Neonatal hair metabolome of birthweight discordant twins is associated with neurobehavioral impairments at 2-3 years of age

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Abstract

Objective: To characterize the metabolic variation in neonatal hair samples associated with intrauterine growth discordance in dichorionic-diamniotic (DCDA) twins and to evaluate the effects of specific metabolic alterations on later neurobehavioural outcomes in infancy. **Design:** Cohort-based case-control study **Setting:** Peking University Third Hospital **Population:** DCDA twins with birth weight discordance(DCDA-D) and birthweight concordance (DCDA-C) within a twin cohort recruited between September 2017 and December 2018 in Beijing, China. **Methods:** A specific hair metabolic profile of 14 pairs of DCDA-D twins was revealed using gas chromatography-mass spectrometry by comparing that of 28 pairs of DCDA-C twins. Pearson's correlation was used to assess the relationship between the neonatal hair metabolome and neurocognitive outcomes, assessed using the Ages and the Infant's Stages Questionnaires, third edition (ASQ-3) at 2 or 3 years of age. **Main outcome measure:** neonatal hair metabolome and long-term neurodevelopment. **Results:** A total of seventeen hair metabolites were significantly different within DCDA-D twin pairs compared to DCDA-C twins. Particularly, reduced levels of cysteine, threonine, and leucine were identified in both the larger and smaller DCDA-D twins compared with DCDA-C twins. The deregulated metabolic pathways including cysteine, methionine, aminoacyl-tRNA, nicotinate, and nicotinamide metabolism biosynthesis pathways in DCDA-D groups were positively correlated with infant neurocognitive development at 2 or 3 years of age, especially in problem-solving domains. **Conclusion:** Neonatal hair metabolic variations in utero of growth discordance in DCDA twins may be associated with poor neurocognitive development. Metabolome profiles of hair may be novel predictors of infant neurodevelopment longitudinally.

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ABSTRACT

Objective : To characterize the metabolic variation in neonatal hair samples associated with intrauterine growth discordance in dichorionic-diamniotic (DCDA) twins and to evaluate the effects of specific metabolic alterations on later neurobehavioural outcomes in infancy.

Design: Cohort-based case-control study

Setting: Peking University Third Hospital

Population: DCDA twins with birth weight discordance(DCDA-D) and birthweight concordance (DCDA-C) within a twin cohort recruited between September 2017 and December 2018 in Beijing, China.

Methods: A specific hair metabolic profile of 14 pairs of DCDA-D twins was revealed using gas chromatography-mass spectrometry by comparing that of 28 pairs of DCDA-C twins. Pearson's correlation was used to assess the relationship between the neonatal hair metabolome and neurocognitive outcomes, assessed using the Ages and the Infant's Stages Questionnaires, third edition (ASQ-3) at 2 or 3 years of age.

Main outcome measure: neonatal hair metabolome and long-term neurodevelopment.

Results: A total of seventeen hair metabolites were significantly different within DCDA-D twin pairs compared to DCDA-C twins. Particularly, reduced levels of cysteine, threonine, and leucine were identified in both the larger and smaller DCDA-D twins compared with DCDA-C twins. The deregulated metabolic pathways including cysteine, methionine, aminoacyl-tRNA, nicotinate, and nicotinamide metabolism biosynthesis pathways in DCDA-D groups were positively correlated with infant neurocognitive development at 2 or 3 years of age, especially in problem-solving domains.

Conclusion: Neonatal hair metabolic variations in utero of growth discordance in DCDA twins may be associated with poor neurocognitive development. Metabolome profiles of hair may be novel predictors of infant neurodevelopment longitudinally.

Keywords: Neonatal hair; dichorionic-diamniotic (DCDA); twins; birth weight (BW) discordance; and neurobehavioral impairment.

Tweetable abstract: Neonatal hair metabolome predicts long-term neurodevelopment of birthweight discordant twins.

1 | INTRODUCTION

Twin pregnancies are associated with a threefold to sevenfold increase in perinatal morbidity and mortality compared to singleton pregnancies,[1] mainly due to higher rates of preterm birth and discordant growth.[2] Intertwin size discordance has been reported to be an independent risk factor for stillbirth and short-term adverse neonatal outcomes especially the smaller twin of birthweight discordance of 20 % or more suffering from a neurodevelopmental disadvantage.[3]

Twin birth weight (BW) discordance is attributable to various genetic or environmental factors containing differences in nutrient supply associated with variation in placental mass, placental insufficiency and umbilical cord insertion. These impact one twin more than the other in a sub-optimal intrauterine environment, which of twins contributed to short- [4] and long-term perinatal outcomes,[5,6] especially the effects on neural development (e.g. induced neurological morbidity).[7]

Several studies have assessed the link between metabolic alterations with BW discordance and abnormal infant neurodevelopment. However, most have focussed on circulating factors in blood– often highly dynamic and not reflective of the cumulative time *in utero* .[8,9] A downregulation of amino acids, including valine, tryptophan, isoleucine, and proline, occurred in the cord blood plasma of selective fetal growth restriction (FGR) in monochorionic-diamniotic (MCDA) twins.[10] Disrupted essential amino acids, such as methionine, phenylalanine and tyrosine in cord plasma were correlated with phenotypic growth discordance of selective FGR.[11] Gut microbial dysbiosis and variation in the faecal metabolome of neonatal twins have also been correlated with long-term neurobehavioural development in selective FGR.[12]

The concentration of endogenous compounds and environmental compounds in hair samples is maintained in an ordered temporal manner as hair grows and therefore the neonatal hair metabolome is considered reflective of the time *in utero* . Maternal exposure in the hair metabolome has been explored to reflect the lower language ability in offspring with 373 infant-mother included.[13]

We previously demonstrated the feasibility of using the neonatal hair metabolome to accurately generate a longitudinal picture of the intrauterine environment over pregnancy.[14] However, to date, no study has attempted to identify specific metabolic differences in the newborn hair metabolome associated with discordant *in utero* growth in dichorionic (DC) twin pregnancies, or explored the link between metabolic variation in hair at birth with later neuro-developmental outcomes.

This study aimed to define hair metabolic perturbations associated with intrauterine growth discordance of DC twin pregnancies and to assess the link between metabolic variation in the hair at birth with infant neurodevelopment at 2-3 years of age.

2 | MATERIALS AND METHODS

2.1 | Experimental Design

This is a cohort-based case-control study, within a twin cohort recruited at the Peking University Third Hospital (Clinicaltrials.gov Identifier: NCT03220750) between September 2017 and December 2018. Chorionicity was established at booking ultrasound prior to 14 weeks' gestation. A total of 201 DCDA twin pregnancies were recruited between 14-28 weeks of gestational age. The exclusion criteria included maternal factors, such as chronic diseases, obstetric complications and delivery complications; fetal factors included

major congenital anomalies, or major fetal structural anomalies, or aneuploidy, other adverse twin pregnancy outcomes, and those lost to follow-up. DC twin neonates were classified into DCDA-C (DCDA twins with birth weight concordance) and DCDA-D (DCDA twins with birth weight discordance) according to consensus-based diagnostic criteria of a within-pair difference in BW exceeding 25%, with one twin <10th centile.[15] The gross examination of placenta was executed carefully by our obstetricians immediately after delivery including discerning the intertwin membrane to confirm chorionicity (determined by antenatal ultrasound), the placental territory and cord insertions attributed to each twin. Maternal and fetal clinical characteristics were measured within 24 hours after delivery.

2.2 | Hair collection and preparation

Hair samples were collected immediately after delivery 0.5 cm proximal from the scalp with processing as per a previously published protocol.[16] All hair samples were stored at -20°C until processing for analysis. Hair samples (3.5mg ± 0.5 mg) were randomized, washed with distilled water and methanol twice. Three internal standards, 20 μL of D4-alanine (Sigma, USA, 10 mM), D5-phenylalanine (Sigma, USA, 10 mM), and D2-tyrosine (Sigma, USA, 10 mM), were added to the hair samples and incubated with 1 ml potassium hydroxide (1 M) at 54 °C for 18 h. Extracts were then neutralized by adding 67 μL sulphuric acid (3 M). To precipitate the salt and protein, 1 ml of methanol was then added, followed by vortexing for 30 seconds and centrifugation at 4000 g for 5 min. The 350 μL of supernatant was concentrated to dryness in a SpeedVac (Labconco, Kansas, USA) at 37 °C for 6 h and stored at - 20degC prior to derivatization. Quality control (QC) samples were also prepared by combining 30 uL of all hair extracts together and following the identical preparation steps to the samples.

2.3 | Gas Chromatography-Mass Spectrometry (GS-MS) data extraction

The dried hair extracts were resuspended in 200 μL of sodium hydroxide (1 M) and chemically derivatized via the methyl chloroformate method based on previous recommendations.[17] All samples were analyzed in a single batch, and derivatized compounds were separated by a GC7890 chromatography system coupled to an MSD5975 with electron impact ionization (70 eV) (Agilent, California, USA). The automated Mass Spectral Deconvolution and Identification System software was utilized for metabolite deconvolution. Subsequently, the metabolite identifications, GS-MS data mining, and data normalization were performed as previously described.[17]

2.4 | Follow-up assessments of physical and neurobehavior development

Infants participated in a follow-up survey 2˜3 years after birth. Telephone interviews with parents were performed to record information on the timing of first walking and speaking. The infant's neurobehavioural development was also assessed through phone interviews using the Questionnaires of Infant Ages and Stages third edition (ASQ-3) encompassed five developmental domains: communication, gross motor, fine motor, problem-solving, and personal social, with a maximum of 60 scores for each domain. Physical evaluation of each twin included height and weight, which were measured based on the WHO's standards, and were conducted by qualified physicians. The height and weight measurements were standardized by two z-scores of height-for-age and weight-for-age according to the WHO Anthro program (V.3.2.2) for children five years of age or younger.

2.5 | Statistical analysis

Student's t-test, non-parametric Mann-Whitney U test, Chi-square test, and Fisher's exact test were performed to investigate maternal clinical characteristics using R programming. The Kruskal-Wallis test and Post hoc comparisons were applied to compare neonatal clinical characteristics between DCDA-C, DCDA-D-L, and DCDA-D-S. The Uniform Manifold Approximation and Projection (UMAP) was used to compare the hair metabolome profiles between three twin groups using uMAP and ggplot2 R-packages. The hair metabolite profiles were adjusted by log transformation and Pareto scaling to ensure the Gaussian distribution of the hair dataset prior to statistical analysis. The adjusted logistic regression models were applied to eliminate the confounding effect of gestation age. Metabolic pathway activities were estimated using

identified metabolites by Metaboanalyst 5.0. Moreover, the metabolic network was *in silico* reconstruction based on KEGG metabolic framework using the Metascape package in Cytoscape (Version 3.9.1). Pearson's correlation was executed to determine the correlation between hair metabolite levels, metabolic pathways, physical development indices, and five developmental domains of ASQ-3. The metabolic heatmaps, pathway activity plots, and circus plots were illustrated by pheatmap, ggplot2, and GOplot R-packages.

3 | RESULTS

We included 42 DC twin pregnancy cases, with the DCDA-D group (14 total pairs) and the DCDA-C group (28 randomly selected pairs matched for maternal age and BMI). The larger BW of each twin pair in the DCDA-D group was referred to as DCDA-D-L, and the smaller BW DCDA-D-S. To evaluate the metabolic perturbations of intrauterine growth discordance of DC twin pregnancies, we performed the following three comparisons: DCDA-D-L and DCDA-D-S twins versus control twins (DCDA-C) respectively as comparison 1 and comparison 2. DCDA-D-S vs DCDA-D-L within pair analysis used for comparison 3 (**Figure 1**).

3.1 | Population characteristics

Demographic details of the study population are described in**Table 1** . Overall, there were no significant differences between these two groups in maternal age, pre-gestational maternal body mass index, weight gain during pregnancy, primigravida, and the method of conception. The gestational age at delivery of DCDA-D pregnancies was significantly earlier (1 week) than DCDA-C pregnancies. The postnatal outcomes of DCDA-C and DCDA-D twins are summarized in **Table 2** . No discrepancies were observed within DCDA-D co-twin pairs (comparison 3) regarding fetal abdominal circumference (AC), fetal head circumference (HC), height, pH in cord blood, and Apgar score at 5 min, whilst significant disparities within co-twins were observed for BW and Apgar scores at 1 min. In addition, the Apgar score at 1 and 5 min, pH in cord blood, and the indicators of fetal development, including AC, HC, height, and birthweight, were profoundly discordant for comparisons 1 and 2.

3.2 | Analysis of hair metabolome profiles in DCDA-C and DCDA-D twins

Uniform Manifold Approximation and Projection (UMAP) of our hair samples demonstrated distinct global metabolomic separation of DCDA-C, DCDA-D-S, and DCDA-D-L twins (**Figure 2A**). DCDA-D-S and DCDA-D-L co-twins more resembled each other, while DCDA-C twins were separated from the larger and smaller DCDA-D twins. In total, nine significant metabolites discriminated DCDA-D-L twins from DCDA-C twins by logistic regression with a p-value less than 0.05 (**Figure 2B,** comparison 1). This included lower levels of 10,13-dimethyltetradecanoic acid, nicotinamide, three amino acids, and two organic compounds. 17 significant hair metabolites contributed to the separation of the DCDA-D-S and DCDA-C twins (**Figure 2B** , comparison 2). This encompassed three amino acids and one amino acid derivative at lower levels in DCDA-D-S twins than in DCDA-C twins, while tryptophan, isoleucine, and most of the organic compounds identified in comparison 2 were found at higher levels in DCDA-D-S twins. A single metabolite, cis-aconitic acid, showed higher levels in DCDA-D-L relative to DCDA-D-S (**Figure 2B** , comparison 3). To find the shared metabolic changes in both larger and smaller DCDA-D co-twins compared to DCDA-C twins, four common differential metabolites in both comparisons 1 and 2 were shortlisted including cysteine, l-leucine, 2-aminobutyric acid, and threonine (**Figure 2C**). Noticeably, the lower concentration of cysteine, threonine, and leucine was detected in both DCDA-D-S and DCDA-D-L groups compared to the DCDA-C group. Meanwhile, 2-aminobutyric acid was the only shortlisted metabolite that displayed the lowest concentration in DCDA-C groups compared to both DCDA-D groups.

3.3 | Pathway enrichment analysis for variable metabolites in DCDA-C and DCDA-D twins.

The majority of metabolic pathways in amino acid, translation, and cofactor/vitamin metabolism were down-regulated in both comparison 1 (DCDA-D-L/DCDA-C) and comparison 2 (DCDA-D-S/DCDA-C) (**Figure 3A**). This included cysteine and methionine metabolism, glutathione metabolism, glycine, serine, and threonine metabolism, taurine and hypotaurine metabolism; one translation pathway was aminoacyl-tRNA biosynthesis; and three cofactor/vitamin metabolism included nicotinate and nicotinamide metabolism, pan-

tothenate and CoA biosynthesis and thiamine metabolism. Intriguingly, two pathways associated with carbohydrate metabolism showed specific downregulation in DCDA-D-S twins compared to the DCDA-C twins (**Figure 3A** , comparison 2). No significant metabolic pathway change was detected in comparison 3 (DCDA-D-S/DCDA-D-L). The shortlisted pathways were linked to their shared metabolites and reconstructed into an *in silico* metabolic network (**Figure 3B**). Cysteine, leucine, and threonine were directly linked to the aminoacyl-tRNA-related metabolites including cysteinyl-tRNA, leucyl-tRNA, and threonine tRNA respectively. The network showed that cysteine was converted into antioxidant glutathione through gamma-glutamyl-L-cysteine. Nicotinamide could lead to the production of coenzymes such as $NADP^+$ and NAD^+. Moreover, the network illustrated that aconitate hydratase catalyzes the isomerization of citrate to isocitrate through cis-aconitate in the TCA cycle (**Figure 3B**). Notably, cysteine was the most interconnected metabolite that participated in seven significant pathways of glutathione metabolism, cofactors and vitamin metabolism, and four different amino acid metabolisms (**Figure 3C**).

3.4 | Physical and neurocognitive development of DCDA-C and DCDA-D infants at 2-3 years of age

Both height and weight of infants in the DCDA-D-L and DCDA-D-S groups were significantly lower than those in the DCDA-C group (**Figure 4A**). This also suggests that the larger twin within DCDA-D pairs is also growth restricted to some degree compared to DCDA-C twins. However, there were no significant dissimilarity in the first-speaking time and the first-walking time among the three groups. The ASQ-3 subscale consists of five developmental domains: communication, fine motor, gross motor, problem-solving, and personal social, with 60 scores potentially as a maximum value. We equally subdivided the 60 scores into four intervals for each domain of the ASQ-3 and assessed infants' neurocognitive development by comparing the low scores (30 scores or less) in each domain between different groups (**Figure 4B**). Both the DCDA-D-L and DCDA-D-S groups showed higher frequencies of low scores (<30 scores) in the fine motor, problem-solving domain, and personal-social domain than the DCDA-C group. Remarkably, the DCDA-D-S group showed much higher frequencies of lower scores (<15 scores) in the fine motor, and personal-social domains than DCDA-D-L and DCDA-C groups (**Figure 4B**).

3.5 | Possible effects of hair metabolic changes associated with DCDA-D twins on physical and neurocognitive development later in life

To further detect the altered metabolic activities in early life associated with poor later physical and neurocognitive development, we performed a correlation analysis between the predicted metabolic activity in the hair of DCDA-D twins and evaluation indices of physical and neurocognitive development in infants 2–3 years postpartum. There was no significant correlation between hair metabolic changes and physical development in infant 2-3 years (**Figure 4C**). Fortunately, we found significantly positive correlations between the problem-solving domain and five metabolic pathways including cysteine and methionine metabolism, aminoacyl-tRNA biosynthesis, glutathione metabolism, nicotinate and nicotinamide metabolism, and pantothenate and CoA biosynthesis in DCDA-D twins ($p<0.05$) (**Figure 4C**). Similarly, upregulated aminoacyl-tRNA biosynthesis also was associated with better fine motor and communication outcomes. Unexpectedly, no significant correlation was observed between the personal-social domain and altered metabolic pathways in our DCDA-D twins (**Figure 4C**). In DCDA-D-S group, the correlation of problem solving and aminoacyl-tRNA biosynthesis, nicotinate and nicotinamide metabolism, and pantothenate and CoA biosynthesis was $r > 0.6$ ($p < 0.05$) (**Figure 4D**).

4 | DISCUSSION

4.1 | Main findings

We identified neonatal hair metabolic variations associated with growth discordance in DCDA twins and related these to longer-term neurobehavioural outcomes. Significantly downregulated levels of cysteine, threonine, and leucine were identified in DCDA-D (both larger and smaller) co-twins relative to DCDA-C twins. In addition, a higher level of cis-aconitic acid was observed in the DCDA-D smaller twins relative to their larger co-twin. Three downregulated metabolic pathways (cysteine and methionine, aminoacyl-tRNA,

nicotinate and nicotinamide metabolism) were correlated with neurocognitive outcomes at 2-3 years of age **(Supplemental figure S1)** .

4.2 | Strengths and limitations

The main strengths of the study are, first, to reveal altered neonatal hair metabolome in response to early intrauterine environment dysbiosis of DCDA twins with growth discordance. Secondly,

we evidenced that hair metabolic variations in utero may be associated with poor neurocognitive development of growth discordance in DCDA twins.

Our findings are subject to the following limitations. Only 14 DCDA-D twin pairs were included in this study because BW discrepancy increases the risk of neonatal complications and infant mortality, making it challenging to follow up both co-twins at 2˜3 years old. A longer follow-up study of larger sample size should be performed to evaluate the metabolic effects of the intrauterine environment reflected by using multiple various biospecimens on the later neurobehavioral development.

4.3 | Interpretation

The association of antioxidant-related metabolites with infant neurocognition at two-year-olds

Our study suggests that the downregulation of antioxidants associated metabolic pathways, including cysteine, methionine and glutathione metabolism are correlated with the impaired infant neurodevelopment in the problem-solving domain (**Figure 4C**). Cysteine is a vital sulfur-containing amino acid that acts as a precursor for the biosynthesis of glutathione and acetyl-CoA.[18] A cysteine-hub metabolic network has been reported to regulate oxidative stress, energy metabolism, and cellular autophagy.[19]Accumulating evidence has indicated that lower cysteine levels may trigger vascular endothelium damage in maternal-placenta-fetal circulation through oxidative stress in FGR.[10,20-23]Our previous publications showed that the metabolic profiles of the umbilical cord, neonatal hair, and meconium were consistently characterized by reduced methionine and cysteine levels in twins with selective FGR.[11,12,14] Emerging studies suggest that H_2S, produced from L-cysteine by cystathionine-β-synthase within the brain, reduces oxidative stress-induced injury and protects against cognitive dysfunction resulting from neuroinflammation.[24] L-cysteine administration significantly suppresses hypoxia-ischemia (HI)-induced neuroinflammation in neonatal mice by releasing H_2S.[25] In addition, low levels of methionine and cysteine may interfere with one-carbon metabolism and inhibit DNA synthesis and methylation, which may subsequently impede embryonic and fetal growth.[26]

Glutathione (GSH), comprising glutamic acid, cysteine, and glycine, is one of the most important antioxidants in the central nervous system.[27,28] Authors suggested that the enhanced GSH to limit oxidative stress could ameliorate neuronal injury and improve motor and cognitive function in mice.[29] Neurons are particularly vulnerable to oxidative stress, given that neuronal survival is dependent on the glutathione redox potential.[30] Animal models of FGR have shown oxidative stress and mitochondrial dysfunction in the brain[31]with decreased concentrations of GSH in various cerebral cortex regions, including the temporoparietal, frontal, and occipital lobes after 90 minutes of hypoxia.[32] This suggested that the cortex may be more vulnerable to injury due to oxidative stress than other regions and this may have long-term neurodevelopmental implications.

As the product of cysteine, acetyl CoA is the substrate of the TCA cycle and lower levels are related to the imbalance of redox homeostasis in mitochondria. Mews *et al* found that acetyl coenzyme A synthetase 2 (ACSS2), an enzyme responsible for producing acetyl coenzyme A, can directly regulate histone acetylation (an epigenetic mark) in mammalian neurons and the expression of genes related to cognition and memory, thus affecting spatial memory.[33] Recent study demonstrated that ACSS2 is a critical regulator of fear-memory formation in mice and in rats.[34] Based on these findings, we speculated that intrauterine cysteine levels and related metabolic pathways identified here may play a role in the neurocognitive development of DCDA-D twins.

Aminoacyl-tRNA biosynthesis and neurodevelopment in DCDA-D twins

Lower cysteine, threonine, leucine, and alanyl-proline, involved in aminoacyl-tRNA biosynthesis, were associated with worse communication, fine motor, and problem-solving in both larger and smaller twins of DCDA-D pairs. Aminoacyl-tRNAs are translation substrates and pivotal in interpreting how genetic nucleotides are translated into amino acids. This is mainly achieved by the direct attachment of an amino acid to the corresponding tRNA by a specific aminoacyl-tRNA synthetase (ARS).[35] An increasing number of studies have linked pathogenic variation in ARS with neurological diseases.[36-39] Children with ARS deficiencies were observed with central nervous system symptoms, intrauterine growth restriction, and failure to thrive. These adverse outcomes of ARS deficiency could have resulted from reduced aminoacylation activity, translational inefficiency, and compromised proliferation.[40] Furthermore, several studies have reported that ARS senses amino acids for mTOR signaling, which plays a critical role in neural development. The PI3K-Akt-mTOR pathway is essential for neurogenesis from neural stem cells and subsequent migration and maturation.[41,42] Liang *et al* . showed the inhibition of the Akt-mTOR signaling pathway, disrupting neurogenesis and inducing autophagy.[43] Thus, we speculate that the downregulation of amino acids, such as cysteine, threonine, and leucine associated with discordant growth *in utero*may be an indicator of dysregulation of aminoacyl-tRNA biosynthesis, which participates in modulating decreased translation and consequently decreased protein production at a crucial time during brain development.

Downregulation of nicotinate and nicotinamide metabolism and neurodevelopment in DCDA-D twins

Nicotinate and nicotinamide are precursors of the coenzyme nicotinamide-adenine dinucleotide (NAD^+) and nicotinamide-adenine dinucleotide phosphate ($NADP^+$). Our study demonstrated that a lower hair concentration of nicotinamide was identified in DCDA-D-L compared to DCDA-C twin (**Figure 2B**). In addition, the downregulation of nicotinate and nicotinamide metabolism was detected in both DCDA-D-L and DCDA-D-S twins compared to DCDA-C twins (**Figure 3A**). Consistently, we also observed that the activity of nicotinate and nicotinamide metabolism was positively correlated with gross motor and problem solving in both smaller and larger DCDA-D twins (**Figure 4C**). It has been reported myelinization and effective synaptic connections in the third trimester are key factors in the formation of coordinated motor development in children.[44] Souza *et al.* reported that maternal vitamin B3 (nicotinamide) intake in pregnancy contributes to increased BW.[45] A previous study showed that dietary nicotinamide prolonged pregnancies and prevented FGR in mice with preeclampsia.[46] Moreover, recent literature identified maternal nicotinamide riboside enhanced offspring development and neurogenesis.[47] Nicotinamide has been identified to be associated with neuron differentiation by reducing the proliferation of neural progenitors and accelerating neuronal maturation, neurite outgrowth, and neurotransmitter expression.[48,49] We speculate that nicotinamide has a protective effect on fetal neurodevelopment during gestation, especially aiding the differentiation and maturation of nerve cells involved in motor and neurocognitive development.

5 | CONCLUSIONS

This study is the first to investigate the association between neonatal hair metabolome and infant neurobehavior development at 2-3 years of age. Altered metabolic pathways including cysteine and methionine, aminoacyl-tRNA, nicotinate, and nicotinamide metabolism may reflect excessive oxidative stress, reduced protein production, and attenuated nervous system development during the critical window of intrauterine brain development. Metabolome profiles of neonatal hair may be of value in understanding the underlying pathophysiology of neurodevelopmental outcomes and novel predictors of infant neurodevelopment longitudinally.

AUTHOR CONTRIBUTIONS

YZ, TH, and YW conceived this study. JY, NH, ZY, JY, ZS, ZX and ZW recruited participants and collected samples. XL and YY performed the metabolomics analysis and analyzed data under the guidance of TH. JY, XL, RS, JMC, YW, TH and YZ reviewed and edited the manuscript. All authors provided critical intellectual content and approved the final manuscript.

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CONFLICT OF INTERESTS

None declared. Completed disclosure of interest forms are available to view online as supporting information.

ETHICS APPROVAL

This research was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of Peking University Third Hospital.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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