Prenatal Exposure to Silver is Associated with an Elevated Risk for Neural Tube Defects: A Case Control Study

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Research

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Abstract

Background: Exposure to copper, silver, and titanium has been reported to be associated with a variety of adverse effects on humans. However, few studies have focused on the fetus, which is vulnerable to environmental insults. We investigated the associations between prenatal exposure to the three metals and risk for fetal neural tube defects (NTDs).

Methods: Placental samples from 408 women with pregnancies affected by NTDs and 593 women with normal pregnancies were collected from 2003 to 2016 in an NTD high-risk area in northern China. Placental metal concentrations were quantified and used as prenatal exposure markers. Multilevel mixed-effects logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between metal concentrations and risk for NTDs. Single and joint effects of the metals on NTDs were evaluated with Bayesian kernel machine regression (BKMR), which can account for correlation, nonlinearity, and interaction between metals.

Results: NTDs had higher concentrations of copper (4.16 μg/g) and silver (0.96 ng/g) than controls (copper: 3.91 μg/g; silver: 0.96 ng/g). Silver was associated with an increased risk for NTDs in a dose-response fashion in single-metal logistic regression, with adjusted ORs (95% CIs) of 1.78 (1.04–3.06) and 1.92 (1.11–3.32) in the second and third tertiles, respectively, compared to the lowest tertile. BKMR revealed toxic effects of silver on NTDs when the concentrations of copper and titanium were fixed at their 25th, 50th, and 75th percentiles, and the association appeared to be linear. No interaction of silver with any of the other two metals was observed. Besides, silver concentration was positively correlated with maternal certain dietary intakes, such as meat or fish, during the periconceptional period.

Conclusions: High silver concentrations in placental tissue are associated with an elevated risk for NTDs in offspring. Maternal diet may be a source of silver exposure.

Background

Neural tube defects (NTDs), including anencephaly and spina bifida, are severe and debilitating congenital malformations of the central nervous system that arise from incomplete closure of the neural tube in early embryonic development [1]. The prevalence of NTDs differs greatly between and within countries, varying from 0.5 to more than 10 per 1,000 pregnancies [2, 3]. Although intensive researches have been conducted, the etiology of NTDs is not yet fully understood. A variety of causes or risk factors have been proposed, involving genetic or environmental origin or a complex combination of their interactions. However, little is known about the role of environmental factors except for folate and some medications. Identification of environmental risk factors is important for NTD prevention because these factors may be modifiable.

Copper is an essential transition metal participating in various biological processes, while an excess of copper can be toxic [4]. Dietary and inhalation are the major sources of copper exposure [5]. Copper excess leads to growth reduction and morphological malformations in fish embryos [6]. Several studies also suggested that excessive copper concentrations in maternal serum or plasma are risk factors for NTDs [7–9]. Silver and titanium are two transition metals with no known essential role in biology [10, 11]. Humans are exposed to them from various sources, such as foods, jewelry, cosmetics, tableware, domestic appliances, and medical devices [12–15]. Oral exposure to elevated silver and titanium could induce abnormal ovarian cell morphology and embryonic development, high incidence of visceral damage, and neonatal deaths in mice and rats [16–18]. To our knowledge two studies have reported the three metals and NTDs. A case-control study from Mexico suggested higher silver in maternal hair may be participating in the development of NTDs [19]; in contrast, another study in China found similar concentrations in hair samples of 191 newborns with NTDs and 261 healthy infants, and it also reported that higher levels of titanium in maternal scalp hair was associated with risk for NTDs [20].

Therefore, the relationships between copper, silver, and titanium and NTD risk in offspring are still needed to elucidate. Furthermore, blood has a short turnover; as such, blood-based markers may only reflect short-term exposure. Maternal hair is prone to external environmental contamination. During pregnancy, placental transfer nutrients and toxicants are of concern about fetal health; thus, the placenta has been considered as suitable biomarker to reflect prenatal exposure of metals, including copper, silver, and titanium [21, 22].

The present study aimed to examine the association between maternal exposure to copper, silver, or titanium during pregnancy and risk for NTDs in offspring by measuring their concentrations in placental tissue from groups of NTD-affected and healthy infants using a case-control design.

Methods

Study participants

Study participants were women enrolled in an ongoing case-control study established in 2003 in five rural counties (Pingding, Xiyang, Shouyang, Taigu, and Zezhou) of Shanxi Province in northern China, as previously described [23, 24]. Briefly, the main purpose of the study is to identify environmental factors for major external structural birth defects, i.e., NTDs, orofacial clefts, congenital hydrocephalus, etc. Eligible cases are pregnant women whose current pregnancies are affected by the above-mentioned malformations of any gestational age in live births, stillbirths, or elective pregnancy terminations. Controls are women with healthy fetuses and loosely matched to cases by gestational age (± 4weeks) and maternal residence (residing in the province during this pregnancy). In the present study, we used data collected from January 2003 to December 2016. To obtain a maximum sample size, we concluded controls including those recruited for other defects, resulting in a sample consisting of 408 NTD cases and 593 controls. This study was approved by the Ethics Committee of Peking University (Beijing, China). All study participants provided informed consent.

At enrollment, we obtained information on sociodemographic and lifestyle factors with a structural questionnaire administrated by local healthcare staff through a face-to-face interview. The following covariates were assessed: maternal age at pregnancy (years), prepregnancy body mass index (kg/m^2), ethnicity (Han/non-Han), parity (one/more), education levels (primary school or lower, junior high school, or high school or higher), occupation (farmer/non-farmer), self-reported history of pregnancy affected by birth defects (yes/no), have had a fever (≥ 38.5°C) or influenza (yes/no), passive smoking (ever/never), folic acid supplementation (yes/no), frequency of selected food groups during the periconceptional period, and gestational age at placenta collection (weeks).

Placenta collection and metal quantification

After delivery or elective termination, the placenta was immediately collected and stored at − 20°C with labeled polyethylene plastic bag. The samples were shipped with dry ice to our laboratory for storage at − 20°C prior to processing. The details of sample preparation have been described elsewhere [25]. Shortly after thawing at 4°C, about 6 g specimens on the fetal side were collected within 3 cm around the insertion point of the umbilical cord, rinsed with deionized water, and freeze-dried 24 h (ALPHA2–4 LD plus, Christ, Germany). About 0.1 g lyophilized placental tissue were successively mixed with nitric acid and deionized water, digested under a high-pressure microwave system (Ultra WAVE, Milestone, Italy), diluted with deionized water, and finally added with internal standard (rhodium and indium) for determination of concentrations of the metals with inductively coupled plasma-mass spectrometers (ICP-MS, 7700x, Agilent, USA). Concentrations of metals were expressed with nanogram or microgram per gram dry weight.

The sensitivity and stability of ICP-MS were calibrated by analyzing the internal reference agent (rhenium, GSB 04–1745–2004). The accuracy of ICP-MS was checked by analyzing the standard reference material with known concentrations of three metals (reference material: pig liver, GBW10051). Quality control and assurance of placental metal concentrations assessment were achieved by using ultra-pure grade chemicals in the whole experiment, 1 blank solution control and 1 replicated measurement in every 30 samples, and masking the operators of the group status of the samples (case or control). The correlation coefficients of the standard curves for copper, silver, and titanium were all over 99.9%. The detection limits of copper, silver, and titanium were 3.2 ng/g, 0.32 ng/g, and 1.07 ng/g, respectively. The detection rates for copper and titanium were 100%, while silver was 79%. The samples with silver levels below the detection limit were imputed as half of the detection limit.

Statistical analysis

Comparisons of baseline characteristics, which were expressed as number (percentage), between NTD and control groups were made with the Chi-square test. Placental concentrations of the metals between NTDs and controls were compared using the Mann-Whitney *U* test for medians and Student *t*-test for geometric means, respectively. The heterogeneity of metal concentrations between five geographical regions was checked by multivariate analysis of variance.

Firstly, we used multilevel mixed-effects logistic regression to evaluate the association between each metal exposure and risk for NTDs. Tertiles of concentrations of metals for all subjects in each geographical region were introduced in the model as categorical variables to compare the second and highest tertiles (non-exposure) with the lowest tertile (exposure). The effect sizes of associations were indicated with odds ratios (ORs) and their 95% confidence intervals (95% CIs). Secondly, we employed Bayesian kernel machine regression (BKMR) to examine: (1) the effect of each metal on NTDs while fixing the remaining two metals to 25th, 50th, or 75th percentile; (2) the visualizable dose-response relationship between metal exposure and NTD risk without coarsening the continuous concentrations; (3) the interactive effects between three metals, which cannot be properly handled with linear regression [26]. 10,000 iterations were applied in BKMR. Both regression models adjusted for maternal occupation, education, gestational age at placenta collection, history of pregnancy affected by birth defects, have had a fever or influenza, passive smoking, and folic acid supplementation during the periconceptional period due to their uneven distributions between the case and the control groups.

Correlations between placental metal concentrations and maternal diet frequencies were assessed by Spearman's coefficient. To evaluate possible confounding by the length of gestation, which was different between the case and control groups, on the associations between placental metal concentrations and risk for NTDs, we performed subgroup analyses with a subset of cases and controls matched by the length of gestation. To assess the robustness of the results, we further repeated our analyses by including only NTD cases without other system malformations.

The BKMR was implemented using R (version 4.0.3; R Development Core Team), and the remaining statistical analyses were conducted using Stata 15.0 software (Stata Corporation, College Station, Texas, USA). A two-sided P-value of < 0.05 was considered statistically significant.

Results

Characteristics of participating NTD cases and controls are presented in Table 1. Virtually (99.5%) of all participants were of Han ethnicity. Generally, study subjects were young, with a mean age of 26.9 years in cases and 26.6 years in the controls. Compared to controls, cases were less likely to complete high school education and to report folic acid supplementation during the periconceptional period, more likely to work as a farmer, to report a history of pregnancy affected by birth defects, to have a fever or influenza, and to report passive smoking during the periconceptional period. Over half (56.4%) of the cases had a gestational age at placenta collection of fewer than 28 weeks, compared to only 2.4% in controls, reflecting the impact of elective termination following prenatal diagnosis in the case group. Most of the women had a normal prepregnancy body mass index (approximately 62%), with no difference being observed between the two groups. Approximately half of the participants (49%) were primigravida, and there was no difference in parity between the cases and controls.

Table 1. Characteristics of neural tube defects and healthy controls in northern China, 2003 to 2016.

Characteristics	Neural tube defects (N=408)	Controls (N=593)	P^a
	n (%)	n (%)	
Age (years)			0.667
<25	168 (41.2)	240 (40.5)	
25–29	117 (28.7)	182 (30.7)	
30–34	79 (19.4)	119 (20.1)	
≥35	42 (10.3)	47 (7.9)	
Prepregnancy body mass index (kg/m^2)			0.804
<18.5	31 (7.6)	48 (8.1)	
18.5–24.9	252 (61.8)	372 (62.7)	
≥25.0	114 (27.9)	162 (27.3)	
Occupation, farmer	315 (77.2)	443 (74.7)	0.019
Education, ≥high school	73 (17.9)	173 (29.2)	<0.001
Gestational age at sample collection, <28 weeks	230 (56.4)	14 (2.4)	<0.001
Parity, 1	283 (47.7)	205 (50.2)	0.066
History pregnancy affected by birth defects, yes	21 (5.1)	9 (1.5)	0.003
Periconceptional affected by fever or influenza, yes	138 (33.8)	75 (12.6)	<0.001
Periconceptional folic acid supplementation, yes	130 (31.9)	264 (44.5)	<0.001
Periconceptional passive smoking, ever	248 (60.8)	234 (39.5)	<0.001

[a] Comparisons between NTD cases and controls were used the Chi-square test.

Descriptive data of metal concentrations in the placental tissue of the study subjects are presented in Table 2. Median concentrations of copper and silver were significantly higher in NTD cases than in controls, while the median concentration of titanium did not show a significant difference. When presented in geometric parameters, similar profiles were exhibited for the two groups. Anencephaly and spinal bifida, the two major subtypes of NTDs, showed a similar pattern for the three metals with total NTDs (Table S1).

Table 2. Concentrations of metals in placental tissue (dry weight) in neural tube defects and healthy controls in northern China, 2003 to 2016.

Metal	Median (Interquartile range)[a]		Geometric mean±Standard deviation[b]	
	NTDs (*N*=408)	Controls (*N*=593)	NTDs (*N*=408)	Controls (*N*=593)
Cu (μg/g)	4.16 (3.65–4.91)*	3.91 (3.47–4.49)	4.42±1.30*	4.10±1.09
Ag (ng/g)	0.96 (0.56–1.57)*	0.71 (0.16–1.29)	1.41±1.84*	0.98±1.06
Ti (μg/g)	0.65 (0.57–0.74)	0.63 (0.57–0.71)	0.69±0.40*	0.65±0.12

NTD, neural tube defect, Cu, copper, Ag, silver, Ti, titanium, *P<0.001.

[a] Comparisons between NTDs and controls were used Mann-Whitney *U* test.

[b] Comparisons between NTDs and controls were used Student's *t*-test.

Variations in metal concentrations were observed among the five counties where the study subjects were recruited (Table S2). The variations by region were then considered with multilevel mixed-effects logistic regression in the following analyses.

The associations between tertile concentrations of each metal and risk for NTDs are displayed in Table 3. In single metal regression models, the highest tertile of copper was associated with a 2.28-fold (1.64–3.17) higher risk for NTDs in univariate analyses. However, this association disappeared when confounding factors were adjusted. For silver, the risk for NTDs increased by 2.02-fold (95% CI 1.45–2.82) and 2.19-fold (95% CI 1.55–3.11) in the second and the highest tertiles compared to the lowest tertile. These associations remained after adjustment for potential confounders, and a dose-response relationship remained with an adjusted OR of 1.78 (95% CI 1.04–3.06) and 1.92 (95% CI 1.11–3.32) for the second and highest tertiles, respectively. No association between titanium levels and NTD risk was observed in either unadjusted or adjusted models. In multiple-metal regression models, however, the association between silver concentrations and NTD risk turned to non-significant after adjustment for potential confounders. Similar patterns of associations were presented for anencephaly and spina bifida (Table S3).

Table 3. Associations between placental tertile concentrations of metals (dry weight) and risk for neural tube defects in northern China, 2003 to 2016.

Metal	OR (95% CI) [a],		Adjusted OR (95% CI) [a, b]	
	One-metal model	Three-metal model	One-metal model	Three-metal model
Cu (μg/g)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	1.34 (0.96–1.87)	1.24 (0.89–1.71)	0.66 (0.39–1.11)	0.64 (0.38–1.08)
	2.28 (1.64–3.17)*	1.93 (1.40–2.67)*	1.24 (0.74–2.06)	1.17 (0.70–1.96)
P_{trend}	<0.001	<0.001	0.451	0.582
Ag (ng/g)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	2.02 (1.45–2.82)*	2.02 (1.46–2.80)*	1.78 (1.04–3.06)*	1.69 (0.98–2.92)
	2.19 (1.55–3.11)*	1.97 (1.41–2.74)*	1.92 (1.11–3.32)*	1.74 (0.99–3.06)
P_{trend}	<0.001	<0.001	0.023	0.063
Ti (μg/g)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
	0.88 (0.64–1.21)	0.85 (0.61–1.17)	0.77 (0.46–1.29)	0.76 (0.45–1.28)
	1.09 (0.79–1.50)	1.01 (0.73–1.40)	1.00 (0.61–1.67)	0.95 (0.57–1.59)
P_{trend}	0.608	0.686	0.970	0.859

Cu, copper, Ag, silver, Ti, titanium, OR, odds ratio, CI, confidence interval, *P<0.05.

[a] Calculated by multilevel mixed-effects logistic regression, geographical region as a random effect.

[b] Adjusted for maternal occupation, education, gestational age at sample collection, history of pregnancy affected by birth defects, have had a fever or influenza, passive smoking, and folic acid supplementation during the periconceptional period.

In BKMR, silver was the only metal that shown risk effects on NTDs, as indicated by its three point estimates, which are greater than the null value of zero and their lower boundaries of credible intervals exclude the null. Moreover, the three point estimates of silver are almost identical, suggesting that silver's effect is independent of the other two metals (Figure 1A). No associations between concentrations of copper and titanium and NTD risks were observed (Figure 1A). As Figure 1B indicates, NTD risks increased linearly with silver concentrations, although the curve flatted at the highest silver concentrations. No clear evidence of association was shown for copper and titanium concentrations and risk for NTDs (Figure 1B). No interaction between metals was observed because the slopes of a specific metal were similar when the other metal was set at the 25th, 50th, or 75th percentile while the third one being held at its median (Figure 1C), and none of the interactive effects was significant as the credible intervals for copper, silver, and titanium encompass the zero null value (Figure 1D). When metals were treated as a mixed exposure mixture, NTD risk increased almost linearly across the whole range of mixture concentrations from the 25th percentile through the 75th percentile (Figure S1).

Correlations between frequencies of maternal food groups during the periconceptional period and placental metal concentrations are shown in Table 4. No correlation was found between maternal diet and copper levels, except for the consumption of meat or fish. Higher consumption frequencies of meat or fish, egg or milk, fresh vegetables and fruits, beans or its products, and tea-drinking were positively correlated with concentrations of silver. In addition, increased intake of egg or milk and fresh vegetables and fruits was positively correlated with titanium concentrations.

Table 4. Correlations between placental metal concentrations (dry weight) and frequencies of maternal food consumption in northern China, 2003 to 2016.

Dietary intake[a]	Copper (μg/g)		Silver (ng/g)		Titanium (μg/g)	
	r	*P*	*r*	*P*	*r*	*P*
Meat or fish	−0.066	0.037	0.189	<0.001	0.511	0.991
Egg or milk	−0.029	0.364	0.217	<0.001	0.094	0.003
Fresh vegetables	−0.033	0.304	0.110	0.001	0.069	0.030
Fresh fruits	−0.007	0.823	0.177	<0.001	0.100	0.002
Bean or its products	−0.007	0.836	0.219	<0.001	0.038	0.232
Tea drinking	−0.062	0.056	0.111	0.001	0.056	0.080

[a] Frequency of dietary intake was classified into three levels, i.e., <1, 1 to 6, and >6 times per week.

Ninety-two pairs were obtained when cases and controls were matched by the length of gestation. Similar patterns of placental concentrations of silver in cases and controls were present with the overall analyses (Table S4). Although the dose-response relationship for silver and NTD risk was not significant, largely because of the reduced sample size, the direction of the association was the same as the total sample (Table S5), suggesting that the observed associations were unlikely to be resulted by confounding of gestation. The results did not change meaningfully when NTDs complicated with other malformations were excluded (Table S6 and Figure S2).

Discussion

Using concentrations of copper, silver, and titanium in placental tissue as prenatal exposure markers, this study revealed that exposure to silver was associated with elevated risk for NTDs in a Chinese population with both the traditional logistic regression and the state-of-the-art BKMR. Silver concentrations were positively correlated with frequencies of several food groups. No interaction between silver and any of the other two metals was observed.

Several previous studies have examined the association between maternal exposure to the three metals during pregnancy and NTDs, and our findings are consistent with some but not all. For example, our finding is comparable to a study from Turkey that found no difference in levels of copper in the amniotic fluid between groups of pregnant women complicated with NTDs and healthy fetuses [27], analogous to other two studies from Australia [28] and China [29], while contrary to other studies [7–9]. Silver concentrations in scalp hair from mothers of newborns with NTDs were significantly higher than in healthy controls in an Iranian study [19], which is in line with the present study. Whereas, maternal hair concentration of silver was not associated with risk for NTDs in a study conducted in the same province as well as another province in northern China [20]. For titanium, however, the previous case-control study using maternal scalp hair suggested that high levels of titanium are possibly correlated with increased risk for NTDs [20]. We further examined the correlation of the three metals' concentrations in two biological specimens of maternal hair and placenta in a subset of samples, but no significant relationship was found (data not shown). This discrepancy may be resulted from the complex mechanism of metals' absorption, metabolism, or excretion.

The results of BKMR are in line with the results of individual metal regression. However, contrary to the multi-metal linear model, the relationship between silver and NTDs presented a significantly positive and linear dose-response pattern in BKMR. Generally, conventional statistical approaches, such as logistic regression, include a set of exposures of interest into one model; however, such an approach may contribute to the distortion of results if correlations exist in the targeted exposures [30]. In this study, there is a correlation between placental silver and titanium (Figure S3, $P < 0.001$), with an r value of 0.24, which may explain why silver concentrations are associated with risk for NTDs in BKMR while not in logistic regression.

No interaction of three metals was observed, implying no synergistic effect or antagonistic effect of each two metals (i.e., copper × silver). Therefore, the use of BKMR in combination with conventional linear regression can improve the robustness of the results and get a deeper insight into the complex relations among multiple exposures regarding their effect on NTDs.

Dietary intake is the primary source of human copper exposure, with approximately 75% from solid food and 25% from drinking water [31]. In this study, consumption frequencies of meat or fish were correlated with copper concentrations in placental tissue. As a naturally occurring metal, silver has a wide variety of applications, allowing exposure through various routes of entry to the body, such as ingestion and skin contact. WHO has reported that daily intakes of silver are about 7 μg/person [32]. Studies from Italy, Canada, and the United Kingdom have also reported levels of silver from daily dietary intake, with 0.4 μg/day [33], 7 μg/day [34], and 27 μg/day [35], respectively. Additionally, the workplace also is a major silver exposure source for humans. However, participants in the current study reported no occupational exposure, and most of the study subjects were recruited from rural areas. And our results showed that concentrations of silver in placental tissue are significantly correlated with frequencies of all food groups included in the questionnaire. Thus, daily dietary silver intake may be the major source of exposure in this population. As a common food additive, titanium ubiquitously exists in foods in the form of titanium oxide, and the content in some dairy products with white colors, such as milk, ranges from 0.10 to 0.26 μg/mL [36]. This may partly explain why placental titanium concentrations were positively correlated with the frequency of the consumption of egg or milk in our population.

Reported exposure levels (dry weight) vary among populations and biological samples. For instance, compared to the data from women in Poland, women in our population had a similar concentration of copper (4.04 μg/g vs. 5.64 μg/g) but a lower placental concentration of silver (3.0 ng/g vs. 0.84 ng/g) [37]. Median placental copper concentrations were also similar to those found in previous studies conducted in Bangladeshi (5.3 μg/g) [38] and Japan (3.91 μg/g) [39]. The mean value for copper in placental tissue in this study (4.23 μg/g) was remarkably lower than the concentration reported in a study from India (70.0 μg/g) [40], similar to those from women in Germany (4.4 μg/g) [41], but higher than those reported from healthy pregnant Spanish women (0.97 μg/g) [42]. However, due to differences in element assessment methodology, it may not be appropriate to make a direct comparison of specific element concentrations across studies.

Strengths of the present study include homogeneous ethnicity of study participants, relatively large sample size, and considerable information on characteristics to adjust as potential confounders in statistical models. Besides, metal concentrations in placental tissue were used as an indicator of environmental exposure in utero, which is believed to be able to reflect longer-term measures compared to maternal or cord blood-based markers [21, 43]. Finally, BKMR was used to explore the effect of each metal while taking others into consideration and interactions between any two of the metals.

Inevitably, there also exist some limitations to our analyses. First, it would be ideal to be able to measure the levels of these elements during the 5th to 7th weeks of gestation, the developmental window of NTDs [44]. However, it is infeasible due to ethical or technical reasons. Second, most cases but fewer controls had gestational age at sample collection less than 28 weeks, which may confound the examined associations. However, sensitivity analyses showed that our results could not be completely explained by the confounding of gestation. Third, we were unable to isolate a causal relationship between placental metal levels and risk for NTDs, as this is a case-control study.

Conclusions

Prenatal exposure to high levels of silver in placental tissue was associated with an increased risk for NTDs in offspring in a dose-response manner, and dietary intake may be one of the major sources of maternal silver exposure. Further studies in other populations are needed to replicate the findings of this study, and animal studies are warranted to elucidate biological mechanisms underlying the observed statistical association.

Abbreviations

NTD, neural tube defects; ICP-MS, inductively coupled plasma-mass spectrometers; OR, odds ratio; CI, confidence interval; BKMR, Bayesian kernel machine regression; Cu, copper; Ag, silver; Ti, titanium.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Peking University (Beijing, China) and all study participants provided informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to ethical and legal reasons but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XP and CW conducted the research, analyzed the data, and drafted the manuscript, DW, SY conducted the research, LJ, ZL, LW, WY and AR designed the research, AR and CY supervised the research, AR revised the manuscript, and had primary responsibility for the final content. All authors read and approved the final manuscript.

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Figures

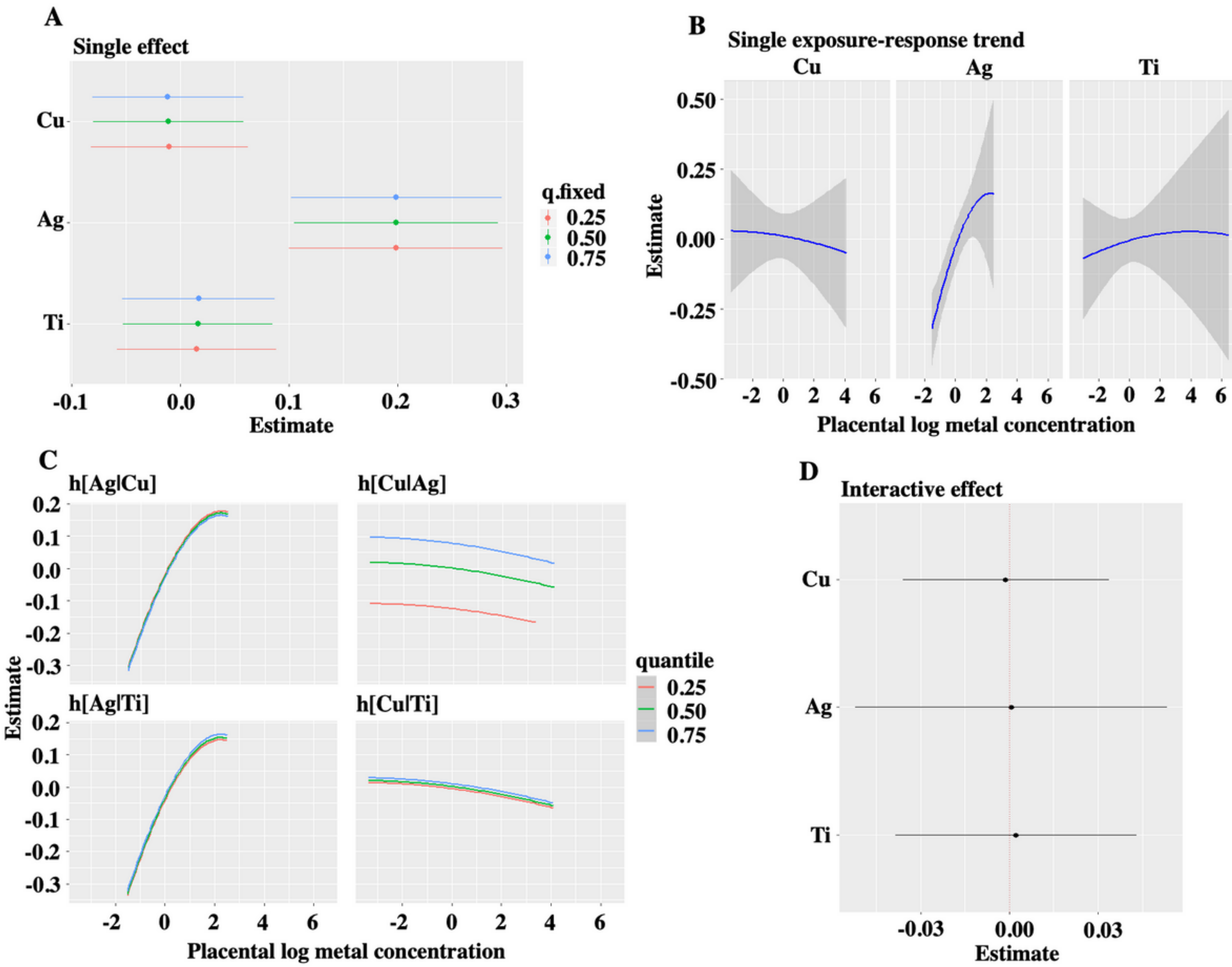

Figure 1

Effect of metals on NTD risk (expressed in βprobit) estimated by Bayesian kernel machine regression. Models were adjusted for maternal occupation, education, gestational age at placenta collection, history of pregnancy affected by birth defects, have had a fever or influenza, passive smoking, and folic acid supplementation during the periconceptional period. (A) Single effect of metal on NTD risk (estimates and 95% credible intervals) by comparing the NTD risk when one metal changes from its 25th percentile to 75th percentile while setting the other two at 25th, 50th, or 75th percentile. (B) Univariate exposure-response function for metal and change in NTD risk while the other two metals are held at their median levels. (C) Bivariate exposure-response functions for each metal: the top left panel shows for silver (Ag) when copper (Cu) is fixed at the 25th, 50th, or 75th percentile, and titanium (Ti) is fixed at its median level. The others were similar to the top left panel. (D) Interactive effect of metals, defined as the change in the single-metal association when the remaining two metals are fixed at their 25th percentile as compared to when they are fixed at their 75th percentile.

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