

Associations of prenatal metal exposure with child neurodevelopment and mediation by perturbation of metabolic pathways

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Prenatal exposure to metals has been associated with impaired neurodevelopment in children, but the detailed molecular mechanisms remain largely unknown. Based on the Wuhan Healthy Baby Cohort, China ($N = 1088$), eleven metals were measured in maternal urine during early pregnancy (13.1 ± 1.1 weeks) and metabolomics profiling was conducted in cord blood. Neurodevelopment was evaluated using the Bayley Scales of Infant Development in 2-year-old children to obtain the mental development index (MDI) and psychomotor development index (PDI). After false discovery rate correction, higher maternal urinary levels of manganese, nickel, aluminum, rubidium, gallium, and the summary score of metals were only significantly associated with lower MDI scores. The weighted quantile sum index of the metal mixture showed a significant inverse association with MDI and PDI scores, with aluminum contributing the most to the associations. Histidine, beta-alanine, purine, and pyrimidine metabolism significantly mediated the above associations, suggesting that disturbances in amino acids, neurotransmitter and neuroendocrine metabolism may be important mediators in contributing to impaired neurodevelopment of children.

The developing brain is particularly vulnerable to environmental pollutants, and even low exposure levels can induce adverse neurodevelopmental outcomes^{1–4}. Maternal exposure to metals, such as lead (Pb) and cadmium (Cd), has been linked to adverse neurodevelopmental outcomes in children, including impaired cognitive development and behavior development^{2,5,6}, as well as an elevated risk of autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD)^{7,8}. However, previous studies have primarily focused on single toxic metals (e.g. Pb, Cd) or mixtures of several metals, and the effects of mixed metal exposure remain inconclusive. Moreover, the biological mechanisms linking prenatal exposure to mixed metals and children's neurodevelopment have not yet been fully established. Previous

studies have suggested that disruptions of homeostatic processes and bioenergetic disturbances caused by environmental exposures in utero, such as increased oxidative stress and lipid oxidation by high maternal levels of aluminum (Al), Cd, Pb, and manganese (Mn)^{9,10}, disturbances of neurotransmission by Al and Mn^{11,12}, mitochondrial dysfunction induced by Pb and Mn^{13,14}, which may be underlying mechanisms leading to adverse neurodevelopmental outcomes^{15,16}. However, the majority of these findings are based on several traditional biomarkers, including 8-hydroxy-2'-deoxyguanosine, 8-hydroxyguanosine, malondialdehyde, and 4-hydroxy-2-nonenal-mercapturic acid^{17,18}, which provide limited insight into complex biological processes.

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Metabolomics, as a high-throughput analytical approach to characterize biological perturbations, has been extensively employed to investigate the influence of environmental exposure on health to enhance our comprehension of pathogenesis^{19–21}. Two previous studies with a small sample size examined the effects of metal exposure during pregnancy on maternal metabolism and then analyzed the biomarkers or metabolic pathways associated with impaired neurodevelopment in children^{22,23}. However, the mediating roles of metabolites or metabolic pathways have not been quantified to explore the causal pathways for the exposure-outcome associations. Metabolomics combined with a meet-in-the-middle (MITM) method and mediation analysis with metabolites and metabolic pathways may facilitate the characterization of the molecular signatures perturbed by metal exposure in utero and understand the pathogenesis of adverse outcomes in early life^{24–26}. In particular, the aforementioned methods can elucidate not only the impact of environmental exposure on individual metabolites that are associated with the outcome but also

the role of the metabolic pathway, thereby providing a more comprehensive understanding of the perturbed biological processes. To the best of our knowledge, no studies have employed this approach to elucidate the potential mechanisms of how prenatal metal exposure affects the cord blood metabolome, and how these responses are linked to children’s neurodevelopment.

Based on a prospective birth cohort in Wuhan, China, we measured the levels of eleven metals in maternal urine from the first trimester, analyzed metabolomics in cord blood, and evaluated the neurodevelopment of 2-year-old children ($N=1088$ mother-child pairs). The Bayley Scales of Infant Development was used to evaluate the children’s neurodevelopment because it is an extensive formal assessment tool that evaluates cognitive and psychomotor development in infants and young children to obtain the motor development index (MDI) and psychomotor development index (PDI)^{27,28}. This study aimed to investigate the associations between prenatal exposure to single and mixed metals and neurocognitive development, and to identify the molecular perturbation and underlying mechanism by applying a comprehensive metabolomics workflow.

Table 1 | Basic characteristics of the study population from a birth cohort study in Wuhan, China ($N=1088$ mother-child pairs)

	Total
Maternal characteristics	
Age at delivery, years	28 (27, 31)
Pre-pregnancy BMI, kg/m ²	20.7 (19.1, 22.8)
Folic acid supplementation during pregnancy	
No	120 (11.0%)
Yes	968 (89.0%)
Maternal education level	
High school or equivalent and below (≤ 12 years)	196 (18.0%)
Bachelor’s degree and above (>12 years)	892 (82.0%)
Annual household income (yuan)	
<100,000	570 (52.4%)
$\geq 100,000$	518 (47.6%)
Parity	
Nulliparous	881 (81.0%)
Multiparous	207 (19.0%)
Neonatal characteristics	
Infant sex	
Female	499 (45.9%)
Male	589 (54.1%)
Gestational age, weeks	39.4 (38.7, 40.1)
Birth weight, g	3345 (3070, 3650)
Low birth weight	
No	1062 (97.6%)
Yes	26 (2.4%)
Preterm delivery	
No	1061 (97.5%)
Yes	27 (2.5%)
MDI	114 (95, 126)
PDI	108 (96, 122)
Cognitive development delay (MDI < 70)	
No	998 (91.7%)
Yes	90 (8.3%)
Psychomotor development delay (PDI < 70)	
No	1056 (97.1%)
Yes	32 (2.9%)

Continuous variables are median (25th, 75th); categorical variables are n (%).
MDI mental development index, PDI psychomotor development index.

Results

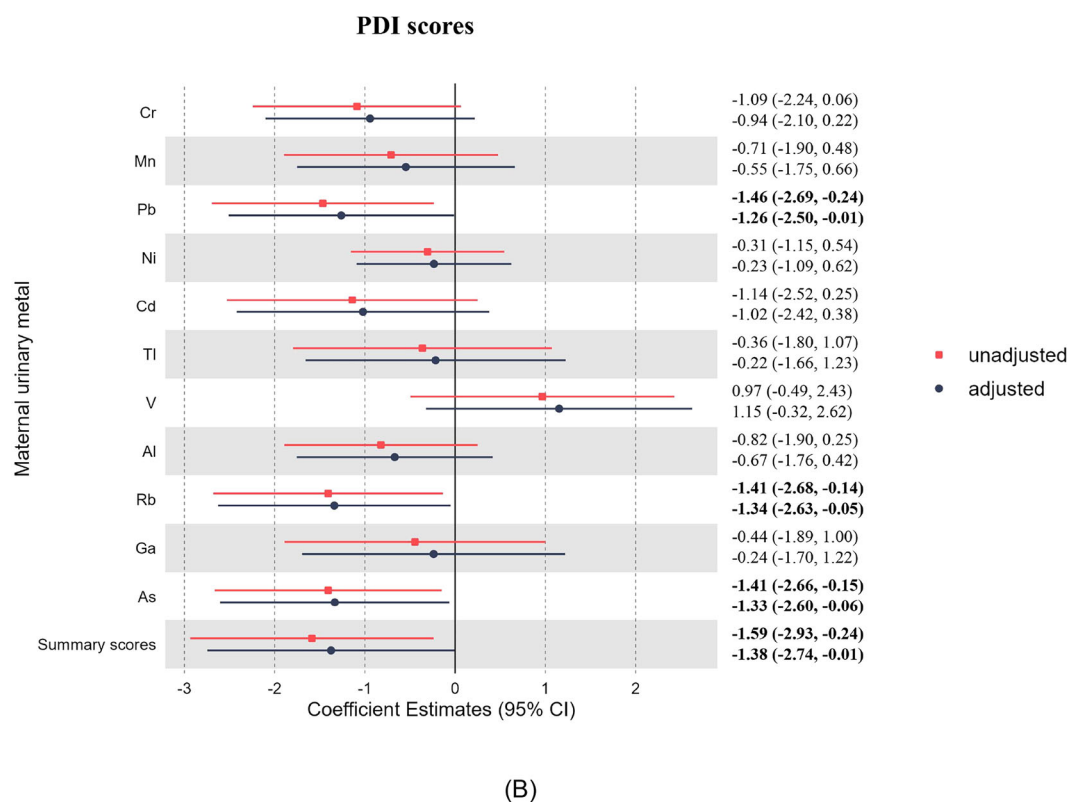
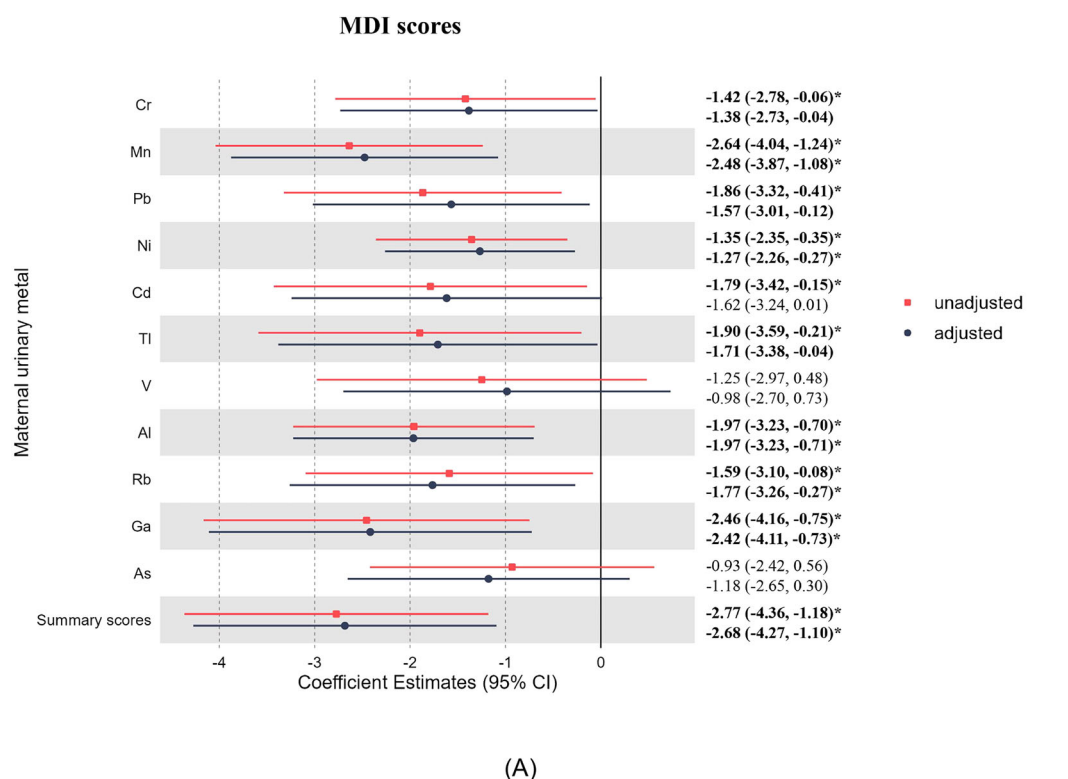
Participant information

As illustrated in Table 1, the median age of mothers at delivery was 28 years. Among the 1088 mothers, 89.0% had folic acid supplementation during pregnancy, 82.0% had a bachelor’s degree or above, 81.0% were primiparous, and 52.4% had an annual household income <100,000 yuan. The mean birth weight of the children was 3345 g, of which 54.1% were boys. The prevalence of low birth weight and preterm birth was 2.4% and 2.5%, respectively. At 2 years of age, the median (25th, 75th percentile) of MDI and PDI scores of the children were 114 (95, 126) and 108 (96, 122), respectively. About 8.3% and 2.9% had moderate to severe cognitive development delay and psychomotor development delay, respectively.

Almost all metals were detected in over 91% of maternal urine samples, but the detection rate of gallium (Ga) was 72% (Table S1). Rubidium (Rb) had the highest median concentration (1653.04 $\mu\text{g/g}$ creatinine), followed by arsenic (As) and Al (20.38 and 20.34 $\mu\text{g/g}$ creatinine, respectively). Thallium (Tl), Cd, vanadium (V), chromium (Cr), Mn, nickel (Ni), and Pb had median creatinine-corrected concentrations ranging from 0.37 to 2.49 $\mu\text{g/g}$ creatinine, while Ga had the lowest median concentration at 0.03 $\mu\text{g/g}$ creatinine. Spearman correlation coefficients between the concentrations of these eleven metals in maternal urine ranged from 0.25 to 0.77 (all $P < 0.05$) (Fig. S1).

Associations between maternal urinary concentrations of metals and children’s MDI/PDI scores

Linear regression models showed significant inverse associations between multiple metals and MDI scores (Fig. 1A). Specifically, after adjusting for covariates, each interquartile range (IQR) increase in maternal urinary concentration of Cr, Mn, Pb, Ni, Tl, Al, Rb and Ga during early pregnancy was significantly linked to a decrease of 1.38 points (95% CI: $-2.73, -0.04$), 2.48 points (95% CI: $-3.87, -1.08$), 1.57 points (95% CI: $-3.01, -0.12$), 1.27 points (95% CI: $-2.26, -0.27$), 1.71 points (95% CI: $-3.38, -0.04$), 1.97 points (95% CI: $-3.23, -0.71$), 1.77 points (95% CI: $-3.26, -0.27$), and 2.42 points (95% CI: $-4.11, -0.73$) in MDI scores, respectively. After multiple testing corrections, the associations of Mn, Ni, Al, Rb, and Ga with lower MDI scores remained significant ($P_{\text{FDR}} < 0.05$). Each IQR increase in the summary score of metals was significantly associated with a decrease of 2.68 points (95% CI: $-4.27, -1.10$, $P_{\text{FDR}} = 0.0056$) in MDI score (Fig. 1A). By using the weighted quantile sum (WQS) analysis, each quartile increase in the WQS index of the metal mixture was associated with a decrease of 3.47 points in MDI score (95% CI: $-5.00, -1.95$, $P < 0.001$), and Al contributed the most to the mixture effect (49.2%), followed by Ga (21.6%), Mn (11.8%), Cd (7.5%), and Cr (3.7%) (Fig. 2A).



For PDI, each IQR increase in maternal urinary concentration of Pb, Rb, and As was significantly associated with a decrease of 1.26 points (95% CI: -2.50, -0.01), 1.34 points (95% CI: -2.63, -0.05), and 1.33 points (95% CI: -2.60, -0.06) in PDI scores (Fig. 1B). However, none of the associations were significant after false discovery rate (FDR) correction ($P_{FDR} > 0.05$). Each IQR increase in the summary score of

metals was significantly associated with a decrease of 1.38 points (95% CI: -2.74, -0.01, $P = 0.049$, $P_{FDR} = 0.15$) in PDI score (Fig. 1B). A significant association was also observed between the WQS index of metal mixture and PDI scores, with one quartile increase in the WQS index being associated with a decrease of 1.88 points in PDI score (95% CI: -3.32, -0.44, $P = 0.011$) (Fig. 2B). Al (30.9%) made the largest

Fig. 1 | Associations between maternal urinary metals and neurodevelopment of 2-year-old children (N = 1088). **A** Linear regression analysis of the associations between maternal urinary metals and MDI scores of children (Per interquartile range increase in the creatinine-adjusted urinary metal concentration ($\mu\text{g/g}$ creatinine)). **B** Linear regression analysis of the associations between maternal urinary metals and PDI scores of children. The x-axis shows the estimated coefficients of the associations between maternal urinary metals and MDI or PDI scores. Data are presented as point estimates (linear regression beta coefficients) and 95% confidence intervals (beta coefficient ± 1.96 (standard error[beta coefficient])).

contribution to the association, followed by Pb (22.6%), As (19.3%), Cd (10.0%), Rb (9.1%).

In the sensitivity analyses that (1) excluding participants with preterm birth and low birth weight, (2) excluding pregnant women who did not take folic acid supplements, (3) excluding tobacco exposure during pregnancy, or (4) adjusting for more covariates (gestation age and birth weight), the results were not materially changed (Table S2). Sex-stratified analyses revealed associations between some metals and Bayley scores were stronger among boys than girls, but the interaction term between metal exposure and sex was not significant (all $P_{\text{sex-int}} > 0.05$, Tables S3 and S4), indicating that child sex did not significantly modify the neurotoxic effect of metals.

Metabolome-wide association study (MWAS) on maternal urinary metals and children's MDI/PDI scores

Of the 438 metabolites detected, 11 metals (Cr, Mn, Pb, Ni, Cd, Ti, V, Al, Rb, Ga, and As) were significantly associated with changes of 145, 113, 70, 28, 15, 26, 91, 104, 89, 79, and 16 metabolites in cord blood, respectively, after adjusting for covariates ($P < 0.05$ and $P_{\text{FDR}} < 0.2$, Fig. 3A, Supplementary Data 1). Higher exposure levels of Mn, Pb, Ni, V, Al, and Ga were mainly linked to the upregulation of amino acids and their metabolites such as carnosine, valine, hydroxylysine, asymmetric dimethylarginine, and targinine, as well as the downregulation of glutamine. Elevated levels of Cr, Mn, Ni, Ti, Al, and Rb were linked with the upregulation of riboflavin levels. The increase in summary score of metal concentrations and WQS index of metal mixture for MDI and PDI were significantly associated with 138, 136, and 115 metabolites, respectively, mainly related to the upregulation of amino acids and their metabolites, phosphatidylcholines (PCs), and phosphatidylethanolamines (PEs) ($P < 0.05$ and $P_{\text{FDR}} < 0.2$, Fig. 3A, Fig. S2, Supplementary Data 1).

In the MWAS on children's MDI/PDI scores, there were 71 and 24 metabolites in cord blood significantly associated with MDI and PDI, respectively ($P < 0.05$ and $P_{\text{FDR}} < 0.2$, Fig. 3B, C, Table S5). Higher levels of carnosine, targinine, bilirubin, lysophosphatidylethanolamine (LPE), lysophosphatidylcholine (LPC), and PEs had significant associations with lower MDI scores. On the contrary, the increase in levels of 10 metabolites, including lysine, glutamine, leucine, alpha-aminobutyric acid, deoxycholic acid glycine conjugate, riboflavin, 4-ethylphenylsulfate, fatty acid (FA) 12:0, sphingomyelin (SM) 38:0, and SM 40:1, showed significant associations with higher MDI scores. The increase in levels of 11 metabolites, including 8 lysophospholipids, 1 amino acid, 1 FA, and 1 phosphatidylinositol (PI) was significantly linked to lower PDI scores. A total of 13 metabolites exhibited a statistically significant correlation with higher PDI scores.

Based on the MITM approach, 56 overlapping metabolites were found in the MWAS on maternal urinary metals and the MWAS on children's MDI scores ($P < 0.05$ and $P_{\text{FDR}} < 0.2$, Fig. 3D). The overlapping metabolites profile consisted of 5 amino acids, 2 bile acids, 4 ceramides, 1 FA, 38 phospholipids, 2 SMs, 1 vitamin and 3 other metabolites. There were 11 overlapping metabolites in the MWAS on both maternal urinary metals and the MWAS on children's PDI scores, including 2 amino acids, 1 phosphatidic acid (PA), 3 PCs, 4 PIs, and 1 phosphatidylglycerol (PG) (Fig. 3E).

Statistical tests were performed with two-sided linear regressions. Adjustments for multiple comparisons were accounted for by calculating P_{FDR} . Estimated coefficients were adjusted for pre-pregnancy BMI, maternal age, parity, folic acid supplementation, maternal education level, infant sex, and cotinine levels in cord blood. Bold indicates statistical significance ($P < 0.05$), and “*” indicates statistical significance ($P_{\text{FDR}} < 0.05$). Source data are provided as a Source Data file. MDI mental development index, PDI psychomotor development index, CI confidence interval, Cr chromium, Mn manganese, Pb lead, Ni nickel, Cd cadmium, Ti thallium, V vanadium, Al aluminum, Rb rubidium, Ga gallium, As arsenic.

Overlapping enriched pathways associated with maternal urinary metals and children's MDI/PDI scores

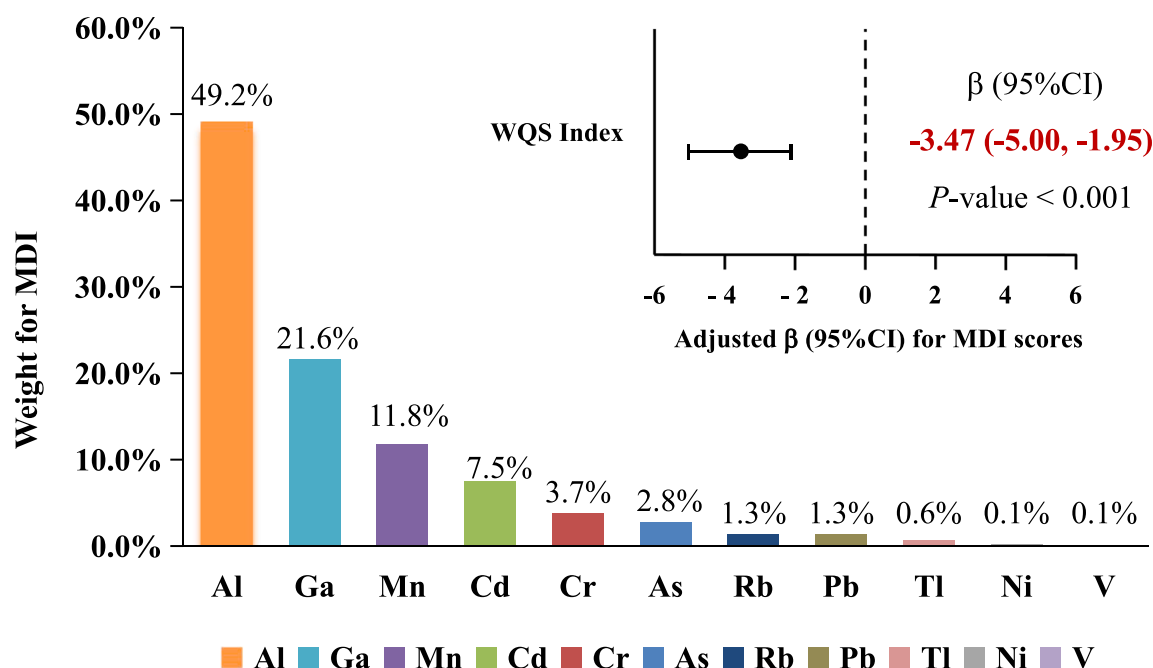
Among the 45 mapped Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, maternal urinary concentrations of Cr, Mn, Pb, Ni, Cd, Ti, V, Al, Rb, Ga, and As were found to be significantly associated with 12, 26, 20, 21, 3, 12, 19, 23, 16, 15, and 14 metabolic pathways, respectively ($P < 0.05$, Fig. 4A, Supplementary Data 2). A hierarchical clustering analysis of the metabolic pathways revealed that Mn, Ni, Pb, and Al clustered together, and the remaining metals (V, Rb, Ti, Ga, As, Cr, and Cd) were grouped. Compared to most of the single metals (except Mn, Ni, and Al), the summary score of metals, the WQS index of metal mixture for MDI and PDI disturbed more metabolic pathways, which were related to significant changes in 21, 26, and 22 pathways, respectively, including amino acid, lipid, and vitamin metabolic pathways ($P < 0.05$, Supplementary Data 2).

The MITM results indicated that 8 metabolic pathways (including lysine degradation; valine, leucine, and isoleucine degradation; valine, leucine, and isoleucine biosynthesis; histidine metabolism; riboflavin metabolism; beta-alanine metabolism; purine metabolism, and pyrimidine metabolism) in cord blood were associated with maternal urinary metals and MDI scores ($P < 0.05$, Fig. 4B). Three pathways (including lysine degradation; valine, leucine, and isoleucine degradation; valine, leucine, and isoleucine biosynthesis) were linked to maternal urinary metals and PDI scores ($P < 0.05$, Fig. 4B).

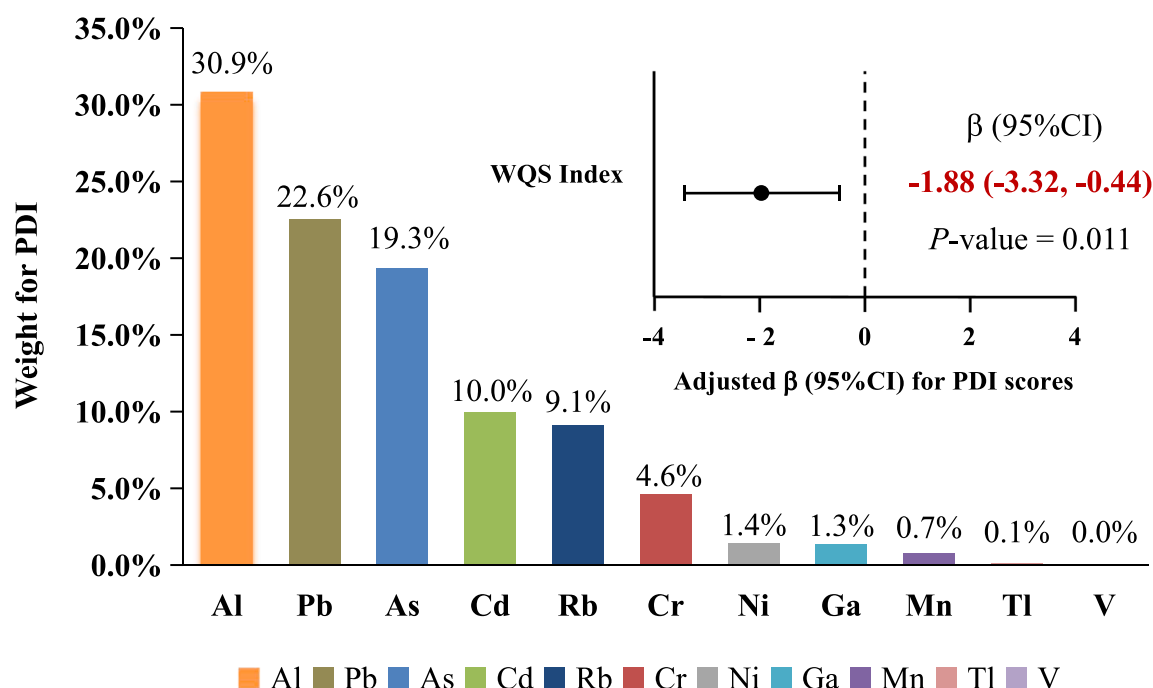
Mediating role of overlapping pathways and metabolites in the association between maternal urinary metals and children's MDI/PDI scores

We evaluated the potential mediation effect of the overlapping enriched pathways and metabolites on the associations between maternal urinary metals and children's MDI/PDI scores. There were three pathway mediators significantly associated with higher levels of metals and lower children's MDI scores, with the most frequently identified being beta-alanine metabolism, followed by histidine metabolism, and riboflavin metabolism. Beta-alanine and histidine metabolic pathways had significant mediation effects on the association of seven metals (Mn, Pb, Ni, V, Al, and Ga), with the mediation effects ranging from 10% to 26% (Table 2, Fig. 5). Among the metabolites detected in the two metabolic pathways, only carnosine significantly mediated a decrease in MDI associated with the six metals, with the mediation effect of 7%, 15%, 15%, 11%, 10%, and 10% for Mn, Pb, V, Al, Rb and Ga, respectively (Table S6). Consistently, the pathways of beta-alanine and histidine metabolism were found to significantly mediate a 13% and 11% change in the association between the summary score of metals and lower MDI, respectively. Additionally, these pathways mediated a 9% and 8% change in the association between the WQS index and lower MDI, respectively. In addition, glutamine, as a common metabolite in purine and pyrimidine metabolic pathways, had significant mediation effects on the association between metals (including Cr, Mn, Pb, V, Al, Rb, Ga, the summary score and WQS index) and lower MDI scores, with mediation effects ranging from 4% to 15% (Table S6).

Additionally, the riboflavin metabolic pathway mediated a -5% to -12% change in the association between metals (including Cr, Mn, Ni, Al, Rb, the summary score, and WQS index) and lower MDI scores. Among the three metabolites detected in this pathway, only riboflavin



(A)



(B)

was found to significantly mediate the association between metals (including Cr, Mn, Al, Rb, the summary score, and WQS index) exposure and lower MDI scores, with a mediation effect of -5% to -10%.

In the mediation analysis of metals with PDI, we found a 10%–26% mediation effect of the lysine degradation pathway on the association

between metals (Cr, Mn, Pb, Ni, Al, Ga, As; the summary score and WQS index) and lower PDI scores (Table S7, Fig. 5). Among the three metabolites detected in this pathway, 5-hydroxylysine was the only one found to significantly mediate the association between exposure to these metals and lower PDI scores, with a mediation effect of 17% to 42% (Table S7).

Fig. 2 | WQS regression analysis of the associations between maternal urinary metals and neurodevelopment of 2-year-old children (N=1088). **A** WQS regression analysis of the associations between maternal urinary metals and MDI scores (Per quantile range increase in WQS index). **B** WQS regression analysis of the associations between maternal urinary metals and PDI scores. Data are presented as point estimates (linear regression beta coefficients) and 95% confidence intervals (beta coefficient ± 1.96 (standard error[beta coefficient])). Statistical tests were

performed with two-sided WQS regressions. Estimated coefficients were adjusted for pre-pregnancy BMI, maternal age, parity, folic acid supplementation, maternal education level, infant sex, and cotinine levels in cord blood. Source data are provided as a Source Data file. WQS weighted quantile sum, MDI mental development index, PDI psychomotor development index, CI confidence interval, Cr chromium, Mn manganese, Pb lead, Ni nickel, Cd cadmium, Tl thallium, V vanadium, Al aluminum, Rb rubidium, Ga gallium, As arsenic.

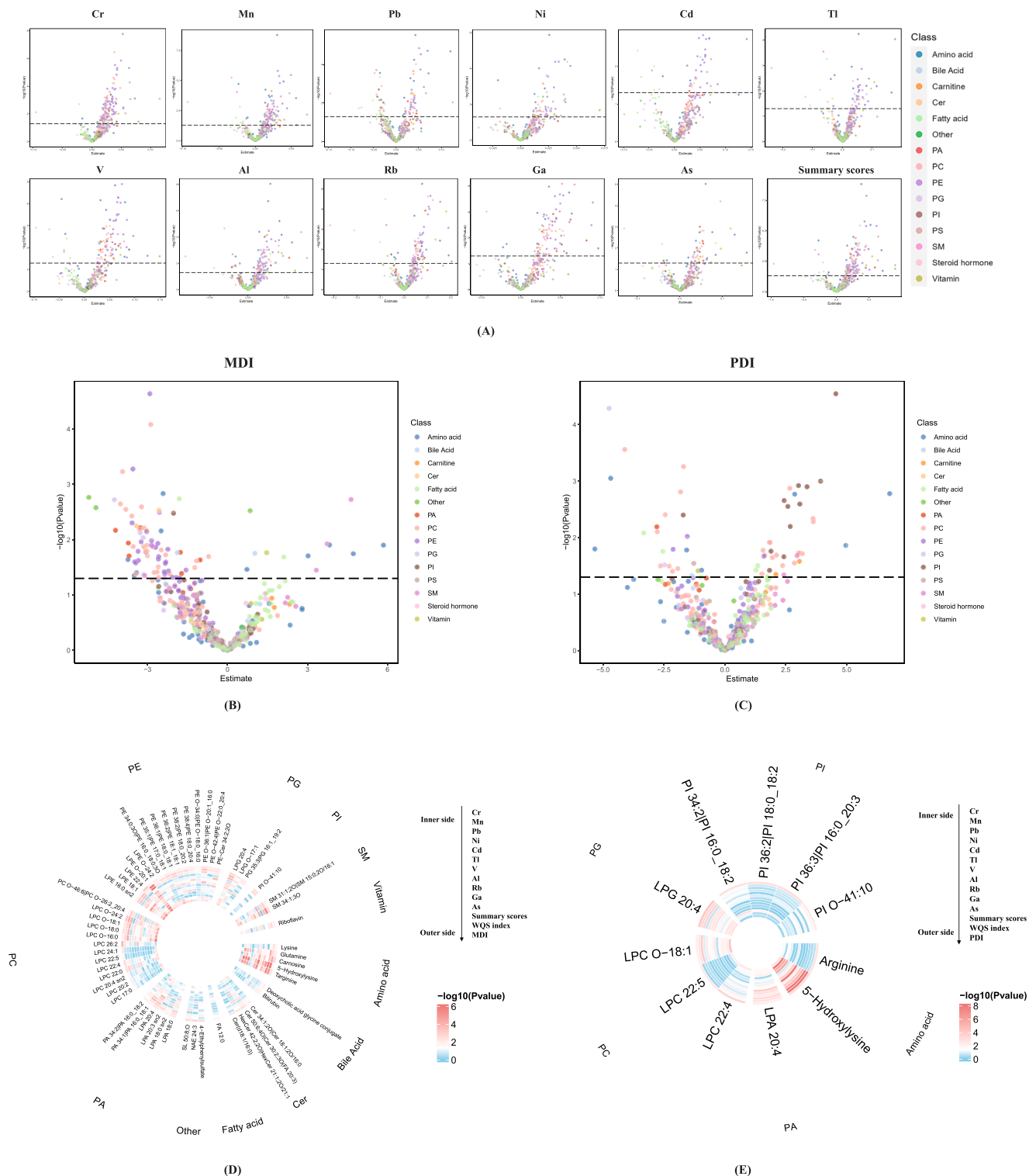


Fig. 3 | Metabolome-wide association study (MWAS) on maternal urinary metals and children's MDI/PDI scores ($N=1088$). **A** Volcano plot of the associations between maternal urinary metals and metabolites in cord plasma samples, analyzed by linear regression models. Statistical tests were performed with two-sided linear regressions. The x-axis shows the estimated coefficient of the association between maternal urinary metals and metabolites in cord plasma samples, and the y-axis shows its value of $-\log_{10}(P\text{-value})$. The significance P -value threshold of 0.05 is shown as a dashed line. Adjustments for multiple comparisons were accounted for by calculating P_{FDR} . Exact P -values and P_{FDR} are provided in Supplementary Data 1. The adjusted covariates for analyzing the association between maternal urinary metal exposure and metabolites included pre-pregnancy BMI, maternal age, parity, folic acid supplementation, maternal education level, infant sex, and cotinine levels in cord blood. **B** Volcano plot of the association between metabolites in cord plasma samples and MDI scores. **C** Volcano plot of the association between metabolites in cord plasma samples and PDI scores. Statistical tests were performed with two-sided linear regressions. The x-axis shows the estimated

coefficient of the association between metabolites in cord plasma samples and MDI/PDI scores, and the y-axis shows its value of $-\log_{10}(P\text{-value})$. The significance P -value threshold of 0.05 is shown as a dashed line. Adjustments for multiple comparisons were accounted for by calculating P_{FDR} . Exact P -values and P_{FDR} are provided in Supplemental Table 6. Different-colored dots represent different types of metabolites. The adjusted covariates for analyzing the association between metabolites and children's MDI/PDI scores were consistent with the above.

D Overlapping metabolites in cord plasma samples that were significantly associated with maternal urinary metal and MDI. **E** Overlapping metabolites in cord plasma samples that were significantly associated with maternal urinary metal and PDI. Source data are provided as a Source Data file. MDI mental development index, PDI psychomotor development index, Cer ceramide, PA phosphatidic acid, PC phosphatidylcholine, PE phosphatidylethanolamine, PG phosphatidylglycerol, PI phosphatidylinositol, SM sphingomyelin, Cr chromium, Mn manganese, Pb lead, Ni nickel, Cd cadmium, Tl thallium, V vanadium, Al Aluminum, Rb rubidium, Ga gallium, As arsenic.

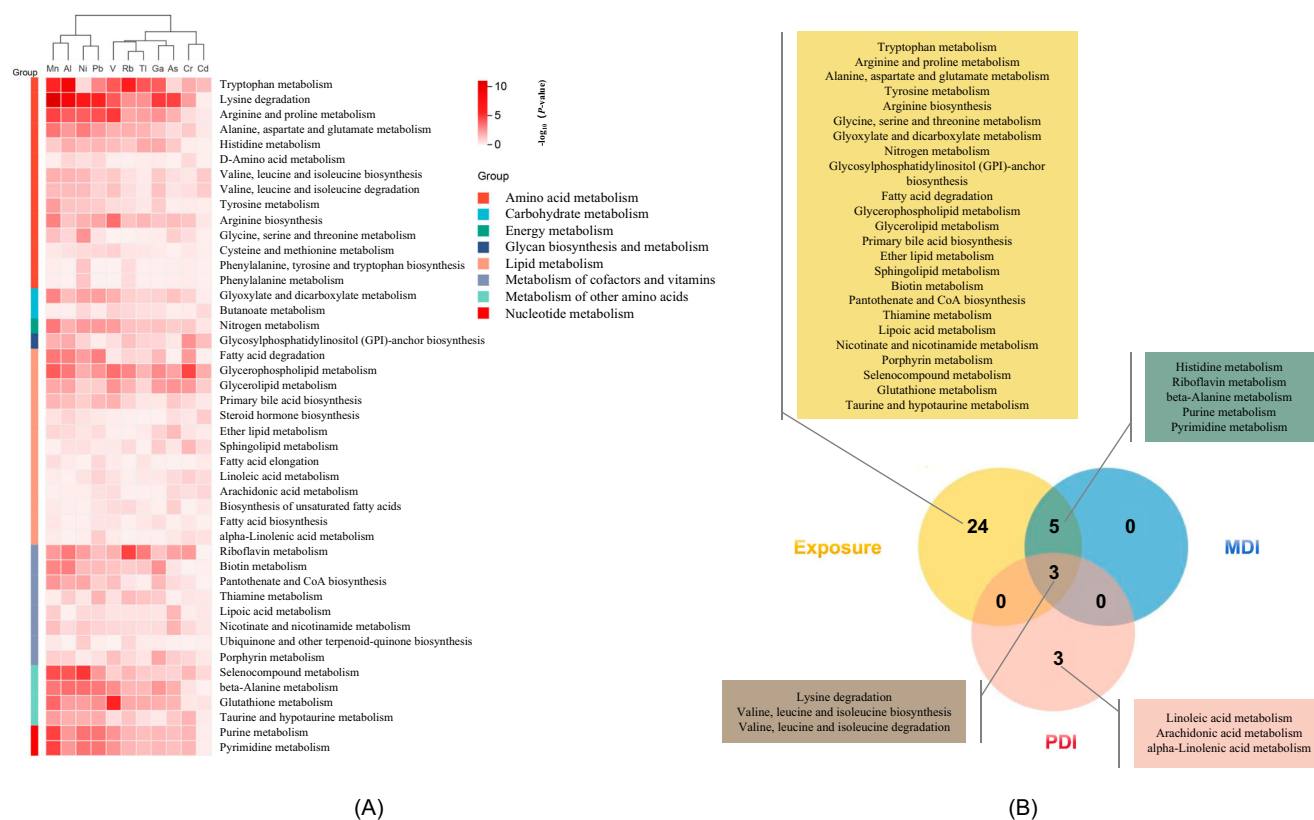


Fig. 4 | Metabolic pathways related to maternal urinary metals and neurodevelopment of 2-year-old children ($N=1088$). **A** Cluster heatmap of metabolic pathways for each maternal urinary metal. Statistical tests were performed with two-sided tests, with statistical significance set at 0.05. The color of the heatmap represents $-\log_{10}(P\text{-value})$ of metabolic pathways. **B** Venn diagrams of significantly enriched KEGG pathway from one or more of the maternal urinary metals, MDI, and

PDI scores ($P < 0.05$). Statistical tests were performed with two-sided tests, with statistical significance set at 0.05. Source data are provided as a Source Data file. MDI mental development index, PDI psychomotor development index, Cr chromium, Mn manganese, Pb lead, Ni nickel, Cd cadmium, Tl thallium, V vanadium, Al aluminum, Rb rubidium, Ga gallium, As arsenic.

In addition to the aforementioned metabolic metabolites, multiple lysophospholipids (e.g., LPA 20:4, LPC O-16:0, LPC O-18:0, LPC O-18:1, LPC O-24:2, LPE 22:4, and LPG 20:4) were significantly mediated the associations between several metals (Cr, Mn, Pb, Ga, the summary score and WQS index) and lower MDI scores, with the mediation effects ranging from 4% to 19% (Table S6). Furthermore, LPA 20:4, LPC O-18:1, and LPG 20:4 also significantly mediated associations between those metals and lower PDI scores, with the mediation effect values ranging from 8% to 25% (Table S8).

The results of the mediator analysis in the sensitivity analyses mentioned above (excluding preterm birth, low birth weight, without

folic acid supplements, and tobacco exposure; adjusting for more covariates) and sex-stratified analyses showed no material changes (Tables S9–S12).

Discussion

Based on a prospective birth cohort, we conducted metabolomics analysis of cord blood to explore the biologic pathways and metabolites that may be involved in the relationship between maternal metal exposure during early pregnancy and children's neurodevelopment at 2 years of age. We found higher levels of single and mixed metals were significantly linked with reduced MDI scores in children. Beta-alanine

Table 2 | Mediation analyses of maternal urinary metal-related metabolic pathways and metabolites on the association between maternal urinary metals and MDI scores of children (N = 1088)

Exposure	Pathway	Indirect effects ^a		P	P _{FDR}	Proportion mediated	Metabolites	Indirect effects ^b		P	P _{FDR}	Proportion mediated
		Estimate (95% CI)						Estimate (95% CI)				
Cr	Riboflavin metabolism	0.09 (0.01, 0.20)	0.005	0.030	0.030	-12%	Riboflavin	0.07 (0.01, 0.17)	0.019	0.093	-9%	
	beta-Alanine metabolism	-0.12 (-0.22, -0.04)	<0.0001	<0.0001	<0.0001	13%	Carnosine	-0.07 (-0.14, -0.01)	0.005	0.130	7%	
	Histidine metabolism	-0.11 (-0.20, -0.04)	0.001	0.003	0.003	11%						
Mn	Riboflavin metabolism	0.05 (0.00, 0.12)	0.032	0.064	0.064	-5%	Riboflavin	0.05 (0.00, 0.11)	0.036	0.130	-5%	
	beta-Alanine metabolism	-0.24 (-0.42, -0.09)	<0.0001	<0.0001	<0.0001	23%	Carnosine	-0.16 (-0.31, -0.04)	0.004	0.136	15%	
	Histidine metabolism	-0.23 (-0.40, -0.08)	<0.0001	<0.0001	<0.0001	21%						
Ni	beta-Alanine metabolism	-0.09 (-0.17, -0.02)	0.011	0.033	0.033	12%	Carnosine	-0.07 (-0.14, -0.01)	0.023	0.204	8%	
	Histidine metabolism	-0.07 (-0.16, 0.00)	0.033	0.066	0.066	10%						
	Riboflavin metabolism	0.07 (0.01, 0.15)	0.006	0.033	0.033	-9%	Riboflavin	0.06 (0.01, 0.14)	0.013	0.204	-8%	
V	beta-Alanine metabolism	-0.24 (-0.44, -0.07)	0.002	0.012	0.012	26%	Carnosine	-0.15 (-0.31, -0.02)	0.017	0.086	15%	
	Histidine metabolism	-0.16 (-0.34, -0.01)	0.034	0.102	0.102	17%						
	beta-Alanine metabolism	-0.10 (-0.18, -0.04)	<0.0001	<0.0001	<0.0001	15%	Carnosine	-0.07 (-0.14, -0.02)	0.005	0.114	11%	
Al	Histidine metabolism	-0.09 (-0.16, -0.03)	0.001	0.003	0.003	14%						
	Riboflavin metabolism	0.06 (0.01, 0.12)	0.005	0.010	0.010	-8%	Riboflavin	0.05 (0.00, 0.10)	0.014	0.118	-7%	
	beta-Alanine metabolism	-0.26 (-0.54, -0.04)	0.023	0.069	0.069	12%	Carnosine	-0.21 (-0.46, -0.03)	0.019	0.157	10%	
Rb	Riboflavin metabolism	0.25 (0.04, 0.52)	0.007	0.042	0.042	-11%	Riboflavin	0.22 (0.02, 0.47)	0.012	0.157	-10%	
	beta-Alanine metabolism	-0.15 (-0.30, -0.03)	0.009	0.054	0.054	11%	Carnosine	-0.14 (-0.28, -0.03)	0.006	0.090	10%	
	Histidine metabolism	-0.13 (-0.28, -0.02)	0.021	0.063	0.063	10%						
As	beta-Alanine metabolism	-0.19 (-0.41, -0.01)	0.037	0.189	0.189	14%	Carnosine	-0.15 (-0.34, -0.01)	0.039	0.435	11%	
	beta-Alanine metabolism	-1.49 (-2.66, -0.53)	<0.0001	<0.0001	<0.0001	13%	Carnosine	-1.05 (-2.10, -0.24)	0.007	0.090	9%	
	Histidine metabolism	-1.26 (-2.36, -0.38)	0.001	0.003	0.003	11%						
Summary scores	Riboflavin metabolism	0.83 (0.13, 1.79)	0.006	0.012	0.012	-7%	Riboflavin	0.76 (0.09, 1.64)	0.013	0.090	-6%	
	beta-Alanine metabolism	-0.33 (-0.59, -0.11)	<0.0001	<0.0001	<0.0001	9%	Carnosine	-0.26 (-0.50, -0.05)	0.008	0.104	7%	
	Histidine metabolism	-0.28 (-0.52, -0.08)	0.001	0.003	0.003	8%						
WQS index	Riboflavin metabolism	0.24 (0.06, 0.48)	0.002	0.004	0.004	-7%	Riboflavin	0.20 (0.03, 0.42)	0.006	0.104	-6%	

Statistical tests were performed with two-sided tests, with statistical significance set at 0.05. Adjustments for multiple comparisons were accounted for by calculating PFDR. Adjusted covariates included pre-pregnancy BMI, maternal age, parity, folic acid supplementation, maternal education level, infant sex, and cotinine levels in cord blood.
MDI: mental development index, Cr chromium, Mn manganese, Pb lead, Ni nickel, V vanadium, Al aluminum, Rb rubidium, Ga gallium, As arsenic.
^aIndirect effects of the pathway between maternal urinary metals and neurodevelopment of 2-year-old children.
^bIndirect effects of metabolites between maternal urinary metals and neurodevelopment of 2-year-old children.

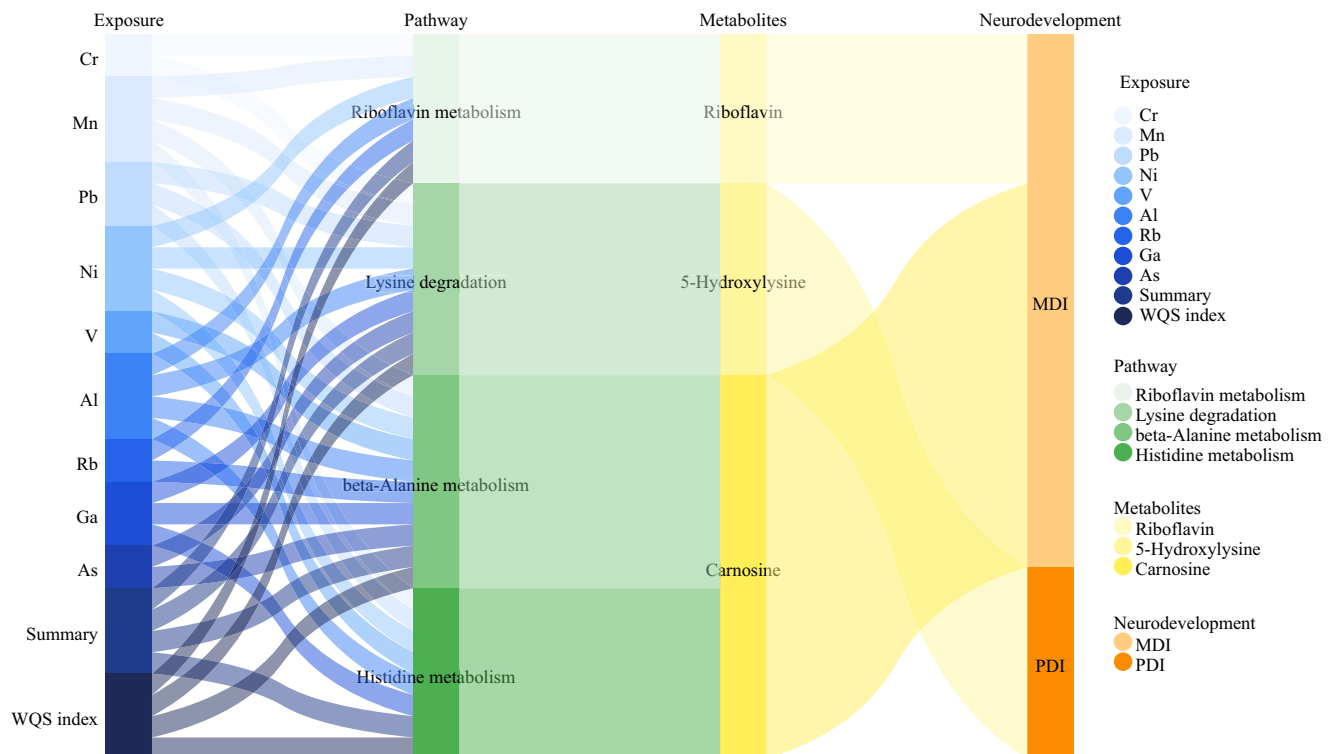


Fig. 5 | Interrelationship among maternal urinary metals, metabolic pathways and metabolites in cord plasma samples, and neurodevelopment of 2-year-old children (N = 1088). A Sankey plot was utilized to examine and visualize the correlation between maternal urinary metal and neurocognitive development of 2-year-old children in the mediation of key cord plasma metabolic pathways and

metabolites. Source data are provided as a Source Data file. MDI mental development index, PDI psychomotor development index, Cr chromium, Mn manganese, Pb lead, Ni nickel, Cd cadmium, Tl thallium, V vanadium, Al aluminum, Rb rubidium, Ga gallium, As arsenic.

and histidine metabolic pathways played intermediate roles in the association between these metals and lower MDI scores. Glutamine, a common metabolite of purine and pyrimidine metabolic pathways, also had significant mediation effects on these associations. Additionally, enhanced riboflavin metabolism may partially mitigate the adverse effects on cognitive development associated with metals.

Compared to the metal concentrations detected in pregnant women worldwide, the median urinary metal concentrations (except V) observed in our study were similar to those pregnant women from other regions in China, such as Nanjing^{29,30} and Taiwan³¹, and were generally comparable to the level reported in Bangladesh³², Mexico³³ and Spain³⁴ (Table S13). However, the median concentrations were approximately 1–2 times higher than those in developed countries such as Greece³⁵, United States^{36–40} and Australia^{41,42}. The median urinary concentration of V was similar to the pregnant women from our birth cohort reported previously⁴³, but was about five times higher than that reported in pregnant women from Nanjing, China²⁹, the United States³⁸, and Australia⁴².

Consistent with previous epidemiological studies, we found that maternal exposure to higher levels of Mn, Ni, Pb, and Al, which are regarded as neurotoxic metals, were linked to lower MDI scores of 2-year-old children, indicating that prenatal exposure to these metals can affect children's cognitive development^{5,44–46}. We also found significant links between maternal exposure to Cr, Tl, Rb, and Ga and lower MDI scores in children, which had been rarely reported. However, we didn't observe significant associations between urinary metals and the categorical outcomes (PDI < 70 or MDI < 70) after multiple corrections. The possible reasons may be that there were not enough cases of neurodevelopmental delay in the study population^{47,48}, and the metal exposure levels observed in the present study could impair neurodevelopment but may not be sufficient to significantly increase the risk of neurodevelopmental delay. Another prospective cohort

study conducted in Anhui, China, which measured metals in maternal serum during pregnancy, also found that higher levels of Tl was associated with poorer cognitive development in children⁴⁹. Nevertheless, it was also worth noting that several researches found no associations between Cr, Tl, and Ga exposure during pregnancy and impaired children's cognitive development^{50–52}. The inconsistent results may be attributable to discrepancies in the study area, study population, sample type, exposure levels, and unmeasured confounding factors. Consistent with the existing epidemiological studies, the impact of prenatal metal exposure on MDI was more pronounced compared to PDI, indicating that the fetal nervous system may be more susceptible to cognitive impairments caused by toxic exposures^{1,53}. This differential impact may be attributable to the disruption of neurotransmitter synthesis, brain cell development, oxidative stress and lipid oxidation caused by metals (such as Mn and Al)^{9,10,54}, which are critical for cognitive functions^{15,55}. Furthermore, a greater number of cord blood metabolites and metabolic pathways were associated with MDI than PDI in our study, further confirming that the impact of metabolic disturbances in utero on cognitive development may be greater than on psychomotor development.

In addition, to explore the cumulative effects of metal exposure on neurodevelopment and verify the possible common metabolic pathways of metal effects on neurodevelopment, we used a summary score to assess the co-exposure of metals⁵⁶, and found the estimate of a decrease in Bayley score associated with the summary score was greater than that of a single metal. The previous study used a summary score to assess the mixture exposure levels of herbicides in adolescents, and also found consistently worse performance on all five neurobehavioral domains assessed⁵⁶, suggesting that exposure to multiple pollutants during pregnancy may exhibit syncretical effects⁵. Furthermore, to minimize the potential for collinearity of the metals, we employed a WQS model and found elevated metal mixture levels

were significantly associated with reduced Bayley scores. Al was identified as the primary neurotoxic biomarker of concern. Similarly, in a previous study that measured prenatal exposure levels of 20 metals in 703 mother-child pairs from Guangxi, China, maternal serum Al was identified as the largest contributor to reduced fine motor and adaptation developmental quotients in children aged 2–3 years⁵. In a prospective birth cohort from Bangladesh, Wei et al.⁵² also observed that among 52 trace elements analyzed, cord serum Al was one of the most significant contributors to decreased cognitive composite scores in children aged 20–40 months. Furthermore, our study identified a greater number of cord blood metabolites and metabolic pathways associated with Al, providing further evidence that Al exposure during pregnancy may lead to a range of metabolic disturbances.

Prenatal exposure to environmental pollutants can disrupt metabolic pathways, rather than just affecting individual metabolites²⁰. Therefore, the metabolic pathway may be more helpful in understanding the potential mechanism mediating the association between maternal exposure levels of metals and children's neurodevelopment⁵⁷. We found that all the mediation effects of pathways were higher than or equal to those mediated solely by metabolites in the association between maternal urinary metals and children's MDI scores. This finding may be attributed to the high correlation between metabolites involved in the same pathway, as well as the similar strength and direction of association between these metabolites and outcomes⁵⁸. Mediator dimension reduction allows all metabolites detected in a pathway to be evaluated as a whole to assess the potential mediation pathway for individual chemical⁵⁷.

In the current research, we observed that the associations between several metals (Mn, Pb, Ni, V, Al, Ga, and the mixed exposure) and impaired cognitive development (decreased MDI scores) in children shared the same two mediating metabolic pathways, including beta-alanine and histidine metabolism, which are two important amino acid metabolic pathways. They play a crucial role in fetal neurodevelopment, particularly in terms of neurotransmitter synthesis, myelin formation, and neuroprotection⁵⁹. Insufficient histidine intake can lower brain histamine levels (an important neurotransmitter) of C57BL/6J male mice, leading to anxiety-like behaviors^{60,61}. Previous case-control studies also showed significant changes in urinary histidine metabolism in children with ASD⁶², with significantly lower levels of urinary beta-alanine and histidine compared to healthy controls^{63,64}. The metabolism of beta-alanine and histidine are also important for the synthesis of carnosine⁶⁵, which serves as the main metabolite mediating the decrease in MDI scores associated with Mn, Pb, V, Al, Rb, Ga, and the mixed metals. Carnosine is a dipeptide that can act as a neuroprotective factor due to its antioxidative properties and chelating ability to divalent metal ions^{65–69}. Carnosine metabolism disorder in childhood has been linked to mental deficiency in children^{70,71}, while carnosine supplementation in childhood may improve communication skills and behavior in children with ASD⁷². Our results indicated that beta-alanine and histidine metabolism as well as carnosine, which were perturbed by maternal exposure to multiple metals during pregnancy, may be an important metabolic molecular mechanism affecting children's cognitive development. This suggests that these pathways and metabolite may be a common target for the wide-ranging effects of environmental factors. Future studies are needed to elucidate the specific mechanisms and explore their potential as a target for the control and prevention of pollutants affecting cognitive development.

In addition, glutamine was observed to act as a co-mediator in the association of multiple metals (including Cr, Mn, Pb, V, Al, Rb, Ga, and mixed exposure) and cognitive developmental damage. Glutamine, as a precursor of neurotransmitters, is directly involved in several critical brain processes, such as energy metabolism, ammonia homeostasis, and neurotransmitter cycling^{73,74}. The results of our study indicated a positive association between glutamine levels and MDI scores.

Furthermore, the findings of several epidemiological studies were in agreement with our results, which demonstrated that plasma glutamine levels in children with ASD were significantly lower than in controls^{75,76}. Glutamine is primarily synthesized and released from astrocytes⁷³. The target metals can transfer across both the placental and blood–brain barriers, and they can accumulate in astrocytes⁷⁷, and impair their homeostatic capabilities, posing a potential threat to fetal neurodevelopment during the most sensitive early stages of life⁷⁸. In vitro cell experiments showed that Mn overexposure could dysregulate astrocytic cycling of glutamine and glutamate by aberrant phosphate-activated glutaminase pathway^{10,79}, and Al could decrease astrocytic glutamate intake⁸⁰, thereby inducing excitotoxicity and secondary neuronal death. Perinatal low-dose Pb exposure in *rats* could cause glutamate and glutamine dyshomeostasis in microglia and astrocytes by downregulating the protein level and activity of glutamine synthase, leading to neuroinflammation and further neuropathological changes⁸¹. For infant neurodevelopment, glutamine dyshomeostasis may play an important role in the mechanisms that underlie the association between metal toxicity and decreased MDI scores in children.

The negative mediation effects of the riboflavin metabolic pathway suggested that Cr, Mn, Ni, Al, and Rb exposure may upregulate the riboflavin metabolic pathway, partially offsetting the effects of metals on reducing MDI scores, which was its mediating role between the mixed metal exposure and impaired cognitive development. Riboflavin metabolism is an important vitamin metabolic pathway, providing important cofactors for energy metabolism, fatty acid oxidation, amino acid and purine metabolism^{82,83}. A cohort study from Japan ($N = 1199$ mother-child pairs) demonstrated that maternal intake of riboflavin during pregnancy may serve as a protective factor against childhood emotional problems in children aged five years⁸⁴. Metals such as Mn, Ni, and Al have been reported to induce oxidative stress in the brain^{9,11,85}, while riboflavin may counteract this stress by enhancing antioxidant enzyme activities and the glutathione redox cycle⁸⁶. Mn has been demonstrated to cause neuronal apoptosis through inducing pro-inflammatory response⁸⁷, but riboflavin has anti-inflammatory effects that can moderate cognitive impairment in *mouse* models of inflammation and Alzheimer's disease⁸⁸. Our observed findings may be attributable to self-regulation mechanisms in individuals, which resemble negative feedback processes occurring before the onset of disease.

Prenatal exposure to metals may reduce MDI and PDI scores by disrupting the metabolism of lysophospholipids. A recent study from Puerto Rico reported associations between metal exposure during pregnancy and maternal lipids ($N = 83$ pregnant women) with significant alterations in lysophospholipids⁸⁹, which was aligned with the findings of the present study. Metals such as Cr, Mn, and Pb, induce oxidative stress through the production of reactive oxygen species, which in turn increase lipid peroxidation of cell membranes^{11,90,91}. Consequently, elevated levels of lysophospholipids, which are products of lipid peroxidation, can contribute to neuronal sheath demyelination and neuronal apoptosis, ultimately impacting brain development^{92,93}. The process of lipid peroxidation has been demonstrated to damage the structure of cell membranes and to reduce the permeability of the blood–brain barrier⁹⁴, which may further exacerbate adverse neurodevelopmental effects. Previous studies have reported elevated levels of lipid peroxidation markers in two-year-old children with ASD and ADHD^{18,95}, and identified increased cord blood lysophospholipid levels as risk factors for ADHD and ASD symptoms⁹⁶. Consistent with these findings, our study also observed that higher levels of lipid peroxidation products were associated with lower Bayley scores. These findings suggest that lipid peroxidation during fetal neurodevelopment may be one of the mechanisms linking prenatal metal exposure and impaired neurodevelopment.

This study has several strengths, including its prospective birth cohort design and comprehensive data collection from pregnancy. The comprehensive metabolomics framework was used to discover the overlapping pathways and metabolites in the association between maternal urinary metals and children's MDI/PDI scores, and allow all metabolites detected in a pathway to be evaluated as a whole to assess the potential mediation pathway. Moreover, our study had a relatively larger sample size in comparison to other investigations on prenatal exposure and metabolomics^{23,89,97}. The present study is subject to several limitations. First, residual confounding may persist in any observational study and may affect the observed associations. Nevertheless, after adjusting for various social and lifestyle variables, our findings remained robust and consistent with previous experimental and human evidence regarding the neurotoxic effects of metals^{1,45}. Second, a single-time measurement of urinary metals may not fully capture true exposure levels during pregnancy. Urinary Cd is a reliable biomarker to reflect body burden over a relatively long period of time⁹⁸, but urinary levels of other metals have moderate to high variability throughout pregnancy⁹⁹, due to the changes in individual physiological characteristics, dietary habits, and environmental exposures. To enhance the reliability of exposure assessments, future studies of repeated measurements of metal concentrations during pregnancy are needed. Third, in this observational exploratory study, the use of looser thresholds for FDR correction in metabolomics analyses may lead to a higher incidence of type I errors, but this was a necessary compromise to balance the likelihood of missed discoveries and the risk of false discoveries^{57,100}. Fourth, although we used a highly sensitive ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) detection method to accurately quantify metabolites reported in the literature as important for fetal growth and development, it cannot fully cover all metabolites¹⁰¹. Complementing the current study with an untargeted metabolomics approach in the future will help to expand metabolite coverage and capture more metabolic pathways that may be relevant to metal neurotoxicity. Fifth, some of the covariates were self-reported, such as annual household income, may be subject to recall bias. Finally, the metabolic pathway of cord blood can serve as a mediator to reveal the mechanism between prenatal metal exposure and neurodevelopmental toxicity in children based on a longitudinal study, and the molecular mechanisms responsible still require further elucidation and validation.

Our study suggests that maternal metal exposure during early pregnancy may affect children's neurodevelopment. Metabolomics analysis indicates that perturbed amino acids, lipids, neurotransmitters, and neuroendocrine metabolism by metal exposure in utero may play important roles in contributing to impaired neurodevelopment in childhood. The upregulation of riboflavin metabolism may help alleviate some adverse impacts on neurodevelopment associated with metals. Further investigation is warranted to validate our findings, explore the underlying metabolic mechanisms, and assess the potential of biomarkers for prediction, therapy, and prevention approaches to mitigate the adverse effects of environmental pollution exposure during pregnancy on neurodevelopmental outcomes in children.

Methods

Study participants

This study was conducted using the Wuhan Health Baby Cohort (WHBC), which was established at the Wuhan Women and Children Medical Care Center in Wuhan, Hubei, China. Details about this cohort have been previously reported⁴³. Pregnant women were recruited if they met the following criteria: (1) Chinese residents of Wuhan city; (2) had a singleton pregnancy and completed a prenatal care examination in early pregnancy (<16 weeks); (3) gave birth at the study hospital; and (4) completed the questionnaires. For this study, 2120 pregnant women who provided urine samples during the first trimester and cord

blood samples were included between March 2014 and March 2016. Subsequently, the newborns with congenital malformations ($N=15$) and fetal metabolic diseases ($N=30$) were excluded. Of the 2075 mother-child pairs, 1100 mothers whose children completed the neurodevelopment assessment at 2 years of age ultimately remained in this study. Participants with missing values for pre-pregnancy body mass index (BMI) ($N=1$) or urinary metal concentration outliers (>five times the standard deviation, $N=11$) were excluded from the study. No statistically significant differences were found in demographic characteristics about the population whether or not they completed the Bayley scales at 2 years of age (Table S14), except that the mothers of children who completed the Bayley scales had a higher pre-pregnancy BMI (20.7 kg/m^2 vs 20.4 kg/m^2 , $P=0.048$). Finally, a total of 1088 mother-child pairs were included in the final analysis. All participants provided informed consent before participating in the study. The research protocol was approved by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology and Wuhan Medical & Healthcare Center for Women and Children.

Neurodevelopment assessment

When the children were around 2 years old (range: 23–26 months), they were invited back to the hospital to evaluate neurodevelopment using the standardized Chinese revision of the Bayley Scales of Infant Development (BSID-CR). BSID is one of the most widely used intelligence scales for infants and young children, and BSID-CR is the Chinese version which has been validated and used in Chinese children to assess cognitive and psychomotor development^{27,28}. Cognition, language and social development are assessed by the mental scale to generate the MDI. Fine and gross motor development is assessed by the psychomotor scale to generate the PDI. Then, MDI and PDI scores are derived by converting the raw scores into standardized scores using age-specific norms. Higher MDI and PDI scores indicate better neurodevelopment. Children with MDI or PDI scores below 70 were classified as moderate to severe cognitive or psychomotor development delay, respectively^{47,48}. All BSID-CR tests were conducted in a quiet room at the hospital by certified psychologists according to standardized guidelines. Video reviews and grading were used for quality control purposes.

Urine collection and metal exposure assessment

During early pregnancy (13.1 ± 1.1 weeks), pregnant women provided spot urine samples in trace element-free containers, which were then stored at -20°C . Urine samples were thawed at room temperature prior to trace metal determination. To prepare the samples for analysis, 2.0 mL of a 1.2% HNO_3 solution was added to a 0.5 mL sample of urine, and the mixture was left to undergo nitrification overnight. The concentrations of Cr, Mn, Pb, Ni, Cd, Tl, V, Al, Rb, Ga, and As were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7900, Agilent Technologies, Santa Clara, CA, USA) in helium mode¹⁰².

To verify the accuracy of the measurements, each batch included the addition of human urine standard reference material as an external quality control sample (SRM2670a, the National Institute of Standards and Technology, Gaithersburg, MD, USA). Each batch included a blank sample containing 1.2% HNO_3 , which was included solely to minimize the potential for contamination. The spike recovery values for urinary metals exhibited a range between 98.81% and 106.44%. The intra-assay coefficients of variation (CV) ranged from 0.29% to 3.22%, while the inter-assay CVs ranged from 0.05% to 7.26%. The limits of detection (LODs) of these metals ranged from 0.001 to $0.106 \mu\text{g/L}$. The concentration of creatinine in the urine samples was quantified using a creatinine kit (Mindray BS-200 CREA Kit, Mindray Bio-medical Electronics Co., Ltd., Shenzhen, China), and the adjusted metal concentrations were ultimately expressed as $\mu\text{g/g}$ creatinine.

Cord blood collection and metabolomic analysis

After the babies were delivered, umbilical cord blood samples were collected in tubes containing ethylenediaminetetraacetic acid (an anticoagulant) by trained nurses. Subsequently, the cord plasma was separated by centrifugation and stored at -80°C in order to facilitate subsequent research. The same sample pretreatment method was used for the analysis of both metabolites and polar lipids¹⁰³. After the plasma was thawed at room temperature, 50 μL of cord plasma and 200 μL of the extract (acetonitrile: methanol = 1:4, v/v), which contained the 61 labeled internal standards (Tables S15 and S16), were added to a 2 mL centrifuge tube. The samples were subjected to a 15-min vortexing process and then centrifuged at a rate of $19,000 \times g$ for 10 min at 4°C . Two hundred μL of the supernatant was collected for analysis. The order for sample preparation and data collection of biological samples were randomized. In this study, cord plasma was collected from 1088 newborns.

We applied the stable isotope dilution internal standard method to quantify cord plasma metabolites using a UPLC-QTRAP-MS system (SCIEX, ExionLC AD-QTRAP 7500) (Table S17). Two different chromatographic columns [Waters ACQUITY UPLC HSS T3 (1.8 μm , 2.1 mm \times 150 mm) and BEH Amide (1.7 μm , 100 mm \times 2.1 mm)] were used in the UPLC-QTRAP-MS analysis. For Waters ACQUITY UPLC HSS T3, the mobile phases A and B were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. The column temperature was 40°C , with a flow rate of 0.4 mL/min. The injected volume was 10 μL . For Waters ACQUITY UPLC BEH Amide, the mobile phases A and B were 0.1% formic acid in water (containing 5 mM ammonium formate) and 0.02% formic acid in acetonitrile, respectively. The column temperature was 40°C , with a flow rate of 0.3 mL/min. The injected volume was 1 μL . The scheduled multiple reaction monitoring (sMRM) mode was used for detection, and positive and negative ionization modes were used simultaneously. The following general MS parameters were utilized: ion spray voltage of +5500/−4500 V, source temperature of 350°C , curtain gas at 40 psi, and ion source gases 1 and 2 at 50 psi each. To optimize the MRM transitions, standard solutions dissolved in a mobile phase (A: B ratio of 1:1 v/v) were injected into the mass spectrometer. Optimized MRM transitions, retention times, chromatographic columns, and the deuterated internal standards of metabolites are shown in Table S17. Data acquisition and processing were performed using the Sciex OS-Q software (Sciex, USA). In every 15 analytical samples, a quality control (QC) sample was added to track the instrumental analysis procedure. The target metabolites (Table S17) selected for detection in this study are those identified in previous literature as critical for fetal growth and development. Alongside the 20 protein amino acids, the assay included some neurotransmitters, tryptophan metabolites, and dipeptides, such as γ -aminobutyric acid, indoleacetic acid, and carnosine, which play important roles in fetal growth, neurodevelopment, immune function, and energy metabolism^{104–108}. After excluding metabolites with poor reproducibility (relative standard deviation of QC > 20%), a total of 45 amino acids and their metabolites were retained. Furthermore, the analysis includes 11 hormones (e.g. thyroxine, cortisol)^{109,110}, 9 carnitines (e.g. acetylcarnitine, palmitoylcarnitine)⁹⁶, 7 bile acids (taurocholic acid, glycocholic acid, etc.)¹¹¹, and 7 vitamins (riboflavin, thiamine)^{112,113}, which are crucial for maintaining fetal hormone balance, energy metabolism, bile acid synthesis, and nutrient supply. Furthermore, five additional metabolites (e.g. betaine) that play a crucial role in fetal neurological and growth development were also considered and analyzed¹¹⁴. All metabolites were detected in over 96% of the cord plasma samples, and concentrations of metabolites below the LOD were imputed using $\text{LOD}/\sqrt{2}$. The coefficients of variation from QC samples ranged from 2 to 20% (Table S17).

We applied the relative quantitative analysis method to quantify cord plasma polar lipids employing a UPLC-QTOF-MS/MS system (SCIEX, ExionLC AD-QTOF X500R). A Phenomenex Kinetex C18 column

(100 mm \times 2.1 mm, 2.6 μm) was used to analyze polar lipids in the UPLC-QTOF-MS/MS system. The column temperature was 55°C , and the flow rate was 0.4 mL/min. Mobile phase A consisted of a mixture of water, methanol, and acetonitrile in a ratio of 1:1:1 (v:v:v) with 5 mM ammonium acetate, while mobile phase B comprised a mixture of isopropanol and acetonitrile in a ratio of 5:1 (v:v) with 5 mM ammonium acetate. Information-dependent acquisition (IDA) was employed for the acquisition of MS/MS data in negative electrospray ionization modes. The following general MS parameters were utilized: a mass range of 50–1200 m/z , ion spray voltage of −4500 V, declustering potential of −80 V, and collision energy at −30 V. Polar lipids identification was carried out using MS-DIAL (version 4.80, <https://systemsomicslab.github.io/comprms/msdial/main.html>). In the identification process, mass tolerances with an accuracy of 0.01 Da for MS and 0.05 Da for MS/MS were employed. The peaks were integrated automatically using Sciex OS software (version 1.6, Sciex), and manual checks and adjustments were made in case of peak misalignments. The K nearest-neighbor method was used to impute missing lipid values. A specific class of lipid molecular species was quantified by normalizing the peak area of individual molecular ions to that of a selected internal standard (Table S16). The CV was calculated for each QC sample across the batches, and metabolites with a CV greater than 30% were excluded. A total of 354 polar lipids were selected and utilized for further analyses.

Covariates

Information on maternal demographic information, including pre-pregnancy weight, age, annual household income, maternal education, and data on folic acid supplementation, was collected by trained nurses through interviews prior to delivery. The pre-pregnancy BMI was calculated by self-reported pre-pregnancy weight and height, which was measured at admission to the hospital. Information regarding parity, newborn sex, birth weight, and fetal metabolic disease was retrieved from medical records. Cord plasma cotinine, a biomarker of tobacco exposure, was analyzed by UPLC-QTRAP-MS as described above for the metabolites, and the optimized MRM transitions, retention times, chromatographic columns, and the deuterated internal standards of cotinine are shown in Table S17.

Statistical analysis

Maternal urinary concentrations of metals below the LODs were imputed with $\text{LOD}/\sqrt{2}$. All urinary concentrations of metals corrected by creatinine were log2-transformed prior to statistical analysis. Furthermore, the correlations between the urinary levels of metals were calculated using Spearman analysis and presented in the form of a heatmap. To demonstrate the calculation of the sample size, the significance level was set at 0.05, the power level at 0.95, and the effect size at 0.15 based on the mean and standard deviation of the estimates reported in the literature^{45,53}. The two-sample *t*-test for power analysis was employed to calculate the sample size using the R software (pwr package), resulting in a sample size of 178. Our sample size ($N = 1088$) is much larger than the required, giving a test power of over 0.99.

We investigated the associations of individual metals in maternal urine and the summary score of metals with children's MDI/PDI scores by fitting linear regressions. Multiple testing was corrected using the Benjamini–Hochberg false discovery rate (FDR) procedure¹¹⁵. Given that metals were log2-transformed, we multiplied the β estimates by $\log(Q3/Q1, 2)$ to determine the change in children's MDI/PDI scores per IQR increase in urinary concentrations, where Q1 and Q3 were the first and third quartile of the metal concentrations, respectively¹¹⁶. We used the multivariable logistic regression model to estimate the associations of individual metals in maternal urine and the summary score of metals with neurodevelopmental delay (PDI < 70 or MDI < 70). Given the lack of statistical significance for the associations with neurodevelopmental delay after multiple corrections (Table S18), subsequent analyses were conducted using the Bayley scores.

To analyze the association between the sum metals and neurodevelopment, the summary score of metal concentrations was calculated by an approach that standardizes and normalizes metal concentrations⁵⁶. The concentrations of each metal were added by one and natural log-transformed. The log-transformed concentrations were divided by the standard deviation of all metals, and then the average was calculated as the summary score. We multiplied the β estimates by the difference between Q3 and Q1 to determine the change in children's MDI/PDI scores per IQR increase in the summary score of metal concentrations. Considering the potential interaction effects between metals, the WQS regression, which can reduce dimensionality and avoid multicollinearity, was conducted to assess the associations between the potentially highly relevant co-exposures and health outcomes¹¹⁷. Briefly, WQS combined the metals into an index by transforming the metals into quartiles and constrained the association to a negative direction, and the result was interpreted as the change in children's MDI/PDI scores associated with one quartile increase in WQS index of the metal mixture. In addition, the weight for each metal expressed as the percentage of that sum to one, was estimated from 1000 bootstraps to identify their contribution to the mixture effect on the outcome.

Based on the biological plausibility and previous studies^{22,23}, a directed acyclic graph was used to determine the covariates (Fig. S3). Finally, the models were adjusted for a set of covariates, including maternal pre-pregnancy BMI (continuous), maternal age at recruitment (continuous), parity (categorical: nulliparous or multiparous), folic acid supplements (categorical: yes or no), infant sex (categorical: male or female), maternal education (categorical: high school or equivalent and below, bachelor's degree and above), and cotinine levels in cord blood (continuous).

We applied a comprehensive metabolomics workflow including a metabolome-wide association study (MWAS), pathway enrichment analysis, and MITM approach, to identify the molecular perturbation¹¹⁸. Specifically, the MWAS was used to explore the associations of cord blood metabolomics with maternal urinary metal concentrations and children's MDI/PDI scores. As the concentrations of metabolites exhibited a right-skewed distribution, a log₂ transformation was employed to normalize the data. The covariates in the MWAS analysis were consistent with the above. The KEGG database for *Homo sapiens* was employed in Metaboanalyst 6.0 (<https://www.metaboanalyst.ca/>) to identify pathways associated with urinary metal concentrations and children's MDI/PDI scores, respectively. Furthermore, we used the MITM approach to discover the overlapping pathways and metabolites.

Next, we used pairwise mediation analysis (R version 4.1.1; “mediation” package⁵⁷) and mediator dimension reduction (R version 4.1.1; “hdmed” and “mediation” package^{57,119}) to explore the mediating effect of overlapping metabolites and pathways, respectively, in the association between maternal urinary metals and children's MDI/PDI scores in order to identify the underlying mechanism (Fig. S4). We hypothesized that the cord plasma metabolites and pathways mediate the relationship between maternal urinary metals and children's MDI/PDI scores. In the analysis of the mediating effect of pathways, the metabolites detected in an overlapping metabolic pathway constitute a mediator group. We employed a Penalized Dimensional Mediation Model to estimate the weights for each metabolite within these mediator groups in a high-dimensional mediation framework. This model facilitated the combination of mediator variables based on their estimated weights, and then the mediating effects of this combined mediating variable in the association between maternal urinary metal exposure and children's MDI/PDI scores were calculated.

Furthermore, in order to ascertain whether adverse perinatal factors or other confounding factors influenced the reliability of the results, the following sensitivity analyses were conducted: (1) excluding children with preterm birth (gestation age <37 weeks) or low birth weight (<2500 g); (2) excluding pregnant women who did not take

folic acid supplements during pregnancy; (3) excluding those with tobacco exposure during pregnancy (cord plasma cotinine concentrations >1.78 ng/mL)^{120,121}; (4) additional adjustment for more covariates (gestation age and birth weight). Sex stratification was also performed to explore the potential sex-specific neurotoxic effect.

All statistical analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA) or R software (version 4.1.1, “pwr”, “gWQS”, “corrplot”, “ggplot2”, “ggforestplot”, “tidyverse”, “ComplexHeatmap”, “circlize”, “hdmed” and “mediation” package). Statistical significance was defined as a two-sided *P*-value less than 0.05. The condition of FDR < 0.2 or larger has previously been employed to evaluate multivariate omics data^{57,100}, with the objective of identifying more meaningful biomarkers. Accordingly, we used an FDR threshold of 0.2 in metabolomic analyses.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. The source data for all figures and tables are included in the Supplementary Information or Source Data file, which accompanies this article. The raw data (e.g., demographic covariates, clinical outcomes, prenatal metal concentrations, and cord blood metabolomics) is available under controlled access to comply with data privacy laws and protect the confidentiality of sensitive participant information. Researchers seeking access may make requests for data by contacting the corresponding author via email (WHBC@zgwhfe.com, april1972@163.com, xus@hainanu.edu.cn or xiawei@hust.edu.cn), or through the official portal of The Wuhan Healthy Baby Cohort (WHBC) study group (https://www.zgwhfe.com/etyy/en/detail_english/12_22_62.html). The request must include a formal proposal outlining the research purpose and methodology. The WHBC team commits to responding within 15 working days. Approved requests require the signing of a Data Use Agreement, which ensures compliance with the legal and regulatory framework of the People's Republic of China, adherence to ethical research practices, and institutional oversight by Wuhan Children's Hospital. Additionally, the agreement stipulates that the data is allocated exclusively for medical and health-related research purposes and prohibits any commercial interests. Source data are provided with this paper.

Code availability

Code is available upon request from the corresponding author, who will respond within 15 business days.

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Author contributions

The study was conceived by Y.X., W.X., and A.Z., who also drafted and revised the manuscript. Y.X., D.Z., G.M., A.Z., and W.X. collected data and samples and measured urinary concentrations of metals and cord blood metabolomic. Y.X., H.X., A.Z., S.X., and W.X. analyzed the data. Co-principal investigators S.X., Y.L., H.X., A.Z., and W.X. conducted research design, quality assurance, and data collecting for the Wuhan Healthy Baby Cohort. Each author reviewed the manuscript, gave insightful comments, and gave their approval.

Competing interests

The authors declare no competing interests.

Additional information

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