bin Jiang—conceptualization, formal analysis, methodology, writing—reviewing and editing.

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## Declarations

**Ethics approval and consent to participate** This study was approved by the institutional review board of Taipei Mackay Memorial Hospital (IRB no. MMH-I-S-596 and IRB no. 13MMHIS088).

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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