12 months?" If participants answered "yes" in the second, third, and fourth surveys, the infant was categorized as having "Persistent AD." If participants answered "yes" once or twice among the three surveys, the infant was categorized as having "Episodic AD." Furthermore, if participants answered "no" in all three surveys, the infant was categorized as having "no history of AD."

Confounders

Data regarding a child's sex, birth weight, and gestational age were obtained from their birth records. Family demographics and lifestyle were investigated in the first survey, including parental age, parental smoking habits, whether the infant was living with grandparents, the number of siblings at birth, and maternal anxiety during child rearing. Socioeconomic status (SES) variables were obtained from the first survey, including the maternal working status at 6 months postpartum, and from the second survey, with respect to parental education. Household income was averaged using data from all the four surveys. Details of the distribution of these confounders are shown in Table 1.

Analyses

Ordered logistic regression was used to investigate associations between feeding pattern or breastfeeding duration and AD, which was categorized on the basis of AD persistence (i.e., no history, episodic, or persistent). For the multivariate analysis, we use three models. Model 1 adjusted for the child's demographics (sex, birth weight, and gestational age); model 2 adjusted for the child's demographics plus family demographics and lifestyle (parental age, parental smoking habits, whether living with grandparents, number of siblings at birth, and maternal anxiety during child rearing); and model 3 adjusted for the child's demographics, family demographics and lifestyle, plus SES (parental education and household income). The associations between breastfeeding duration and AD were also investigated using ordered logistic regression.

For sensitivity analysis to avoid the bidirectional association between breastfeeding and AD (e.g., young children with allergic symptoms were more likely breastfed by mothers who believe breastfeeding would prevent further exacerbation of allergic symptoms [15,18]), we focused on subjects without allergic symptoms (physician-diagnosed AD or bronchial asthma, based on questionnaire response) during the age 6–12 months ($N = 31,322$) and investigated the association between feeding pattern or breastfeeding duration and AD at the age 18–42 months using ordered logistic regression because breastfeeding is finished after the age 18 months in most cases. All analyses were performed using STATA SE statistical package, version 12 (Stata Corp., College Station, TX).

Results

In total, 1402 (3.6%) and 8787 (22.7%) of young children had persistent and episodic AD up to the age 42 months, respectively. Furthermore, 8679 (22.4%), 27,861 (71.9%), and 2217 (5.7%) of children were exclusively, partially, or not breastfed for the first 6 months of life. More than half of the children were breastfed for 6 months or more. With respect to crude association, exclusive breastfeeding and a breastfeeding duration of 6+ months were more likely present among infants with persistent AD (26.0% and 62.3%, respectively) compared with infants with no history of AD (21.7% and 55.0%, respectively). In addition, sex, maternal age, paternal smoking habits, living with grandparents, older siblings, maternal anxiety during child rearing, maternal education, paternal education, maternal work status, and average household income were associated with AD status (Table 1).

The odds ratio (OR) of feeding pattern during the first 6 months of life with respect to having AD from the age 6 to 42 months is shown in Table 2. Exclusive and partially breastfed infants were 1.35 and 1.18 times significantly more likely to have AD (95% confidence interval [CI], 1.21–1.51; and 1.07–1.31, respectively) than infants fed formula alone, after adjusting for the child's demographics. The P for trend demonstrated significance ($P < .001$). Further adjustment for family demographics and lifestyle (model 2) and SES (model 3) attenuated the point estimate (OR of exclusive breastfeeding, 1.33 and 1.26 for models 2 and 3, respectively), but P for trend remains significant ($P < .001$ for both).

Similarly, the OR of breastfeeding duration during the first 6 months of life and the development of AD from the age 6 to 42 months, derived from ordered logistic regression, is shown in Table 3. Infants who were breastfed for at least 6 months were 1.32 times more likely to have AD (95% CI, 1.11–1.38) than infants fed formula alone after adjustment for the children's demographics (model 1). The OR was lower if the breastfeeding duration was shorter (OR, 1.13 and 1.03 for 3–5 months and 1–2 months, respectively), yielding a strong dose-response association (P for trend < .001). Further adjustment for family demographics and lifestyle (model 2) and SES (model 3) attenuated the point estimate (OR of 6 months breastfeeding: 1.30 and 1.24 for models 2 and 3, respectively), but P for trend remains significant ($P < .001$ for both).

Before sensitivity analysis, we investigated the association between young children with and without allergic symptoms from the age 6 to 18 months with respect to the distribution of feeding pattern and breastfeeding duration and found that young children with allergic symptoms from the age 6 to 18 months are more likely to be breastfed and for a longer duration ($P = .001$ and $P < .001$; data not shown). Therefore, further sensitivity analysis (i.e., among young children without allergic symptoms from the age 6 to 18 months) was performed. We then confirmed that exclusively breastfed infants were 1.35 times more likely to have AD (95% CI, 1.13–1.60) than infants fed formula alone, after adjustment for covariates measured in this study (model 1; Table 4). Furthermore, young children breastfed for at least 6 months were 1.33 times more likely to have AD (95% CI, 1.13–1.57) than infants fed formula alone, after adjustment for covariates (model 1; Table 5). Both ORs were slightly attenuated in model 2 and model 3 but remained significant. P for trend also demonstrated significance for both feeding pattern and breastfeeding duration, suggesting a dose-response association ($P < .001$ in models 1, 2, and 3).

Discussion

We discovered that breastfeeding for 6 months of life is associated with an increased risk of AD up to the age 42 months, using a nationwide, population-based, prospective large birth cohort study in Japan. More specifically, young children breastfed exclusively or for at least 6 months were 1.26 or 1.24 times more likely to have AD up to the age 42 months compared with formula-fed young children, respectively. Furthermore, among children without allergic symptoms from the age 6 to 18 months, we found positive associations between breastfeeding (terminated before the age 18 months in most cases) and occurrence of AD after the age 18 months, suggesting that reverse causation (to have AD → breastfeeding) is unlikely. Our study meets 11 of 12 criteria suggested by Kramer [14], although there was one unmet criterion, that is, the assessment of effects in children at high risk of AD, which was not possible due to lack of information on parental allergic history.

Table 1
Characteristics of study population ($N = 38,757$)

Characteristics	No history of AD ($n = 28,568$, 73.7%)	Episodic AD ($n = 8787$, 22.7%)*	Persistent AD ($n = 1402$, 3.6%)†	P (χ^2 test)
	n (%)	n (%)	n (%)	
Feeding pattern				
Formula only	1715 (6.0)	433 (4.9)	69 (4.9)	<.001
Partial breastfeeding	20,648 (72.3)	6244 (71.1)	969 (69.1)	
Exclusive breastfeeding	6,205 (21.7)	2110 (24.0)	364 (26.0)	
Breastfeeding duration (mo)				
Never	1715 (6.0)	433 (4.9)	69 (4.9)	<.001
1–2	5484 (19.2)	1474 (16.8)	200 (14.3)	
3–5	5652 (19.8)	1600 (18.2)	260 (18.5)	
6+	15,717 (55.0)	5280 (60.1)	873 (62.3)	
Sex				
Male	14,643 (51.3)	4653 (53.0)	809 (57.7)	<.001
Female	13,925 (48.7)	4134 (47.1)	593 (42.3)	
Birth weight (g)				
<2500	2100 (7.4)	595 (6.8)	85 (6.1)	.15
2500–4000	26,132 (91.5)	8096 (92.2)	1303 (92.9)	
4000+	329 (1.2)	95 (1.1)	14 (1.0)	
Gestational age (wk)				
<37	1181 (4.1)	317 (3.6)	46 (3.3)	.11
37–42	27,123 (95.0)	8386 (95.5)	1341 (95.7)	
42+	246 (0.9)	82 (0.9)	15 (1.1)	
Maternal age (when the child was aged 6 mo)				
<30	12,416 (43.5)	3759 (42.8)	568 (40.5)	.03
30–39	15,530 (54.4)	4860 (55.3)	794 (56.6)	
40+	622 (2.2)	168 (1.9)	40 (2.9)	
Paternal age (when the child was aged 6 mo)				
<30	8621 (30.2)	2637 (30.0)	432 (30.8)	.52
30–39	16,818 (58.9)	5217 (59.4)	835 (59.6)	
40+	3129 (11.0)	933 (10.6)	135 (9.6)	
Mother's smoking habits (when the child was aged 6 mo)				
No	24,154 (84.9)	7494 (85.7)	1205 (86.4)	.09
Yes	4285 (15.1)	1254 (14.3)	189 (13.6)	
Father's smoking habits (when the child was aged 6 mo)				
No	10,659 (38.0)	3431 (39.7)	544 (39.7)	.01
Yes	17,417 (62.0)	5206 (60.3)	828 (60.4)	
Living with grandparents (when the child was aged 6 mo)				
No	22,356 (78.3)	6983 (79.5)	1083 (77.3)	.03
Yes	6212 (21.7)	1804 (20.5)	319 (22.8)	
Older siblings				
0	14,168 (49.6)	4485 (51.0)	649 (46.3)	.002
1	10,457 (36.6)	3188 (36.3)	553 (39.4)	
2+	3943 (13.8)	1114 (12.7)	200 (14.3)	
Maternal anxiety during child rearing (when the child was aged 6 mo)				
Scarcely not	11,095 (38.9)	2994 (34.1)	426 (30.4)	<.001
Some	15,658 (54.9)	5169 (59.0)	838 (59.8)	
Very much	1750 (6.1)	606 (6.9)	137 (9.8)	
Maternal education				
<High school	1396 (4.9)	370 (4.2)	58 (4.2)	<.001
High school	11,270 (39.7)	3098 (35.4)	477 (34.3)	
Some college	11,845 (41.7)	3829 (43.8)	611 (43.9)	
College+	3899 (13.7)	1449 (16.6)	246 (17.7)	
Paternal education				
<High school	2174 (7.7)	586 (6.8)	98 (7.1)	<.001
High school	11,162 (39.7)	3251 (37.5)	553 (40.0)	
Some college	4439 (15.8)	1407 (16.2)	201 (14.6)	
College+	10,358 (36.8)	3420 (39.5)	529 (38.3)	
Maternal work status (when the child was aged 6 mo)				
Not working	21,070 (74.6)	6484 (74.5)	994 (71.5)	.01
Full-time work, with childcare leave	2892 (10.2)	976 (11.2)	169 (12.2)	
Full-time work, without childcare leave	1546 (5.5)	427 (4.9)	79 (5.7)	
Other	2752 (9.7)	819 (9.4)	149 (10.7)	
Average income per year (the first, second, and fourth survey); million yen				
<2.5	2505 (8.8)	602 (6.9)	135 (9.6)	<.001
2.5–5.0	12,030 (42.1)	3648 (41.5)	576 (41.1)	
5.0–7.5	9444 (33.1)	3028 (34.5)	451 (32.2)	
7.5–10	3047 (10.7)	1029 (11.7)	153 (10.9)	
10+	1542 (5.4)	480 (5.5)	87 (6.2)	

* Episodic AD cases were defined as those diagnosed with AD at least once from the age 6 to 42 mo.
† Persistent AD cases were defined as those diagnosed with AD every year from the age 6 to 42 mo.

The present study is consistent with the findings from Japan that used cross-sectional design [19,20] and prospective design [9]. Because the previous prospective study in Japan [9] was based on a relatively small sample size ($N = 763$) and a low follow-up rate (76.1%), they concluded a null association between breastfeeding and AD, although the point estimate of OR was positive and similar

Table 2
ORs for breastfeeding patterns during the first 6 mo with respect to AD from the age 6 to 42 mo using ordered logistic regression analysis ($N = 38,757$)

Feeding pattern	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Formula only	Reference	Reference	Reference
Partial breastfeeding	1.18 (1.07–1.31)*	1.16 (1.05–1.29)*	1.11 (1.00–1.24)
Exclusive breastfeeding	1.35 (1.21–1.51)†	1.33 (1.19–1.49)†	1.26 (1.12–1.41)†
P for trend	<.001	<.001	<.001

Model 1: adjusted for child's sex, birth weight, and gestational age. Model 2: adjusted for variables used in model 1 plus maternal age, paternal age, maternal smoking habit, paternal smoking habit, living with grandparent(s), older siblings, and maternal anxiety during child rearing. Model 3: adjusted for variables used in model 2 plus maternal education, paternal education, maternal work status at 6 mo postpartum, and average household income.
* $P < .01$.
† $P < .001$.

to our study (OR, 1.27; 95% CI, 0.83–1.95) [9]. Furthermore, the prevalence of AD in the present study (26.3% up to the age 42 months) is similar to that of the previous study in Japan [9] (18.6% up to the age 24 months), although there were differences in the follow-up period and diagnostic criteria (our study used physician diagnosis, whereas previous study [9] used the International Study of Asthma and Allergies in Childhood [ISAAC] questionnaire). Therefore, because our study is a larger prospective study, we add to the literature that the association between breastfeeding and AD is generalizable in Japan.

Another study also discovered positive association between breastfeeding and AD among children without atopic heredity in Finland [17], in Denmark [10], and in a case-control study in New Zealand [8], where fish consumption is relatively high as in Japan [21], suggesting that maternal fish intake may explain the association between breastfeeding and AD, especially among children without allergic heredity. A previous study reported that maternal fish intake during pregnancy was associated with AD and asthma in infants [22]. Fish contains polychlorinated biphenyls (PCBs), and PCBs are transferred to human milk [23,24]. A study in Netherland showed that breastfed infants showed 3.6 times higher plasma PCB levels than formula-fed infants [25]. Because PCB influence the immune system [26,27], breastfeeding may play a role in inducing AD through exposure to PCB.

Alternatively, the hygiene hypothesis [28] may explain the association between breastfeeding and AD. That is, breastfeeding may be a risk factor for AD because it reduces the effect of exposure to bacteria or endotoxins [29] on the immune system, such that the infant does not fully develop mature immune response mechanisms such as the shift from Th-2 dominance in infants to Th-1 in later childhood. Because we have adjusted for the number of siblings as a proxy of the hygiene situation in the house and the association remains significant, the hygiene hypothesis is not likely to explain the association. However, because we do not have information regarding infections at the age 6 months, it is not possible to adjust for infection at the age 6 months to confirm an independent effect of breastfeeding on AD. In addition, breastfeeding may affect the balance of gut flora, which is associated with the development of AD [30–32]. Further study is needed to elucidate the mechanism underlying how breastfeeding is associated with the development of AD.

Other studies have reported a protective effect of breastfeeding on AD [4–6]. A previous study that reported a protective effect of breastfeeding on AD, assessed at 12 months [4], precluding a longer protective effect of breastfeeding on AD. Another observational study reported that a protective effect of breastfeeding on AD was found for the early onset of AD (age < 2 years) but not for a later onset of AD [5]. Furthermore, a longer follow-up study reported that breastfeeding is no longer protective for AD in subjects aged 9–21 years [29].

Several limitations need to be addressed. First, because AD was assessed as doctor-diagnosed AD (which is not always based on the same criteria), misclassification might occur. Previous studies ascertaining AD prevalence use the ISAAC questionnaire [33], which reports higher prevalence than physician-diagnosed prevalence [34]. Thus, our AD classification may capture more severe cases than the ISAAC assessment, suggesting our OR would be an underestimate of the association. Second, because we did not have data regarding parental allergic history, it was not possible to adjust or stratify the data for parental allergic history in this investigation of the association between breastfeeding and AD. The Longitudinal Survey of Babies in the 21st century is aiming to investigate the status of children born in 2001 to develop countermeasures to the falling birth rate. Parental allergic history was not included in the survey questionnaire. Third, the definition of exclusive breastfeeding in this survey differs from the World Health Organization definition [35]. We are not collecting data on the use of solid foods, water, or other liquids, so the number of infants in the exclusive breastfeeding category may be overestimated. Fourth, although the sample size was large, it was not a representative sample of the Japanese population because it included only infants born in January and July, and the months of birth were not adjusted due to the lack of data. Thus, possible differences related to seasonal variation of birth may have been missed.

Table 3
ORs for breastfeeding duration during the first 6 mo with respect to AD from the age 6 to 42 mo using ordered logistic regression analysis ($N = 38,757$)

Breastfeeding duration (mo)	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Never	Reference	Reference	Reference
1–2	1.03 (0.92–1.16)	1.02 (0.91–1.14)	0.99 (0.88–1.12)
3–5	1.12 (1.00–1.25)	1.10 (0.98–1.24)	1.06 (0.95–1.19)
6+	1.32 (1.19–1.47)*	1.30 (1.17–1.45)*	1.24 (1.11–1.38)*
P for trend	<.001	<.001	<.001

Model 1: adjusted for child's sex, birth weight, and gestational age. Model 2: adjusted for variables used in model 1 plus maternal age, paternal age, maternal smoking habit, paternal smoking habit, living with grandparent(s), older siblings, and maternal anxiety during child rearing. Model 3: adjusted for variables used in model 2 plus maternal education, paternal education, maternal work status at 6 mo postpartum, and average household income.
* $P < .001$.

Table 4
ORs for feeding pattern with respect to AD from the age 18 to 42 mo among children without allergic symptoms from the age 6 to 18 mo using logistic regression ($N = 31,322$)

Feeding pattern	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Formula only	Reference	Reference	Reference
Partial breastfeeding	1.20 (1.02–1.42)*	1.20 (1.01–1.41)*	1.15 (0.97–1.36)
Exclusive breastfeeding	1.35 (1.13–1.60)†	1.35 (1.13–1.61)†	1.29 (1.08–1.55)†
P for trend	<.001	<.001	.001

Model 1: adjusted for child's sex, birth weight, and gestational age. Model 2: adjusted for variables used in model 1 plus maternal age, paternal age, maternal smoking habit, paternal smoking habit, living with grandparent(s), older siblings, and maternal anxiety during child rearing. Model 3: adjusted for variables used in model 2 plus maternal education, paternal education, maternal work status at 6 mo postpartum, and average household income.
* $P < .05$.
† $P < .01$.

Table 5
ORs for breastfeeding duration with respect to AD from the age 18 to 42 mo among children without allergic symptoms from the age 6 to 18 mo using logistic regression ($N = 31,322$)

Breastfeeding duration (mo)	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Never	Reference	Reference	Reference
1–2	1.06 (0.89–1.27)	1.06 (0.88–1.27)	1.03 (0.85–1.24)
3–5	1.13 (0.95–1.35)	1.14 (0.95–1.36)	1.09 (0.91–1.31)
6+	1.33 (1.13–1.57)*	1.33 (1.13–1.58)*	1.28 (1.08–1.52)*
P for trend	<.001	<.001	<.001

Model 1: adjusted for child's sex, birth weight, and gestational age. Model 2: adjusted for variables used in model 1 plus maternal age, paternal age, maternal smoking habit, paternal smoking habit, living with grandparent(s), older siblings, and maternal anxiety during child rearing. Model 3: adjusted for variables used in model 2 plus maternal education, paternal education, maternal work status at 6 mo postpartum, and average household income.
* $P < .01$.

Conclusions

In conclusion, breastfeeding is associated with an increased risk of AD up to the age 42 months, using a nationwide, population-based, prospective large birth cohort study in Japan. Moreover, among children without allergic symptoms from the age 6 to 18 months, we found positive associations between breastfeeding and occurrences of AD after the age 18 months. Further study is needed to elucidate the mechanism by which breastfeeding was associated with the development of AD.

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Home environment and prenatal exposure to lead, arsenic and zinc on the neurodevelopment of six-month-old infants living in Chitwan Valley, Nepal

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ABSTRACT

Background: We have previously reported the inverse associations between *in utero* levels of lead (Pb), arsenic (As) (i.e., toxic elements), and neurodevelopmental indicators (i.e., motor and state regulation cluster score) measured by the Brazelton Neonatal Behavioral Assessment Scale, third edition (NBAS III) in this cohort at birth. Using additional follow-up, this study investigated the effects of cord blood levels of Pb, As, and zinc (Zn) (an essential element) and the postnatal environment on the neurodevelopment of 6-month-old infants in Chitwan Valley, Nepal.

Methods: In total, 100 mother–infant pairs were recruited from Chitwan District, Nepal. Pb, As, and Zn concentrations in cord blood were measured. Postnatal raising environment (i.e., HOME score or home environment hereafter) was evaluated using the Home Observation for Measurement of Environment (HOME) scale. Neurodevelopment of infants at 6 months (n = 94) was assessed according to the Bayley Scale of Infant Development, second edition (BSID II). Multivariable regression adjusting for covariates was performed to determine the associations of *in utero* levels of toxic and essential elements and the home environment with neurodevelopment scores.

Results: Cord blood levels of Pb, As, and Zn were not associated with any BSID II cluster scores in 6-month-old infants. The total HOME score was positively associated with the Psychomotor Development Index (PDI) score (coefficient = 0.59, 95% confidence interval [CI] = 0.04 to 1.13).

Conclusion: In this cohort, detrimental effects of *in utero* Pb and As on neurodevelopmental indicators observed at birth did not persist at 6 months of age, while it showed an association between the neurodevelopment and home environment.

1. Introduction

Enormous efforts have been devoted to clarify the determinants of human neurodevelopment. There is substantial evidence from animal studies indicating the associations between *in utero* exposure to toxic elements (e.g., Pb and As) and *in utero* deficiencies of essential elements (e.g., Zn), and fetal neurodevelopment [e.g., Bhatnagar and Natchu, 2004; Rodriguez et al., 2002; Toscano and Guilarte, 2005; Wright and Baccarelli, 2007]. However, the results of epidemiological studies on the associations between *in utero* exposure to toxic elements and *in utero* deficiencies of essential elements, and later neurodevelopment are inconsistent.

From 2002 to 2004, a workgroup of the Centers for Disease Control and Prevention (CDC) Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) reviewed 23 scientific studies including 16 separate populations and found that intelligence quotient (IQ) and general cognitive index outcomes among children aged less than 5 years are associated with blood Pb levels (BLLs) less than 100 μg/L. The workgroup concludes that there is no safe level for blood Pb in children (CDC ACCLPP, 2007). In their review, Gilbert and Weiss (2006) propose that the BLL that should prompt public health actions is 20 μg/L. Although the majority of the earlier studies report detrimental effects of Pb in later neurodevelopment, a number of studies do not report signs of neurodevelopmental deficit when the mean BLL exceeded 44 μg/L (Minder et al., 1998), 71 μg/L (Prpic-Majic et al., 2000), or 100–160 μg/L (Ernhart and Greene, 1990; Harvey, 1986; Lansdown et al., 1986; Smith et al., 1983). Further, such data from Asian studies are, still, scarce (Kile et al., 2009; Patel et al., 2006).

Despite the evidence of a cross-sectional association between postnatal As exposure and neurodevelopment, the effect of prenatal As exposure on later neurodevelopment remains unclear. The lack of association between maternal As level during pregnancy (i.e., urinary As level during the first, second, and third trimesters as a proxy of prenatal exposure) and neurodevelopmental indicators (BSID II) at 7, 18 and 60 (among boys only) months was reported in Bangladesh (Hamadani et al., 2010, 2011; Tofail et al., 2009). However, no study has investigated the association between cord blood As level, which is considered a better bioindicator of prenatal exposure (Parajuli et al., 2013), and the neurodevelopment of 6-month-old infants, which is an important developmental milestone including mental and psychomotor development (CDC, 2012).

Regarding the association between *in utero* Zn levels and neurodevelopment, positive association was reported between maternal Zn intake during pregnancy and lactation period with a neurodevelopmental indicator, the habituation cluster of the Brazelton scale, at 6 months of age (Kirksey et al., 1991). Randomized controlled trials show that Zn supplementation to pregnant mothers and infants improved the locomotor development scores of infants in Newfoundland (Friel et al., 1993), fine and gross motor skills of children in China (Sandstead et al., 1998), and mental and psychomotor development scores of infants in Chile (Castillo-Duran et al., 2001). In contrast, several studies failed to detect such effects (Black et al., 2004b; Tamura et al., 2003; Taneja et al., 2005). The inconsistencies among these studies might be attributable to different levels of Zn deficiency, which can be attributed to different settings and populations.

In the present study, we targeted the Chitwan Valley in lowland (Terai) Nepal, because we assumed that relatively large amounts of Pb and As are circulating in the soil, water, air, and living organisms that is enough to cause neurodevelopmental deficits (Parajuli et al., 2012). Inverse associations between cord blood levels of Pb and As, and neurodevelopmental indicators (i.e., motor and state regulation cluster score, respectively) measured by the Brazelton Neonatal Behavioral Assessment Scale, third edition (NBAS III) are reported in children in this cohort at birth (Parajuli et al., 2013). The Chitwan District is located at the junction of a highway from Kathmandu and an east–west highway where many vehicles emit Pb into the environment (Shrestha et al., 2003). In addition, the district is a hotspot of As contamination (Pokhrel et al., 2009). In addition to the problem of toxic elements, owing to Zn deficit in soil of lowland Terai (Harrington et al., 1989; Pokharel, 1997), Zn deficiency is reported to be a health problem in the region (Andersen, 2007; Christian et al., 2006). Thus, because of these environmental conditions, it is anticipated that the associations between neurodevelopment and toxic and essential elements will be relatively more detectable in the Chitwan District than in developed countries where exposure to toxic and essential element intakes is well regulated. The objectives of the present study were to investigate the effects of *in utero* exposure to Pb, As (toxic elements), and Zn (an essential element) and home environment on the neurodevelopment scores of 6-month-old infants in Chitwan District, Nepal.

2. Methods

2.1. Study sample

The eligibility criteria in the present study were as follows: residence in Chitwan District for at least 2 years, at term pregnancy when the mothers visited the hospital (more than 37 weeks of gestation), age of 18–40 years, *per vaginam*, singleton, and no reports of diabetes, hypertension, or preeclampsia. In total, 200 pregnant mothers were approached between September and October 2008 in the Bharatpur General Hospital of Chitwan District. Among them, 119 were eligible. Mothers were informed of the background and objectives of the study, what they would experience during the study process, the benefits, and the potential risks, although none were expected. Finally, 100 women signed a letter of informed consent (participation rate, 84%). The study protocol was approved by the ethics committees of the Graduate School of Medicine, the University of Tokyo (approval no #2244) and of the Bharatpur General Hospital, Chitwan, Nepal.

2.2. Measurements of levels of cord blood elements

Cord blood was collected from the placenta by midwives according to the routine aseptic procedure. Cord blood (10 mL) was collected into a trace metal-free cryovial containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Cord blood samples were stored in a freezer at −20 °C for less than 1 month, transported to the laboratory in Tokyo while kept frozen with dry ice, and stored in a deep freezer at −78 °C until analysis.

Pb, As, and Zn levels in the cord blood samples were measured at the Department of Human Ecology at the University of Tokyo which has a clean room to minimize contamination. The methods and research findings from this cohort have been published previously (Parajuli et al., 2012). The certified reference material (CRM), "Seronorm" trace elements whole blood level-1, lot MR 4206 (Sero AS, Billingstad, Norway), was used. The observed values for each element were within the certified range. Randomly selected cord blood samples (20%) were analyzed twice for all elements. For all the elements measured, there was no statistical difference between the two measurements, and the correlations between them ranged from 0.90 to 0.95, depending on the element.

Of the 100 cord blood samples, 94 samples were used to measure Pb, As, and Zn levels. The other 6 cord blood samples were not used due to lack of identity information (i.e., subjects ID) on the sampling vial tag. In addition, due to limited sample volume (as one blood sample was taken from each newborn cord, but the blood sample was measured twice), cord blood Pb levels could not be re-measured in 15 samples; thus, the data of the Pb levels of 79 cord blood samples were used for analysis.

2.3. Interview on the day of delivery

The following information was collected from mothers after delivery by interview: mother's age, parity, gender of baby, gestational age, time and date of delivery, educational level, annual family income, smoking during pregnancy, and alcohol consumption during pregnancy.

2.4. Postnatal home environment

The author (RPP) visited the home of each mother–infant pair after approximately 6 months (192.8 ± 13.2 days after their baby was born) after delivery and evaluated the postnatal home environment according to the HOME scale (Caldwell and Bradley, 1984). The scale includes 45 items and is the total evaluation (i.e., by both observation and interview) of parental response to child's behavior, acceptance of child, organization of environment, learning material, parental involvement with the child, and opportunities of variety for baby. Therefore, the possible scores range from 0 to 45, with scores <25 indicating a "less stimulating" home environment (Torres-Sanchez et al., 2007). Of the 100 mothers enrolled in the cohort, the home environment of 94 homes was evaluated.

2.5. Anthropometry of mothers and infants at birth and at 6 months

The height and weight of the mothers were recorded just before delivery. Height was measured to the nearest 0.1 cm. Body weight was recorded to the nearest 0.1 kg using a portable digital scale (Model BF-046 WH; Tanita, Tokyo Japan). Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). The birth weight of the newborns was obtained from hospital records.

Abbreviations: Pb, lead; As, arsenic; Zn, zinc; CDC, Centers for Disease Control and Prevention; ACCLPP, Advisory Committee on Childhood Lead Poisoning Prevention; IQ, intelligence quotient; EDTA, ethylenediaminetetraacetic acid; BLL, blood Pb level; USA, United States of America; BMI, body mass index; NBAS III, Neonatal Behavioral Assessment Scale, third edition; BSID II, Bayley Scale of Infant Development, second edition; MDI, Mental Development Index; PDI, Psychomotor Development Index; HOME scale, Home Observation for Measurement of Environment scale.

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The height and weight of the mothers and infants were measured 6 - months after birth. Height was measured to the nearest 0.1 cm. Body weight was recorded to the nearest 0.1 kg using a portable digital scale (Model BF-046 WH; Tanita, Tokyo Japan). BMI was calculated as described above.

2.6. Neurodevelopmental indicators of infants at 6 months

The second edition of the Bayley Scale of Infant Development (BSID II) (Bayley, 1993) was used to assess the neurodevelopmental status of 6-month-old infants. The BSID II is frequently used in the field of neurotoxicology (Eskenazi et al., 2006; Jedrychowski et al., 2007, 2009a,b, 2010; Ribas-Fito et al., 2003; Surkan et al., 2008; Torres-Sanchez et al., 2007, 2009). The BSID II comprises 2 neurodevelopmental indicators: the Mental Development Index (MDI) and the Psychomotor Development Index (PDI). The MDI reflects the infant's levels of cognitive function, language, and personal and social development. The PDI reflects the gross and fine motor functions.

The BSID II was administered to the infants at 6 ± 1 month of age, and the age of the infants (in days) was recorded. The author (RPP), blinded to the exposure status, conducted the BSID II on all infants in their own homes. The infants were assessed in their own home because it was judged to be convenient for both caregiver and infant (i.e., no shyness because of the familiar environment). Uniform assessment conditions including early information of the assessment, limited presence of family members and siblings, friendly demeanor of the tester to the infants, moderate light at the place of assessment, and no hunger or sleepiness among the infants were maintained. In order to be consistent with previous studies (Torres-Sanchez et al., 2007; Hamadani et al., 2010, 2011; Tofail et al., 2009) BSID II and HOME scale were used in this research design.

2.7. Statistical analysis

First, the distributions of all variables were examined for normality. The cord blood levels of toxic and essential elements, and annual family income were log-transformed.

Bivariable and multivariable regression analyses were conducted to examine the associations between neurodevelopment indicators (MDI and PDI) and toxic (Pb and As) and essential (Zn) elements, and HOME scale scores. Other covariates known to be correlated with neurodevelopmental indicators including the mother's age (Tian et al., 2009), parity (Jedrychowski et al., 2010), family income (Black et al., 2000), mother's educational level (Janssen et al., 2008; Jedrychowski et al., 2010; Surkan et al., 2008; Wu et al., 2008), mother's BMI just before birth and 6 months after birth (Tofail et al., 2009), weight of the infant at birth and 6 months after birth (Badr et al., 2009; Black et al., 2004a,b; Tofail et al., 2009; Torres-Sanchez et al., 2007; Wu et al., 2008), gestational age (Badr et al., 2009; Hutton et al., 1997; Simic et al., 2009; Tofail et al., 2009) and the infant's age at the time of BSID II assessment (Torres-Sanchez et al., 2007) were evaluated whether they influence the exposure–outcome associations or not. Only gestational age in weeks and the infant's age at the time of BSID II assessment were found to influence the exposure–outcome associations (i.e., confounders). Thus, only these two confounders were entered into the multivariable linear regression model for adjustment for a minimally adjusted model (Model 3). In addition, unadjusted and mutually adjusted associations between explanatory variables, confounding variables and covariates, and response variables (Model 1 and Model 2, respectively) were also evaluated.

The distribution of the covariate age (in days) at BSID II assessment was normal. Smoking during pregnancy (n = 5) and alcohol consumption during pregnancy (n = 4) were not analyzed owing to low n values.

The level of significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 11.5 (SPSS Inc., Tokyo, Japan).

3. Results

3.1. Characteristics of mothers and infants

Table 1 summarizes the characteristics of mother–infant pairs at birth and 6 months after birth. The maternal, household, and newborn characteristics of this cohort have been published previously (Parajuli et al., 2012). The indicators of the nutritional status of newborns (i.e., birth weight and height) did not differ by sex. In contrast, the body weight and height of the infants 6 months after birth differed significantly by sex: boys were on average 512 g heavier and 1.7 cm taller than girls ($p < 0.05$, data not shown). The mean body weight of the infants in the present study 6 months after birth was 7021 g (5500 to 10,100 g). The BMI of the mothers decreased significantly (from 23.2 to 21.8) by 6 months after birth (paired t-test, $p < 0.001$). All (i.e., 100%) cord blood samples (n = 94 for As and Zn, n = 79 for Pb) had detectable levels of As, Zn, and Pb. The mean HOME score was 29.3 (range, 16 to 42); the HOME scores were normally distributed.

3.2. Neurodevelopmental indicators of infants at 6 months

The distributions of the BSID II cluster scores including the MDI and PDI are shown in Fig. 1. All infants were evaluated by the BSID II 6 months after birth (mean assessment age, 192.8 days). The index scores of the cohort were normally distributed. The BSID II scores of this cohort were similar to those in the studies in other countries (Black et al., 2004a,b; Chiriboga et al., 2007; Eskenazi et al., 2006; Nakajima et al., 2006; Simic et al., 2009; Torres-Sanchez et al., 2007; Wang et al., 2005). None of the cluster scores differed with respect to gender ($p > 0.05$, data not shown). According to the criteria provided by the BSID II manual (Bayley, 1993), the MDI of all infants fell into the normal range (i.e., 85 to 114), while the PDI of 3 infants was categorized as "mildly delayed development" (i.e., 70–84) (data not shown).

Table 1
Characteristics of the mothers and infants who participated in the study.

Characteristics	Mean or N (SD or %)	Range
Mother's characteristics at birth (n = 100)		
Age (years)	22.9 (3.7)	18 to 37
Primipara	66 (66)	
Educational level (years)	9.2 (3.8)	0 to 17
BMI (kg/m^2)	23.2 (2.9)	16.8 to 32.7
Newborn babies' characteristics (n = 100)		
Gestational age (weeks)	38.9 (1.4)	37.0 to 43.0
Sex of baby (male)	47 (47)	
Birth weight (g)	3029 (438)	2200 to 4000
Cord blood elements (median)		
Pb (μg/L) (n = 79)	20.6	6.83 to 220.8
As (μg/L) (n = 94)	1.33	0.51 to 9.58
Zn (μg/L) (n = 94)	2112	1299 to 6430
Mother's characteristics at 6 months after birth (n = 94)		
Mother's BMI (kg/m^2)	21.8 (3.3)	15.8 to 33.5
Duration of exclusive breast feeding (months)	5.30 (1.33)	0.00 to 6.00
Household characteristics		
Annual family income in US dollars (USD)	2529 (2882)	150 to 17,250
Total HOME scale score (n = 94)	29.3 (5.3)	16 to 42
Infant's characteristics at 6 months after birth (n = 94)		
Body weight (g)	7261 (865)	5500 to10100
Length-for-age Z score (HAZ) (94)	0.19 (1.44)	−3.08 to 3.72
Weight-for-length Z score (WHZ) (94)	−0.77 (1.59)	−4.01 to 4.07
Age at BSID II assessment (in days) (n = 100)	192.8 (13.2)	168 to 217
Scores on BSID II clusters		
Mental Development Index (MDI) score	105.1 (4.9)	93 to 113
Psychomotor Development Index (PDI) score	101.7 (9.3)	81 to 126

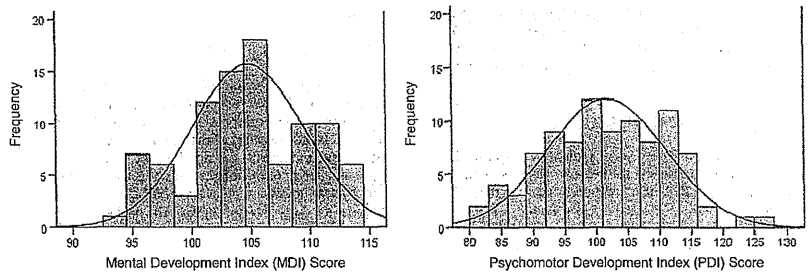

Fig. 1. BSID II cluster scores (MDI and PDI) with normal distribution curve are shown in the figure.

3.3. Associations between cord blood levels of As, Pb, and Zn and BSID II scores

Table 2 summarizes the associations among cord blood levels of As, Pb, and Zn and BSID II scores. In the bivariable regression model, only cord blood levels of Zn were positively associated with MDI score (coefficient = 9.31, 95% CI = 0.75 to 17.87). Pb and As cord blood levels were not associated with any BSID II cluster score. Total HOME scale scores were positively associated with PDI scores (coefficient = 0.37, 95% CI = 0.02 to 0.72). Gestational age was positively associated with PDI scores, while age at BSID II assessment was inversely associated with MDI scores.

In the multivariable regression model, the significant bivariable correlation between cord blood levels of Zn and MDI score disappeared (coefficient = 7.45, 95% CI = −2.53 to 17.44). Pb and As cord blood levels were not associated with any BSID II cluster score. Home environment was positively associated with PDI score (coefficient = 0.59, 95% CI = 0.04 to 1.13). In addition, minimally adjusted model (Model 3) and fully adjusted model (Model 2) showed similar results. Older infants scored lower in the MDI and PDI clusters than their younger

Table 2
Association of demographic and *in utero* chemical and home environmental variables with MDI and PDI scores of BSID II at 6 months from birth.

Response variables	Mental Development Index score			Psychomotor Development Index score		
Explanatory variables	Model 1[a]	Model 2[b]	Model 3[c]	Model 1[a]	Model 2[b]	Model 3[c]
Cord blood level (log μg/L)	Estimated change (95% confidence interval) in the value of the response variable for a one unit increase of the explanatory variable					
Lead (Pb) (n = 79)	0.75	1.95	2.02	−3.17	−1.31	−1.06
	(−2.76 to 4.25)	(−1.54 to 5.44)	(−1.32 to 5.37)	(−9.94 to 3.60)	(−8.38 to 5.75)	(−7.70 to 5.58)
Arsenic (As) (n = 94)	1.55	−0.04	1.01	−3.16	−1.63	−2.02
	(−3.55 to 6.64)	(−6.25 to 6.16)	(−4.53 to 6.55)	(−13.05 to 6.74)	(−14.20 to 10.93)	(−13.02 to 8.98)
Zinc (Zn) (n = 94)	9.31	7.45	7.41	7.44	7.58	7.88
	(0.75 to 17.87)	(−2.53 to 17.44)	(−1.82 to 16.64)	(−9.52 to 24.40)	(−12.64 to 27.81)	(−10.46 to 26.22)
HOME score at 6 months	0.03	0.23	0.20	**0.37**	**0.59**	**0.54**
Confounders	(−0.16 to 0.21)	(−0.03 to 0.50)	(−0.03 to 0.42)	**(0.02 to 0.72)**	**(0.04 to 1.13)**	**(0.10 to 0.98)**
Gestational age (week)	0.53	0.27	0.37	**1.42**	1.12	1.05
	(−0.21 to 1.26)	(−0.57 to 1.11)	(−0.41 to 1.16)	**(0.01 to 2.84)**	(−0.58 to 2.82)	(−0.51 to 2.61)
Age at BSID II assessment (days)	**−0.12**	**−0.16**	**−0.15**	−0.13	**−0.21**	**−0.21**
Covariates	**(−0.19 to −0.05)**	**(−0.25 to −0.07)**	**(−0.24 to −0.06)**	(−0.27 to 0.01)	**(−0.40 to −0.03)**	**(−0.38 to −0.03)**
Mother's age (years)	−0.01	−0.09		0.07	−0.01	
	(−0.28 to 0.26)	(−0.53 to 0.35)		(−0.45 to 0.59)	(−0.91 to 0.88)	
Parity	0.38	0.35		0.25	0.93	
	(−0.75 to 1.50)	(−1.68 to 2.38)		(−1.95 to 2.44)	(−3.19 to 5.04)	
Mother's educational level (years)	−0.09	−0.10		0.21	0.12	
	(−0.34 to 0.16)	(−0.47 to 0.26)		(−0.27 to 0.70)	(−0.62 to 0.86)	
Log annual family income (USD)	0.40	−0.87		0.50	−1.99	
	(−2.25 to 3.05)	(−3.94 to 2.21)		(−4.64 to 5.65)	(−8.23 to 4.24)	
Mother's BMI (kg/m^2) just before delivery	0.17	0.35		0.05	−0.25	
	(−0.17 to 0.50)	(−0.30 to 1.00)		(−0.60 to 0.70)	(−1.56 to 1.07)	
Mother's BMI (kg/m^2) at 6 months	0.06	−0.28		0.08	0.09	
	(−0.24 to 0.36)	(−0.86 to 0.31)		(−0.50 to 0.66)	(−1.10 to 1.28)	
Birth weight (kg)	1.48	1.25		1.14	2.22	
	(−0.75 to 3.70)	(−1.44 to 3.94)		(−3.21 to 5.50)	(−3.23 to 7.67)	
Weight of infants at 6 months (kg)	0.47	0.31		0.64	−0.19	
	(−0.65 to 1.59)	(−1.08 to 1.70)		(−1.54 to 2.83)	(−3.00 to 2.62)	
R^2 (adjusted R^2)		0.249 (0.085)	0.202 (0.135)		0.183 (0.004)	0.165 (0.095)

Bivariable and multivariable associations between *in utero* chemical and home environmental variables and covariates with BSID II clusters are shown in the table.
Mental Development Index (MDI) score.
Psychomotor Development Index (PDI) score.
Bold values indicate significance of the test of association.
[a] Bivariable regression analysis was conducted to see the unadjusted association between explanatory variables, confounding variables and covariates, and response variables.
[b] Multivariable regression model was conducted to see the mutually adjusted effect of explanatory variables, confounding variables and covariates, and response variables.
[c] All the "explanatory variables (*in utero* Pb, As, and Zn levels and HOME score)" and "confounders (i.e., gestational age and the infant's age at the time of BSID II assessment)" were forced into the single multivariable regression model of each BSID II cluster for adjustment.

counterparts. In separate analysis (n = 94) with 15 samples which were measured only once for Pb, indicated few minor changes in results, though overall picture had not been changed much in both models of MDI and PDI (data not shown).

4. Discussion

The cord blood levels of Pb, As, and Zn were not associated with any BSID II cluster score in 6-month-old infants in the multivariable model. The adjusted multivariable model failed to show the significant association between cord blood levels of Zn and MDI scores apparent in the bivariable model. Infants with relatively superior HOME scores (discussed in later section) exhibited better gross and fine motor functions (i.e., PDI scores) than those with less stimulating HOME scores.

4.1. Associations between cord blood levels of toxic elements and BSID II cluster scores

The cord blood levels of Pb and As were not associated with any BSID II cluster scores evaluated at 6 months. In contrast, inverse associations between prenatal Pb levels and BSID II scores at 6 months are reported in a Saudi Arabian cohort (Al-Saleh et al., 2009), an American cohort (Dietrich et al., 1990), and a cohort in Shanghai (Shen et al., 1998). The discrepancy between the present and previous results might be attributable to different exposure levels between cohorts. The median cord blood Pb level in the present cohort (20.6 μg/L) is substantially lower than that reported in the Saudi Arabian (33.6 μg/L), American (80 μg/L), and Shanghai cohorts (90 μg/L). In addition, lack of power (i.e., power ranges from 0.06 to 0.32) because of small sample size (n = 79) might be responsible for the insignificant association in the present cohort compared to relatively larger sample sizes of the previous studies with inverse associations [Saudi Arabian cohort (n = 653), Shanghai cohort (n = 348) and American cohort (n = 283)].

The cord blood levels of Pb and As are reported to be significantly inversely associated with the motor and state regulation cluster score (NBAS III) of infants evaluated at birth (Parajuli et al., 2013). There are several possible explanations for these results. It is possible that the effect diminishes over time during the postnatal period (Claus Henn et al., 2012). For example, most of the As burden from an infant's body is excreted via urine, because urinary As concentrations decrease during the first 4 months after birth (80 μg/L during the first 2 days of life to <30 μg/L at 4 months of age) (Concha et al., 1998). Furthermore, the neuroplasticity of the immature brain is another possible reason underlying the diminution of the effect over time (Bulas and Glass, 2005). Similar mechanisms can be speculated for Pb as well.

4.2. Associations between cord blood levels of Zn and BSID II cluster scores

The cord blood levels of Zn were not associated with any BSID II cluster scores. Similar to earlier studies (Black et al., 2004b; Tamura et al., 2003; Taneja et al., 2005), infants with higher *in utero* Zn levels did not exhibit better neurodevelopment scores in the present study. This may be because of the good nutritional status manifesting birth weight as a proxy, because the subtle positive effects of Zn on the MDI in the bivariable model diminished after adjusting for covariates including birth weight. Furthermore, in a separate analysis, the significant association between the MDI and Zn levels did not change after adjusting for HOME scores; only the coefficient of association between log Zn and the MDI increased from 9.31 to 9.68, while the *p*-value decreased from 0.033 to 0.029.

4.3. Associations between HOME scale and BSID II cluster scores

The HOME scale score was positively associated with gross and fine motor development (i.e., the PDI) 6 months after birth, which is concordant with a report in Mexico (Torres-Sanchez et al., 2007) but not the one in the United States (Eskenazi et al., 2006). The mean HOME score of 6-month-old infants in the present study was similar to those in studies in the Mexican cohort (mean: 30.1, SD: 4.06 (Torres-Sanchez et al., 2007) and mean: 30.4, SD: 4.06 (Torres-Sanchez et al., 2009)). However, in the present study, 25% of the babies lived in "less stimulating" (i.e., total HOME score <25) home environments, which is comparable to the Mexican cohort (20.1%) (Torres-Sanchez et al., 2007, 2009). Additionally, only 8%–9% of infants in the American cohort (Eskenazi et al., 2006) lived in similar environments. The positive association between HOME scale score and neurodevelopmental indicators is consistently reported among 18–24-month-old Latino children in the United States (Badr Zahr, 2001) and native German children (Walkowiak et al., 2001). In the Nepalese cohort, we can speculate that parents had higher "response to child's behavior," "acceptance of child," and "parental involvement with the child" scores because of the extended family culture in Nepal (Advameg Inc., 2013; Lamar Soutter Library, 2013) that involves living together after marriage and harmony within the neighborhood. In addition, because of cultural assimilation among different cultural and religious groups (Levine, 1987) such as the Hindus and Buddhists, Nepalese children are used to celebrating many festivals together, which involve different family visits. Such frequent family visits with babies might provide the babies with better "opportunities of variety for babies," which was described in the HOME cluster scores. However, "organization of environment" and "learning material for babies" might not be ideal as assumed by the HOME scale, which was developed in a Western context in the U.S. Approximately 25% of infants in the present sample were living in "less stimulating" (i.e., total HOME score <25) home environments. Therefore, in Nepal, "organization of environment" and "learning material for babies" in the HOME cluster contributed to lower PDI scores.

5. Limitations

The present study has several limitations that should be considered. First, the small sample size and hospital-based sampling limit the generalizability of the findings. Second, several potential confounders were not assessed, including prenatal exposure to mercury and pesticides (Torres-Sanchez et al., 2007, 2009); these should be included in future studies. Third, the applicability of the HOME scale in a Nepali context is another potential problem. Five out of the 45 items in the HOME scale, such as "availability of specific toys", might decrease HOME scores, because such toys are not common in Nepal. Despite this, the HOME scale is frequently used in developing countries (Baker-Henningham et al., 2009; Black et al., 2004a,b; Lozoff et al., 1995). In addition, the procedure in which BSID II and HOME scale assessments were conducted in a single home visit might bring some bias into the results.

Nonetheless, this study has a number of strengths. The present cohort is the first longitudinal birth cohort from Nepal for which a considerable amount of information about environmental exposure and potential confounders was collected. Our study population had relatively higher average and wider ranges of As and Pb levels than those of populations with similar background exposure levels in developed countries. Since the BSID II was performed in the infants' homes, this avoided possible underperformance by infants caused by a novel assessment environment. Future epidemiological research should be conducted to evaluate the effects of *in utero* and postnatal exposures of toxic and essential elements on neurodevelopment in other populations from developing countries with different exposure and deficiency levels.

6. Conclusions

In a birth cohort study in the Terai region of lowland Nepal, the cord blood levels of Pb, As, and Zn were not associated with the BSID II cluster scores of 6-month-old infants. The home environment was significantly associated with PDI scores. Our study, which is the first of this kind

conducted in lowland Nepal, failed to detect the association between the neurodevelopment at 6 months and the low level prenatal exposures to Pb and As, while it showed an association between the neurodevelopment and home environment.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ntt.2013.12.006.

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