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Prenatal exposure to $PM_{2.5}$ specific constituents and child neurodevelopmental delay: The role of maternal metabolites and mediterranean diet

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ABSTRACT

Prenatal exposure to fine particulate matter ($PM_{2.5}$) constituents has been associated with impaired neurodevelopment, yet constituent-specific perturbations in maternal metabolism during pregnancy and their associations with child neurodevelopmental outcomes remain unclear. Among 6661 mother–child pairs in the Maternal and Infant Health Cohort in Hefei (MIH-Hefei), we estimated associations between prenatal exposure to major $PM_{2.5}$ constituents and clinically diagnosed neurodevelopmental delay (NDD) using generalized linear regression and multi-pollutant mixture models. Maternal metabolomic profiles were analyzed to identify pathways associated with each constituent and with NDD, and a meet-in-the-middle (MITM) framework was used to detect overlapping pathways linking exposure and outcome. Effect modification by maternal Mediterranean diet (Med) adherence was tested via interaction terms. The results indicated that higher prenatal exposure to several $PM_{2.5}$ constituents was associated with increased NDD risk (ORs (95% CIs) for Q4 vs Q1 were: sulfate (SO_4^{2-}), nitrate (NO_3^-), ammonium (NH_4^+), organic matter (OM), and black carbon (BC): 1.376 [1.026, 1.844], 1.650 [1.204, 2.261], 1.431[1.064,1.923], 1.583 [1.166, 2.148], 1.772 [1.314, 2.389]), and mixture models showed consistent positive associations. Metabolomics and MITM have revealed potential links between the constituent-pathway-outcome axis. Among $PM_{2.5}$ constituents, OM and SO_4^{2-} showed the strongest associations with perturbations in four convergent pathways (including glycine, serine, and threonine metabolism and nutrient/energy metabolism pathways). Greater Med adherence modestly attenuated these adverse associations. Overall, specific $PM_{2.5}$ constituents may increase NDD risk partly through maternal metabolic dysregulation and Mediterranean-style dietary patterns during pregnancy may offer limited mitigation.

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1. Introduction

Approximately 10% of children worldwide experience neurodevelopmental delay (NDD), which can have broad and lasting consequences for learning, social functioning, and activities of daily living (WHO, 2023). Of particular concern, the prevalence of childhood NDD continues to increase globally (Jia et al., 2025; Maenner et al., 2023). NDD is a multifactorial condition shaped by genetic susceptibility and modifiable influences, including environmental exposures and maternal nutrition (Cloutier et al., 2025; Horner et al., 2025; Wang et al., 2022). These modifiable influences may be particularly consequential during pregnancy, a critical window for brain development (Rice and Barone, 2000), when maternal external exposures and internal physiological states may shape offspring neurodevelopment (Jing et al., 2025; Kong et al., 2025).

Fine particulate matter ($PM_{2.5}$) is a recognized air pollutant associated with a variety of adverse health outcomes, and growing evidence suggests that its toxicity depends not only on particle mass but also on its chemical composition (Burnett et al., 2018). In the Shanghai birth cohort, $PM_{2.5}$ constituents were inversely associated with developmental abilities across multiple domains assessed by the Ages and Stages Questionnaire (Lei et al., 2022), and additional evidence suggests that fetal metabolites may partially mediate these associations (Lu et al., 2024). Previous studies have shown that the active ingredients in $PM_{2.5}$, after inhalation and deposition, can trigger oxidative stress and inflammatory responses, thereby affecting mitochondrial activity, lipid metabolism, and other metabolic processes (Guo et al., 2017; Kurlawala et al., 2023). Pregnancy is a metabolically adaptive state in which maternal glucose, lipid, and amino-acid metabolism are dynamically regulated to meet fetal growth demands (Bowman et al., 2021). In this context, $PM_{2.5}$-related systemic responses may interfere with pregnancy-specific metabolic adaptations and alter the maternal metabolome and intrauterine milieu, including placental signaling, vascular function, and nutrient transport (Rosenfeld, 2021; Yan et al., 2019). Maternal metabolic perturbation may therefore represent an upstream biological interface linking constituent-specific $PM_{2.5}$ exposure to fetal metabolic programming and neurodevelopmental vulnerability.

However, some important gaps remain. First, most previous studies have assessed child neurodevelopment using screening scales, with limited evidence on clinically diagnosed neurodevelopmental delay. Second, existing metabolomic evidence has mainly relied on fetal or cord blood signals, which reflect downstream responses and do not adequately capture pregnancy-specific maternal metabolic alterations that may represent earlier, potentially modifiable biological changes. Third, constituent-specific exposure, maternal metabolomic perturbation, and neurodevelopmental outcomes have rarely been evaluated within a single analytical framework, and preventive strategies during pregnancy have not been evaluated.

Maternal metabolism is influenced by both environmental factors and nutrient intake. As a predominantly plant-forward dietary pattern, the Mediterranean (Med) dietary pattern provides high-quality protein and carbohydrates needed for fetal neurodevelopment (Vassilopoulou et al., 2025) and may help maintain a favorable metabolic milieu during pregnancy (Tosti et al., 2018). Recent studies in pregnant populations have shown that adherence to a Med dietary pattern is associated with distinct maternal metabolomic signatures, particularly involving lipid- and amino acid-related metabolites (Che et al., 2024; Chen et al., 2023), and can affect antioxidant defense, inflammation regulation, and energy homeostasis (Schwingshackl et al., 2020). Moreover, our previous work suggests that diet may modify the potentially harmful effects of environmental exposures (Zu et al., 2024). Nevertheless, whether the Med diet modifies the association between $PM_{2.5}$ constituents and offspring neurodevelopment through maternal metabolic environment regulation remains unclear.

Accordingly, this study was designed with the following objectives. First, to evaluate the associations of prenatal exposure to major $PM_{2.5}$ constituents with clinically diagnosed NDD in offspring. Second, to characterize maternal metabolomic pathways associated with each major $PM_{2.5}$ constituent during pregnancy. Third, to identify overlapping pathways jointly associated with constituent exposure and NDD, thereby highlighting candidate biological pathways linking exposure to outcome. Fourth, to assess the potential effect-modifying role of adherence to the Med dietary pattern in this association.

2. Methods

2.1. Study design

Our study was nested within the Maternal and Infant Health Cohort in Hefei (MIH-Hefei), China. Details of data collection and participant recruitment have been reported previously (Liu et al., 2021; Ma et al., 2024). Eligible participants were required to meet the following criteria: age 18–45 years, gestational age between 16 and 23 weeks at recruitment, lived in Hefei for at least two years, and a naturally conceived singleton pregnancy.

This study aimed to assess the association between maternal exposure to $PM_{2.5}$-specific constituents during pregnancy and neurodevelopmental delay in children. Based on the study objectives, we applied exclusion criteria during three phases: pregnancy, delivery, and a 36-month postpartum follow-up period. These criteria primarily included excluding pregnant women if they lacked detailed residential addresses, had unavailable pregnancy outcome data, or had incomplete neurodevelopmental assessments of their offspring. After applying these criteria, 6661 complete mother-child pairs were ultimately included for association analysis. The recruitment and screening process for mother-child pairs is detailed in Figure S1.

2.2. $PM_{2.5}$ and its specific constituents exposure assessment

Prenatal exposure to ambient $PM_{2.5}$ and its specific constituents was assessed in two stages. First, maternal residential addresses were collected during face-to-face interviews using a standardized questionnaire that also obtained sociodemographic information. Each reported address was then geocoded to latitude and longitude using the Amap (Gaode) mapping service (https://www.amap.com/). Second, individual exposure concentrations were estimated by linking these coordinates to the ChinaHighAirPollutant (CHAP) dataset, which provides daily concentrations of $PM_{2.5}$ and constituent concentrations at 1 km resolution across China from 2015 to 2021 (https://weijing-rs.github.io/product.html). This dataset integrated routine ground monitoring observations, satellite aerosol retrievals, atmospheric reanalysis and model simulations, emission inventories, meteorological variables, and land surface covariates within machine learning or deep learning frameworks to generate spatially continuous daily exposure estimates. More details can be found elsewhere (Jing et al., 2021; Wei et al., 2023a). The $PM_{2.5}$ specific constituents considered in this study included sulfate (SO_4^{2-}), nitrate (NO_3^-), ammonium (NH_4^+), organic matter (OM), black carbon (BC), and chloride (Cl^-) (Wei et al., 2023a, 2023b).

Gestational age was determined based on the first day of the last menstrual period and verified using ultrasound. Pregnancy was categorized into three phases: weeks 1–13, 14–27, and 28 until delivery, representing the first, second, and third trimesters, respectively. Daily concentrations of individual $PM_{2.5}$ constituents were assigned to each participant based on geocoded residential addresses. For each exposure window (full pregnancy and three trimesters), the average concentration of each constituent was calculated as a gestational day-weighted mean. Specifically, the mean was derived from all valid daily exposure records within the corresponding window, with each gestational day equally weighted and calculated according to each participant's actual days of exposure.

2.3. Neurodevelopmental delay in children

Information on neurodevelopmental status was obtained from the Hefei child health care registry. Children were screened at 5–7, 11–13, and 24–36 months of age using the Denver Prescreening Developmental Questionnaire (DPDQ), a parent-completed tool derived from the Denver Developmental Screening Test and validated for children aged 0–6 years. Those suspected of having developmental problems were further evaluated by trained pediatricians using the Gesell Developmental Schedules (GDS). Based on the child's performance in each test domain, the examiner determined a developmental age, and a Developmental Quotient (DQ) was then calculated as DQ = developmental age/chronological age × 100. DQ ≤ 85 was classified as developmental delay. In this study, children diagnosed with developmental delay at any one of the three follow-up assessments were defined as having neurodevelopmental delay.

2.4. Plasma metabolomics data acquisition

Peripheral venous blood samples were collected from pregnant women in late pregnancy (32–34 gestational weeks). Plasma was separated from whole blood within the standard time window, aliquoted, and stored at −80 °C until extraction and analysis. In total, 1000 plasma samples were randomly selected for metabolomic profiling; because of missing or insufficient specimens, 219 samples were not analyzed, and untargeted metabolomics data were ultimately available for 781 plasma samples.

For sample preparation, 100 μL of plasma was mixed with four volumes of extraction solvent (acetonitrile: methanol = 1:1, v/v). Samples were vortexed for 30 s, sonicated in an ice–water bath for 10 min, incubated at −40 °C for one hour, and then centrifuged at 12,000 rpm for 15 min at 4 °C. Transfer 100 μL of the resulting supernatant to a clean vial for analysis. Subsequently, untargeted plasma metabolomic profiles were acquired using a Vanquish high-performance liquid chromatography system (Thermo Fisher Scientific). Finally, the raw data were converted to the mzXML format using ProteoWizard and processed with an in-house program, which was developed using R and based on XCMS, for peak detection, extraction, alignment, and integration. The R package and the BiotreeDB (V3.0) were applied in metabolite identification. Relevant details are presented in the Supplementary Methods.

2.5. Assessment of dietary pattern score

Dietary intake was assessed at mid-pregnancy (16–23 gestational weeks) using a semi-quantitative food frequency questionnaire (FFQ) covering the previous month. Participants reported their usual consumption frequency for each food item. Frequency categories (never/rarely, 1–2 times/week, 3–6 times/week, >1 time/day) were converted to 0, 0.2, 0.6, and 1 times/day, respectively. Portion sizes were recorded using natural units (e.g., one tomato) or standard weight/volume measures.

A Mediterranean diet adherence score was then constructed according to a modified method proposed by Bédard et al. (Dai et al., 2022). From the 39 food groups predefined in this research, we identified those aligning with the Mediterranean diet and grouped them into six favorable categories (vegetables; fruits and nuts; legumes; fish; cereals; milk and dairy products) and one unfavorable category (meat). Each participant's weekly consumption for these groups was determined and measured against the population median. For the favorable groups, a score of 1 was given for intake above the median and 0 for intake at or below the median, while the scoring for meat was inverted. The sum of these seven group scores yielded a total Mediterranean Diet (MD) score from 0 to 7, where a higher value reflected stronger adherence. For further analysis, MD scores were classified as either low (0–3) or high (4–7).

2.6. Covariates

Potential confounders associated with $PM_{2.5}$ constituents and NDD in children were identified using a directed acyclic graph (DAG) to illustrate these associations (Fig. S2). In the multivariable models, we accounted for a range of potential confounders, including maternal sociodemographic, pregnancy-related, birth outcome, and postnatal characteristics. Maternal sociodemographic factors comprised age (continuous), pre-pregnancy BMI [continuous, calculated as weight (kg)/height (m)^2], educational level (junior high school, high school, or bachelor's degree and above), occupation (unemployed, manual labor, or non-manual labor), and monthly household income (<8000 vs ≥8000 CNY). In addition, lifestyle was considered by including the frequency of moderate physical activity in the past three months (<3 vs ≥3 days/week), assessed using the International Physical Activity Questionnaire; moderate physical activity was defined as engaging in activities such as badminton, or brisk walking for at least 30 min per day. To further reduce confounding, we also adjusted for key pregnancy-related factors that may influence child neurodevelopment, including parity (<3 vs ≥3 children), passive smoking during pregnancy (ever vs never), folic acid supplementation (yes/no), gestational diabetes mellitus (yes/no), and vaginitis (yes/no). Birth outcomes were obtained from medical records, including child sex, birth weight (g), and gestational age at birth (weeks; continuous).

To further account for postnatal influences on infant neurodevelopment, additional variables were collected from follow-up questionnaires administered at 6 and 12 months postpartum. These included maternal postpartum depression (yes/no), duration of exclusive breastfeeding (months; continuous), feeding difficulties (yes/no), infant sleep problems (yes/no), infant fever ≥ 38.5 °C (yes/no), and whether parents were the primary caregivers (yes/no). Feeding difficulty at 6 months was assessed using a 23-item parental feeding questionnaire. Consistent with prior work, a total score > 34 (above the 75th percentile) was used to define feeding difficulty. Infant sleep problems at 6 months were determined using the Brief Infant Sleep Questionnaire (BISQ). Additional methodological details for these instruments and classifications have been reported in our previous research (Ma et al., 2024).

2.7. Statistical analysis

2.7.1. Data preprocessing and descriptive analysis

We conducted descriptive analyses of maternal sociodemographic, pregnancy, and postpartum characteristics. These variables were compared between children with and without neurodevelopmental delay using Student's t-test for continuous variables and the χ^2 test for categorical variables. For exposure assessment, the distributions of $PM_{2.5}$-specific constituents' levels during each trimester and across the entire pregnancy were summarized using the median and interquartile range [median (IQR)]. In addition, Spearman correlation coefficients were calculated to evaluate the pairwise correlations of $PM_{2.5}$ constitutes exposures across trimesters and for the whole pregnancy. For metabolomics data, we first quantified the proportion of missing values for each metabolite and excluded those with > 50% missingness. For metabolites with ≤ 50% missingness, we impute missing values by multiplying the minimum value by a random number between 0.1 and 0.5 (Zhao et al., 2024). In subsequent correlation analysis, $PM_{2.5}$ constitutes during each trimester and the entire pregnancy, and all metabolites after imputation were standardized.

2.7.2. Estimation of the associations between $PM_{2.5}$ specific constituents and NDD

To evaluate the association between prenatal exposure to $PM_{2.5}$ constituents and neurodevelopmental delay in children, we applied three complementary modeling approaches. First, we constructed multivariable logistic regression models to estimate odds ratios (ORs)

and 95% confidence intervals (CIs) for NDD per quartile increase in the average $PM_{2.5}$ constituents' concentrations over the entire pregnancy. Exposures were categorized into quartiles, which were entered into the models as ordinal variables to estimate the change in risk associated with each quartile increase. These models were adjusted for a set of potential confounders, including maternal sociodemographic characteristics (age, education, occupation, and household income), pregnancy-related factors (parity, passive smoking during pregnancy, and folic acid supplementation), birth outcome factors (infant sex) and postpartum characteristics (poor sleeping in infants, exclusive breastfeeding, infant feeding difficulties, fever in infants, parents as primary caregivers and postpartum depression). Second, to explore critical exposure windows, we fitted multiple logistic regression models to estimate the association of each $PM_{2.5}$ constituents (per quartile increase) during the first, second, and third trimesters with the risk of neurodevelopmental delay. The same set of covariates was included in all trimester-specific models. Third, given the high correlations among $PM_{2.5}$ constituents, we employed mixture modeling approaches to assess joint effects while reducing dimensionality and mitigating multicollinearity. Weighted quantile sum (WQS) regression was used to estimate the overall effect of the $PM_{2.5}$ constituents' mixture on neurodevelopmental delay and to derive weights reflecting the relative contribution of each constituent to the mixture effect. WQS regression was implemented using the gWQS package in R. For each gestational window, the 6 $PM_{2.5}$ constituents were categorized into quartiles ($q = 4$). The dataset was randomly split into a training set (40%) and a validation set (60%). Constituent weights, constrained to sum to one, were estimated using 1000 bootstrap samples, and the direction of the WQS index was prespecified as positive. Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were calculated to evaluate model fit.

Finally, we applied quantile-based g-computation (qg-comp) models, implemented using the qgcomp package in R, to further quantify and visualize the joint effects of the $PM_{2.5}$ constituents' mixture across the entire pregnancy and within each trimester on NDD. We fitted qgcomp.boot models with a binomial distribution and $B = 1000$ non-parametric bootstrap iterations to estimate the overall mixture effect and corresponding 95% CIs. The 95% CIs were constructed using the percentile method based on the bootstrap distribution of effect estimates. The qgcomp OR represents the change in NDD risk associated with a one-quantile simultaneous increase in all $PM_{2.5}$ constituents. All exposures were categorized into 4 quantiles ($q = 4$) before modeling. Model fit was also assessed using AIC and BIC.

2.7.3. Overlapping metabolic pathways identification

We applied a comprehensive metabolomics workflow, integrating a metabolome-wide association study (MWAS), LASSO (Least Absolute Shrinkage and Selection Operator), pathway enrichment analysis, and the MITM (meet-in-the-middle) approach, to identify overlapping metabolic pathways potentially linking prenatal $PM_{2.5}$-specific constituents' exposure with neurodevelopmental delay.

First, MWAS was conducted using multivariable linear regression models, adjusting for the same set of covariates as in the main regression analyses, to assess the associations between each $PM_{2.5}$ constituents and individual metabolite levels. P values were corrected for multiple testing using the false discovery rate (FDR), and metabolites meeting the FDR-adjusted significance threshold were retained for subsequent steps.

Second, for metabolites that showed significant associations with a given $PM_{2.5}$ constituent in MWAS, we applied LASSO regression (glmnet package in R) to select representative metabolomic biomarkers for each constituent. The penalty parameter λ was determined using the cv.glmnet function with 10-fold cross-validation, to obtain a more stable and stringent selection of associated metabolites. For the 6 $PM_{2.5}$ constituents, we then took the union of all selected metabolites to define the set of $PM_{2.5}$ constituents–related metabolites.

Third, pathway analyses were carried out using MetaboAnalyst 6.0 with the KEGG (Kyoto Encyclopedia of Genes and Genomes) Homo sapiens pathway library to identify metabolic pathways associated with the set of $PM_{2.5}$ constituents–related metabolites and with neurodevelopmental delay, respectively. For each exposed constituent, we further performed pathway annotation and enrichment analysis on differential metabolites and assessed the contribution of each constituent to the overall enrichment pattern at the pathway level. Furthermore, we applied the MITM approach to identify overlapping pathways and shared metabolites between $PM_{2.5}$ constituents–related and neurodevelopment-related metabolite sets, thereby exploring potential metabolic mechanisms. We further calculated differential abundance (DA) scores to summarize the overall directional trend of each pathway. DA scores were calculated as $(N_{up}\text{-}N_{down})\ /N_{total}$ (Luo et al., 2026).

2.7.4. The regulatory role of Med diet in associations

To investigate the potential regulatory role of maternal Med diet adherence in the relationships of $PM_{2.5}$ constituents' exposure and intermediate metabolites with ND, we evaluated the direct association between Med diet adherence and the risk of ND using multivariable logistic regression models. ORs and 95% CIs were estimated after adjustment for the same set of maternal sociodemographic, pregnancy, and postpartum covariates as in the main exposure models. To further assess whether Med diet modified the effect of $PM_{2.5}$ constituents' exposure on NDD, we introduced multiplicative interaction terms between Med diet category and each $PM_{2.5}$ constituent into the regression models. The significance of effect modification was evaluated by testing the cross-product terms.

Subsequently, to investigate the effects of the Med diet on intermediate metabolites identified in the previous workflow, we performed propensity matching on the Med diet group based on maternal sociodemographic characteristics to balance these covariates. After matching, we compared the mean levels of selected metabolites between Med diet groups using t-tests. The differences in metabolite profiles by Med diet adherence were visualized using bar plots, thereby illustrating the potential regulatory influence of Med diet on key metabolic intermediates within the $PM_{2.5}$-specific constituents-NDD pathway.

2.7.5. Sensitivity analyses

Several sensitivity and stratified analyses were conducted to assess the robustness of the associations between prenatal exposure to $PM_{2.5}$ and its constituents and the risk of NDD. First, temperature and humidity were additionally included as covariates in the main individual-exposure and joint-exposure models. Second, to reduce the potential influence of postnatal severe illness or health-care utilization, we repeated the analyses after excluding 535 children who had been hospitalized between birth and completion of the NDD survey. Third, potential effect modification was evaluated by stratifying participants according to infant sex and maternal pre-pregnancy BMI. Maternal pre-pregnancy BMI was categorized as normal BMI, defined as 18.5–24 kg/m^2, and abnormal BMI, defined as outside this range. *P* values for interaction were calculated to assess heterogeneity across strata.

3. Results

3.1. Characteristics of participants and neurodevelopmental delay

As illustrated in Table 1, this study included 6661 mother-child pairs, of whom approximately 7.1% of the children were diagnosed with neurodevelopmental delay. Among these mothers, maternal characteristics showed a mean (± SD) age of 29.63 ± 4.27 years and a pre-pregnancy BMI of 21.39 ± 2.89 kg/m^2 (Table 1). Most of them have a bachelor's degree or higher (65.0%), took folic acid supplements during pregnancy (93.0%), or engaged in mental work (60.7%). However, only the job category showed differences among the neurodevelopmental delay groups. The mean birthweight and gestational age of the children were 3406 (± 437) g and 39.35 (1.34) weeks, respectively, and 51.9% of them were boys. For exclusive breastfeeding (months), the mean months

Table 1
Characteristics of the study population (n = 6661).

Characteristics	All (n = 6661)	Non-NDD (n = 6186)	NDD (n = 475)	*P* value[b]
Demographics and Pregnancy-related[a]				
Age (years)	29.63 (4.27)	29.61 (4.26)	29.82 (4.35)	0.905
Pre-pregnancy BMI (kg/m^2)	21.39 (2.89)	21.38 (2.88)	21.54 (3.03)	0.232
Education				0.288
Junior high school	816 (12.3)	754 (12.2)	62 (13.1)	
High school	1518 (22.8)	1398 (22.6)	120 (25.3)	
Bachelor's degree and above	4327 (65.0)	4034 (65.2)	293 (61.7)	
Job category				0.016
No job	1991 (29.9)	1825 (29.5)	166 (34.9)	
Mental labour	4040 (60.7)	3764 (60.8)	276 (58.1)	
Manual labour	630 (9.5)	597 (9.7)	33 (6.9)	
Physical activity (≥ 3 days/week)	2985 (44.8)	2781 (45.0)	204 (42.9)	0.423
Family income (≥ 8000 CNY/month)	3405 (51.1)	3180 (51.4)	225 (47.4)	0.099
Parity (≥ 3 times)	1826 (27.4)	1676 (27.1)	150 (31.6))	0.040
Passive smoking	2717 (40.8)	2524 (40.8)	193 (40.6)	0.981
Folic acid supplement	6198 (93.0)	5760 (93.1)	438 (92.2)	0.514
GDM	1064 (16.0)	980 (15.8)	84 (17.7)	0.322
Vaginitis	687 (10.3)	648 (10.5)	39 (8.2)	0.137
Birth outcome				
Gestational age (weeks)	39.35 (1.34)	39.35 (1.32)	39.30 (1.54)	0.453
Gender (male)	3457 (51.9)	3183 (51.5)	274 (57.7)	0.016
Birth weight (g)	3406 (437)	3408 (433)	3385 (484)	0.304
Postnatal factors				
Postpartum depression	1149 (17.2)	1058 (17.1)	91 (19.2)	0.253
Exclusive breastfeeding	8.59 (3.81)	8.71 (3.77)	8.01 (3.96)	0.006
Feeding difficulties	1242 (18.6)	1138 (18.4)	104 (21.9)	0.059
Poor sleeping in infants	1438 (21.6)	1342 (21.7)	96 (20.2)	0.449
Fever in infants ≥ 38.5°	3438 (51.6)	3180 (51.4)	258 (54.3)	0.221
Parents as primary caregivers	4218 (63.3)	3922 (63.4)	296 (62.3)	0.636

Note: NDD, Neurodevelopmental delay; BMI, body mass index; GDM, gestational diabetes mellitus. Continuous data are presented as mean (standard deviation), and categorical data as number (percentage).

[a] All variables were collected at 24–28 weeks' gestation

[b] *P* value is from analysis of variance (for means) or chi-square (for proportions).

at the non-NDD and NDD groups were 8.71 and 8.01, respectively, and the difference was statistically significant. No statistically significant differences were found in other characteristics.

3.2. Distribution of $PM_{2.5}$ specific constituents

Table S1 and Figure S3 illustrate the distribution and correlation matrix of 6 $PM_{2.5}$-specific constituents during each trimester and throughout the entire pregnancy. Overall, concentrations of specific constituents were roughly comparable across trimesters. The median (Inter-quartile range) concentrations of SO_4^{2-}, NO_3^-, NH_4^+, OM, BC, and Cl^- during the whole pregnancy were 10.14 (9.16, 10.80) μg/m^3, 12.45 (12.03, 14.60) μg/m^3, 6.97 (6.14, 7.66) μg/m^3, 18.16 (14.68, 21.65) μg/m^3, 3.58 (3.15, 4.02) μg/m^3, and 1.40 (1.19, 1.64) μg/m^3, respectively.

3.3. Associations of $PM_{2.5}$ and its specific constituents with neurodevelopment delay

The risk of NDD across quartiles of $PM_{2.5}$ and its specific constituents is presented in Fig. 1A and Table S2. In the fully adjusted model, compared with the lowest quartile (Q1), the risk of NDD was significantly increased for the highest quartile (Q4) of BC (OR = 1.772, 95%*CI*: 1.314–2.389), NO_3^- (OR = 1.650, 95%*CI*: 1.204–2.261), NH_4^+ (OR = 1.431, 95%*CI*: 1.064–1.923), SO_4^{2-} (OR = 1.376, 95%*CI*: 1.026–1.844), OM (OR = 1.583, 95%*CI*: 1.166–2.148), and $PM_{2.5}$ (OR = 1.667, 95%*CI*: 1.231–2.258), with all *P*-values < 0.05. In analyses of third-trimester (T3) exposure, similar patterns were observed, and T3 appeared to represent a particularly sensitive window. For BC, OM, and NH_4^+, the associations with NDD remained statistically significant even at the second and third exposure quartiles. In contrast, although Cl^- and SO_4^{2-} showed consistently positive associations with NDD across all quartiles, these estimates did not reach statistical significance.

In covariate-adjusted quantile-based g-computation models, joint exposure to the 6 $PM_{2.5}$ specific constituents during the third trimester and over the entire pregnancy was positively associated with the risk of NDD (OR $_{T3}$ = 1.186, 95%*CI*: 1.034–1.358; OR $_{\text{entire pregnancy}}$ = 1.174, 95%*CI*: 1.043–1.321). Furthermore, results from the WQS model indicated that, among the six specific $PM_{2.5}$ constituents, OM and BC were the main contributors to NDD, with weights of 0.626 and 0.273, respectively (Fig. 1B, C). For the third trimester, NO_3^- emerged as an additional major contributor (weight = 0.415). However, no statistically significant associations between the $PM_{2.5}$ constituent mixture and NDD were observed in the joint exposure models for the first and second trimesters (Fig S4−6 and Table S3). Our findings were substantially unchanged when we additionally adjusted for ambient temperature and relative humidity in both individual-exposure and joint-exposure models, when we excluded 535 children who experienced hospitalization from birth to NDD assessment, and when we applied WQS regression to verify the joint effects of $PM_{2.5}$ constituents. In stratified analyses by infant sex, the positive associations were generally more evident among boys than among girls. A significant interaction was observed for $PM_{2.5}$ exposure by infant sex ($P_{\text{for interaction}}$ = 0.046), while the interaction terms for most single-exposure constituents were not statistically significant. Stratified analysis based on maternal pre-pregnancy BMI showed that the associations between groups were roughly equivalent, and no significant interaction was observed. Detailed results are provided in Tables S4–S6.

3.4. Variation of the metabolites in response to the $PM_{2.5}$-specific constituents

Based on the multivariable linear regression results, the number of metabolites significantly associated with each of the 6 $PM_{2.5}$-specific constituents ranged from 821 to 1213 out of the 2626 profiled metabolites (P_{FDR} < 0.05; Fig. 2A and Table S7−12). To obtain a more restricted set of $PM_{2.5}$ constituent–related metabolites, we then applied a two-step procedure combining multivariable regression with LASSO regression, which yielded 82–184 metabolites for each of the six specific constituents (Fig. 2A and Tables S13−18). We further took the union of all metabolites selected in the two-step procedure to define the $PM_{2.5}$ constituents–related metabolite set, comprising 388 metabolites (Fig. 2B). Subsequently, we performed KEGG pathway enrichment analysis on metabolites that were significantly associated with 388 $PM_{2.5}$-specific constituents' metabolites. Among the 88 KEGG pathways that could be mapped, $PM_{2.5}$ constituent–related metabolites were significantly enriched in 15 metabolic pathways (P_{FDR} < 0.1; Fig. 2C and Table S19). The top three enriched pathways were glycine, serine, and threonine metabolism, ABC transporters, and glycerophospholipid metabolism.

3.5. Overlapping enriched pathways associated with both $PM_{2.5}$-specific constituents with neurodevelopment delay

For each of the 6 $PM_{2.5}$-specific constituents, metabolites that remained significantly associated in both the MWAS and subsequent LASSO selection were submitted to KEGG pathway enrichment analysis. Maternal exposure to SO_4^{2-}, NH_4^+, OM, BC, and Cl^- was significantly associated with 25, 8, 3, 10, and 6 metabolic pathways, respectively (P_{FDR} < 0.1; Fig. S7 and Tables S20−25). In addition, we conducted a separate MWAS for NDD and performed KEGG enrichment on the NDD-associated metabolites, identifying 19 significantly enriched

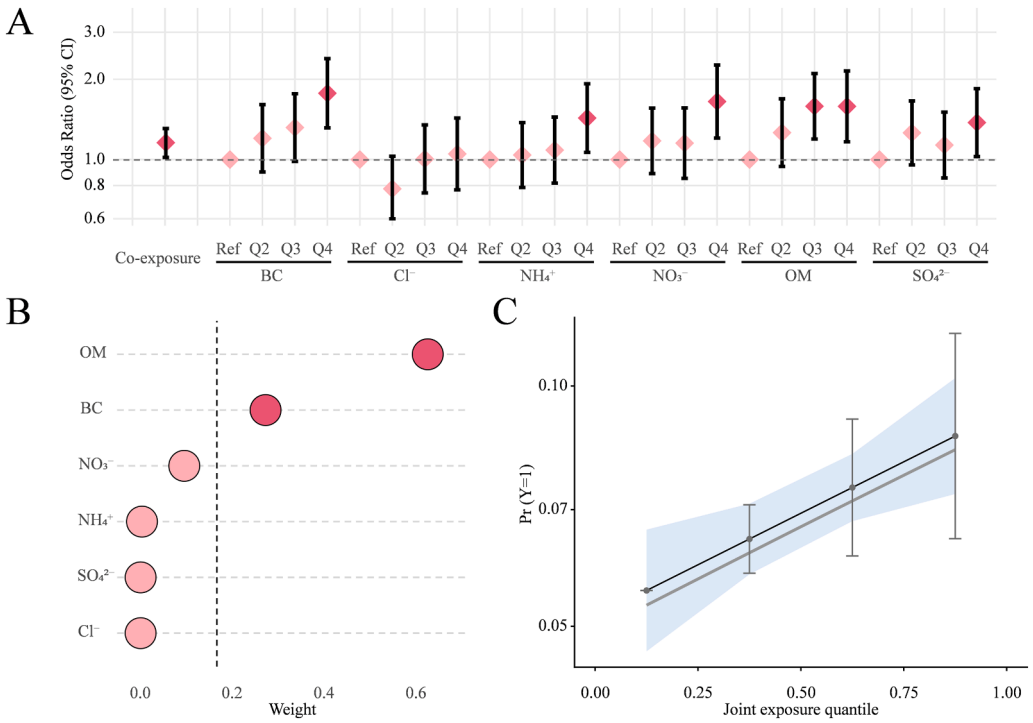


Fig. 1. Associations between maternal exposure to $PM_{2.5}$ specific constituents during the entire pregnancy and child neurodevelopment delay. Abbreviations: $PM_{2.5}$, fine particulate matter; CIs, confidence intervals; WQS, weighted quantile sum; qgcomp, quantile g-computation; NDD, neurodevelopmental delay; SO_4^{2-}, sulfate; NO_3^-, nitrate; NH_4^+, ammonium; OM, organic matter; BC, black carbon; Cl^-, chloride. A) Logistic regression analysis of the associations between $PM_{2.5}$ constituents and child NDD; The y-axis shows the odds ratio of the associations between maternal $PM_{2.5}$ constituents and child NDD (the reference level is the first quartile), data are presented as point estimates and 95%CIs, and odds ratios and 95%CIs for co-exposures estimated using the qgcomp method; B) Weights of $PM_{2.5}$ constituents during throughout pregnancy in the WQS model; C) The qgcomp visualizes the joint effects of $PM_{2.5}$ constituents on neurodevelopmental delay in children, the qgcomp process was repeatedly sampled using the bootstrap method to estimate CIs. Each model was adjusted for maternal age, family income, education, parity, job category, passive smoking, folic acid supplement, infant sex, poor sleeping in infants, exclusive breastfeeding, infant feeding difficulties, fever in infants, parents as primary caregivers and postpartum depression.

pathways among the 89 KEGG pathways that could be mapped ($P_{FDR} <$ 0.1; Tables S26−27). On this basis, Fig. 3A further dissects the 15 KEGG pathways enriched among the 388 $PM_{2.5}$ constituent–related metabolites and illustrates, for each pathway, which of the six specific constituents (SO_4^{2-}, NO_3^-, NH_4^+, OM, BC, and Cl^-) predominantly drive the observed enrichment.

We then applied a meet-in-the-middle approach to identify overlapping enriched pathways between the $PM_{2.5}$ constituents–related metabolite set and the NDD-related metabolite set (Fig. 3B). Four metabolic pathways, including glycine, serine, and threonine metabolism, ABC transporters, biosynthesis of unsaturated fatty acids, and protein digestion and absorption, were shared between the two metabolite sets. Among the 388 $PM_{2.5}$ constituent-related metabolites, 17 were mapped to these four shared pathways, with ABC transporters containing the largest number of mapped metabolites (Fig. S8A). Pathway-level DA scores were positive for all four pathways, indicating an overall upward trend of $PM_{2.5}$ constituent-related metabolites within these pathways (Fig. S8B). In addition, the heatmap of β coefficients further illustrated the direction and magnitude of the associations between individual $PM_{2.5}$ constituents and the core differential metabolites mapped to these pathways (Fig. S8C). Taken together, these findings suggest that SO_4^{2-} and OM are the key $PM_{2.5}$ constituents driving perturbations in these shared metabolic pathways.

3.6. Modifying the role of Mediterranean diet

Higher adherence to the Mediterranean diet was significantly inversely associated with the risk of NDD (OR = 0.803, 95% CI: 0.647–0.998) after adjustment for pre-specified covariates. We also observed that higher Mediterranean diet adherence attenuated the positive associations between high $PM_{2.5}$ constituents' exposure and NDD to varying degrees, particularly for Cl^-, OM, and SO_4^{2-} ($P_{\text{for interaction}} <$ 0.05), suggesting a modifying effect of diet on the exposure–response relationship (Fig. 4A, Table S28).

After propensity score matching, baseline characteristics were well balanced across Mediterranean diet adherence groups, with no statistically significant between-group differences observed in the matched sample (Table S29). However, among the 47 "meet-in-the-middle" metabolites jointly associated with $PM_{2.5}$ constituents and NDD (Table S30), post-matching comparisons identified five metabolites with statistically significant differences: two metabolites (6-ethyl-2,4-dihydroxy-3-methylbenzaldehyde and 2-ketobutyric acid) were positively associated with higher Med adherence, whereas three metabolites (phthalic anhydride, 1-hydroxy-2-naphthoic acid, and 12-hydroxydodecanoic acid) showed inverse associations. The directions of these diet–metabolite associations were opposite to those observed for $PM_{2.5}$ constituents with the same metabolites, supporting a potential counteracting role of the Med (Fig. 4B, Tables S31).

4. Discussion

This study examined the associations between prenatal exposure to major $PM_{2.5}$-specific constituents and offspring NDD in the MIH-Hefei cohort. Our findings indicate that higher prenatal exposure to specific $PM_{2.5}$ constituents is associated with an increased risk of NDD, and quantile g-computation analyses further corroborated this association at the mixture level, with OM and BC emerging as the dominant contributors. Subsequent MITM analyses provided evidence that four metabolic pathways may mediate these relationships, with SO_4^{2-} and OM appearing to be key exposure constituents driving pathway perturbations. In

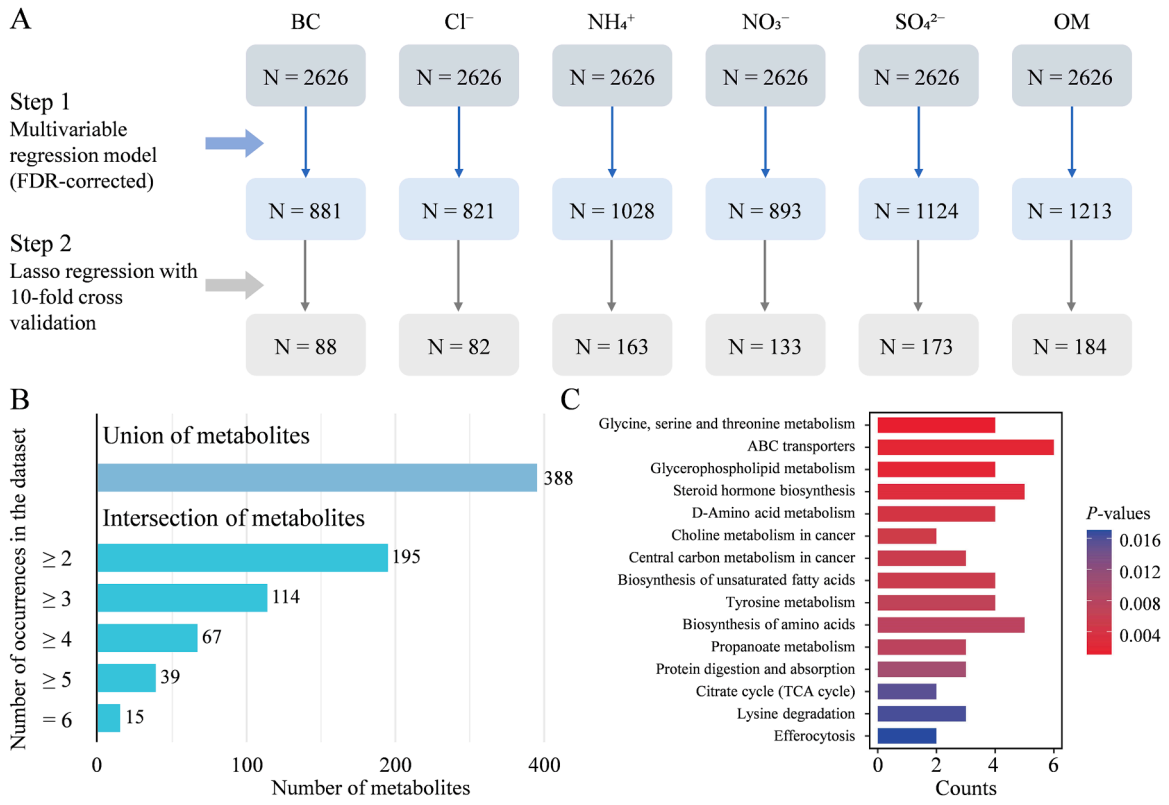


Fig. 2. Screening and functional characterization of maternal metabolites linked to $PM_{2.5}$ specific constituents. Abbreviations: $PM_{2.5}$, fine particulate matter; KEGG, Kyoto Encyclopedia of Genes and Genomes; SO_4^{2-}, sulfate; NO_3^-, nitrate; NH_4^+, ammonium; OM, organic matter; BC, black carbon; Cl^-, chloride. A) The selection processes for related metabolites of exposure to $PM_{2.5}$ constituents during the entire pregnancy; B) The bar graph shows the union and intersection of metabolites significantly correlated with exposure to $PM_{2.5}$ constituents during the entire pregnancy. C) KEGG pathways enriched 388 metabolites that were significantly associated with $PM_{2.5}$ constituents.

addition, higher maternal adherence to a Med modestly attenuated the adverse effects of $PM_{2.5}$ constituents on NDD. This modifying effect may be due to Med improving the metabolic environment, particularly 2-ketobutyric acid, 1-hydroxy−2-naphthoic acid, and 12-hydroxydodecanoic acid.

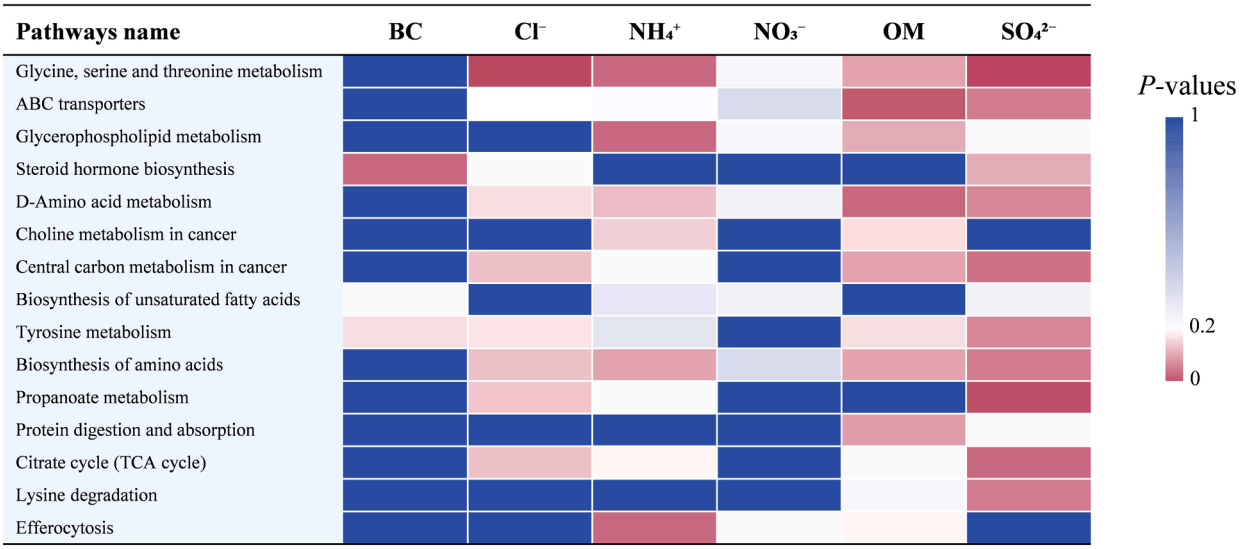
Consistent with our findings, several epidemiological studies have also reported inverse associations between gestational exposure to $PM_{2.5}$-specific constituents and offspring neurodevelopment and cognitive function, with outcomes assessed from birth through early childhood (e.g., at 1 or 5 years of age) (Cloutier et al., 2025; Lei et al., 2022; Lu et al., 2024). Nevertheless, there is still a scarcity of direct evidence linking $PM_{2.5}$-specific constituents with clinical diagnoses. As far as we are aware, this study is the first to investigate prenatal exposure to $PM_{2.5}$ constituents in association with NDD diagnoses based on clinically validated diagnostic records. Our findings highlight OM and BC as the primary contributors to the associations observed.

The prominent contribution of OM is consistent with epidemiologic and experimental data. In a large pregnancy cohort from Southern California, higher prenatal OM exposure was associated with an increased risk of ASD in offspring (Yu et al., 2023). Additionally, in vitro studies provide further support for a biological mechanism, indicating that exposure to OM can alter DNA hydroxymethylation and affect mRNA expression of neuronal genes within neuronal cells (Wei et al., 2017). Furthermore, from a toxicological perspective, OM represents a complex mixture, and polycyclic aromatic hydrocarbons (PAHs) are typical OM-related constituents. Those constituents' exposure has been implicated in disturbed placental function and more direct interference with fetal brain developmental processes (Holme et al., 2024).

Although prenatal BC exposure is associated with adverse neurodevelopmental outcomes in children, the metabolic pathways it affects are not the primary enriched signals in our MITM analysis. We speculate that metabolic disorders may not be the main biological pathways through which BC functions in this context. Results of transvaginal neuroultrasound assessment of fetal brain morphological development in late pregnancy showed that higher BC exposure levels were significantly associated with increased width of the anterior horn of the lateral ventricle and the medullary cistern, as well as decreased depth of the sylvian fissure. Collectively, these fetal neuroanatomical alterations may reflect delayed or inhibited maturation of cerebral cortical folding, which could lead to developmental delays in children's neurological development (Gomez-Herrera et al., 2025; Wu et al., 2022).

We also noted differences in WQS-derived constituent weights across trimesters, which can be explained by both statistical and biological factors. Statistically, WQS weights represent the relative contribution of each constituent within a specific exposure window and are sensitive to trimester-specific exposure distributions and correlations among $PM_{2.5}$ constituents. Given collinearity among $PM_{2.5}$ constituents, weights may be redistributed across correlated components, and small changes in model estimates may alter weight rankings (Tanner et al., 2019). Nevertheless, OM and BC remained among the top three contributors across the entire pregnancy and all trimester-specific models, suggesting their relatively important roles. Biologically, the chemical composition and source profiles of $PM_{2.5}$ may vary across time, meteorological conditions, and emission patterns (Liu et al., 2024). In addition, fetal brain development is characterized by stage-specific processes, including early neurogenesis, neuronal migration and cortical organization in mid-pregnancy, and rapid cortical folding, synaptogenesis, and neural circuit formation in late pregnancy (Ball et al., 2024; Borsani et al., 2019; El Marroun et al., 2020; Sun et al., 2024). These differences may contribute to window-specific susceptibility to $PM_{2.5}$ constituents. Therefore, variation in weights does not necessarily indicate inconsistency; these weights should still be interpreted as exploratory indicators

A



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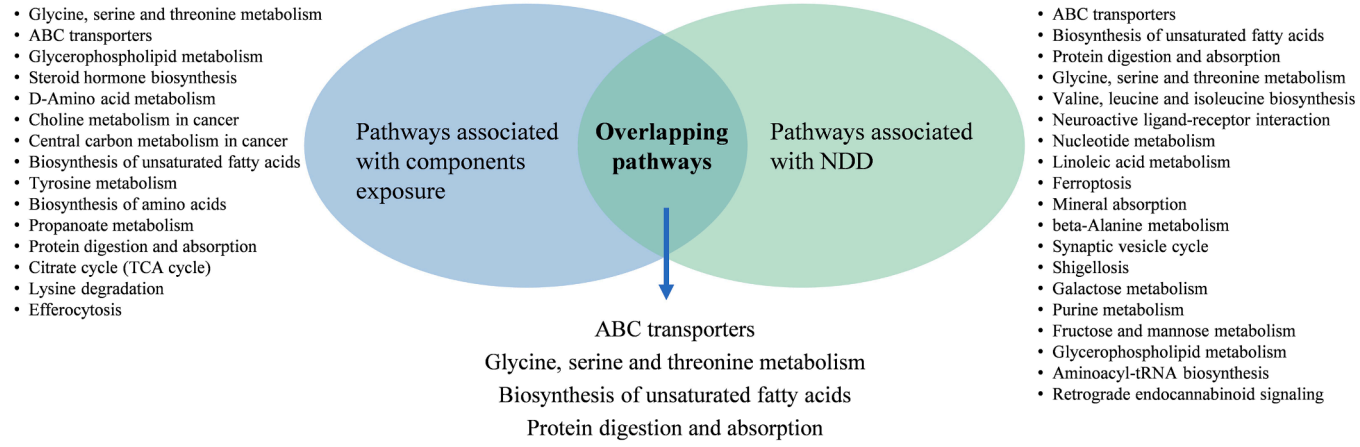

Fig. 3. The overview of enriched metabolite sets related to $PM_{2.5}$ constituents during the entire pregnancy and NDD in children. Abbreviations: $PM_{2.5}$, fine particulate matter; NDD, neurodevelopmental delay; SO_4^{2-}, sulfate; NO_3^-, nitrate; NH_4^+, ammonium; OM, organic matter; BC, black carbon; Cl^-, chloride. A) Heat map of *P*-values of the enriched pathways significantly associated with $PM_{2.5}$ specific constituents. Each cell was colored by the *P*-value of the association of each metabolic pathway; B) The names of overlapping pathways.

and validated in independent cohorts.

Our research further indicates that the third trimester could represent a particularly sensitive period for exposure to air pollution, as notable impacts were detected exclusively in this timeframe. Similarly, Lei et al. found that third-trimester exposure to $PM_{2.5}$ constituents was negatively correlated with cognitive and motor outcomes in infants, based on data from 2435 mother–infant pairs in Shanghai, China (Lei et al., 2022). This finding is biologically plausible given that the brain connectome undergoes dramatic remodeling, with cortical folds forming and synaptic connections developing rapidly during the third trimester, thus making it more sensitive to environmental insults (El Marroun et al., 2020; Sun et al., 2024). Additionally, stratified analysis suggested that these associations may be more pronounced in boys. This finding aligns with prior evidence that males may be more vulnerable to neurodevelopmental toxicants. A systematic review and meta-analysis reported that prenatal exposure to multiple developmental neurotoxicants was linked with lower general and nonverbal IQ, especially among males (Goodman et al., 2023). Mechanistically, greater inflammatory tone and immune vulnerability in male placentas may weaken their resilience under environmental stress (Guma and Chakravarty, 2025; McCarthy, 2019), potentially affecting fetal neurodevelopment (Ceasrine et al., 2022). These sex-specific differences highlight the need for stratified interpretation of environmental exposure effects.

Although the exact biological functions of the identified metabolic pathways remain incompletely understood, which limits definitive mechanistic interpretation, our findings are broadly consistent with emerging evidence. Previous metabolomics studies of prenatal air-pollution exposure have also reported enrichment of the glycine, serine, and threonine metabolism pathway (Hood et al., 2022; Yan et al., 2019). Serine and glycine are key substrates in folate-mediated one-carbon metabolism. During pregnancy, this pathway supports maternal–placental–fetal metabolic adaptation by providing one-carbon units for purine and thymidylate synthesis and for S-adenosylmethionine-dependent methylation reactions. Therefore, perturbation of glycine, serine, and threonine metabolism may affect not only amino-acid homeostasis but also nucleotide synthesis, methyl-donor availability, and redox balance. These processes are particularly relevant to fetal brain development because rapid proliferation and differentiation of neural

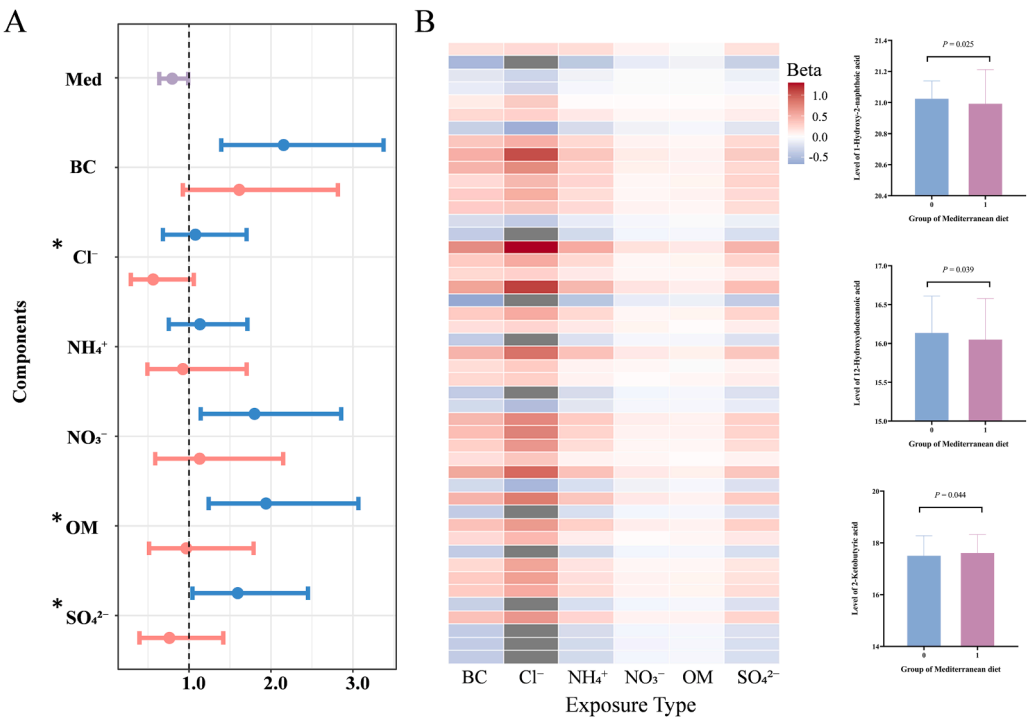

Fig. 4. The moderating effect of Med adherence on the association between $PM_{2.5}$ specific constituents and NDD. Abbreviations: Med, Mediterranean diet; $PM_{2.5}$, Fine particulate matter; NDD, neurodevelopmental delay; SO_4^{2-}, sulfate; NO_3^-, nitrate; NH_4^+, ammonium; OM, organic matter; BC, black carbon; Cl^-, chloride. A) Stratified by Med adherence, the association between SO_4^{2-}, NO_3^-, NH_4^+, OM, BC, and Cl^- and NDD (comparing the fourth quartile with the first quartile). Blue indicates low Med score, red indicates high Med score, purple represents the association between Med and NDD in children (n = 6266), and * indicates a statistically significant interaction. Covariate adjustments for all models were consistent with the main model. Numeric values are presented in Table S28. B) Linear regression model coefficients between average $PM_{2.5}$ specific constituents' levels throughout pregnancy and metabolites identified based on meet-in-the-middle. All models were adjusted for demographics and pregnancy-related characteristics. Numeric values are presented in Table S30. C) Levels of metabolites identified based on meet-in-the-middle in different Med groups after propensity score matching.

progenitor cells require adequate nucleotide supply, while methylation-dependent epigenetic regulation helps control the expression of neurodevelopment-related genes (Kadam et al., 2024; Kalhan, 2016; Korsmo and Jiang, 2021). In addition, glycine participates in glutathione synthesis, and disruption of serine/glycine homeostasis may weaken antioxidant capacity in the maternal–placental environment, thereby increasing fetal vulnerability to oxidative and inflammatory stress (Wu et al., 2015, 2004). Furthermore, animal studies suggest that disturbed serine homeostasis is implicated in brain development and that serine, which can be obtained from the diet or resynthesized from glucose, may ameliorate brain development (Handzlik et al., 2023). These studies provide a biologically plausible context for the pathway perturbations observed in our study.

In addition to glycine, serine, and threonine metabolism, the enrichment of ABC transporters, biosynthesis of unsaturated fatty acids, and protein digestion and absorption further suggest disturbances in maternal–placental nutrient handling and fetal substrate supply, all of which are critical for fetal brain development. ABC transporter-related metabolites may reflect altered transport of endogenous metabolites and xenobiotics at the maternal–placental interface, because placental ABC transporters contribute to fetal protection against potentially harmful compounds (Joshi et al., 2016; Yamashita and Markert, 2021). Biosynthesis of unsaturated fatty acids is also biologically relevant, as long-chain polyunsaturated fatty acids, especially DHA, are important components of neuronal membranes and contribute to neurogenesis, synaptic development, myelination, and signal transduction during fetal brain maturation (Martinat et al., 2021). In late pregnancy, the fetal brain has increasing lipid requirements; therefore, disruptions in maternal lipid metabolism or fatty acid availability may compromise neuronal membrane formation and neural circuit development. Protein digestion and absorption may indicate altered maternal amino-acid availability (Georgieff, 2007). Pregnancy increases protein and amino-acid requirements to support maternal adaptation, placental growth, and fetal tissue development, particularly in late gestation (Elango and Ball, 2016). Because amino acids serve as substrates for fetal protein synthesis, neurotransmitter precursors, and growth-related signaling, altered amino-acid supply may affect neuronal proliferation, synaptogenesis, and brain growth. Overall, these pathways may jointly reflect disturbances in the maternal–placental metabolic environment that influence the delivery of lipids, amino acids, and other substrates required for fetal brain growth and maturation.

Our findings indicate that higher adherence to a Med during pregnancy may mitigate the adverse impact of $PM_{2.5}$-specific constituents on offspring NDD. A large Norwegian cohort reported that higher maternal and child diet quality was associated with a lower risk of attention-deficit disorder in children (Borge et al., 2021), and a randomized controlled trial showed that increased fish intake during pregnancy benefited infant neurodevelopment (Markhus et al., 2021). Other studies have highlighted that diets rich in omega−3 fatty acids, such as vegetables, nuts, and carbohydrates, are recognized and recommended for supporting fetal neural development (Gomez-Pinilla, 2008; Lv et al., 2022; McCann and Ames, 2005), and these food categories are the main dietary style of the Mediterranean diet. In addition, by improving intestinal function, enhancing mucosal immunity, and optimizing lipid metabolism, Med compliance can improve maternal metabolism and inflammatory status, which creates a more favorable intrauterine environment for brain development (Guma and Chakravarty, 2025; Rio-Aige et al., 2025; Zaragoza-Marti et al., 2022). For the fetus, our previous research suggests that umbilical cord blood C-peptide may play a mediating role. The fetus may also directly benefit from the hypothetical anti-inflammatory, antioxidant, and immunomodulatory properties of Med-related nutrients (Dos Santos Silva et al., 2024; Zeng and Brown,

2025).

Another key finding of our study is the further identification of five metabolites that may be important regulatory mediators of the link between the Mediterranean diet's modification of air pollution and neurodevelopment. Among these, 2-ketobutyric acid is closely linked to energy metabolism, immune modulation, and anti-inflammatory responses (Jiao et al., 2025; Lesner et al., 2020), and it is also diet-responsive (Quan et al., 2021). We speculate that higher adherence to the Mediterranean diet may upregulate 2-ketobutyric acid and thereby engage cysteine and methionine metabolism (Jain et al., 2024), with downstream consequences for mitochondrial redox balance and integration with the tricarboxylic acid (TCA) cycle (Niu et al., 2025), a central hub for substrate utilization and brain energy homeostasis (Chen et al., 2022), potentially supporting neurovascular unit (NVU) integrity. In contrast, 1-hydroxy−2-naphthoic acid, a key intermediate in the degradation of environmental hydrocarbon pollutants (Golubev et al., 2024; Macchi et al., 2017), decreased in the high-Mediterranean-diet group, which may indicate reduced exogenous pollutant burden and/or enhanced antioxidant and anti-inflammatory capacity that mitigates pollution-related oxidative stress (Kim et al., 2011). Finally, 12-hydroxydodecanoic acid is an ω-oxidation–related metabolite (Shet et al., 1996) that typically increases when β-oxidation load rises or when oxidative stress and lipid homeostasis are perturbed (Hardwick, 2008; Ranea-Robles and Houten, 2023); its inverse association with the Mediterranean diet is consistent with improved lipid handling and AMPK-linked metabolic reprogramming that promotes fatty-acid oxidation (Barrea et al., 2025).

Our study extends previous research in several important aspects. First, by using a prospective cohort design, trimester-specific exposure assessment, and clinically validated diagnostic records, our study provides more clinically relevant evidence. Second, although prior studies have reported associations between prenatal air pollution exposure and adverse neurodevelopmental outcomes (Cloutier et al., 2025; Holme et al., 2024; Lei et al., 2022), few have incorporated maternal metabolomics to examine the underlying biological pathways. The inclusion of late-pregnancy maternal plasma metabolomics in our study enabled us to link $PM_{2.5}$ constituent exposure to perturbations in amino acid, lipid, nutrient transport, and energy homeostasis-related pathways, thereby strengthening the biological plausibility of the observed associations (Hood et al., 2022; Yan et al., 2019). Third, previous studies on maternal diet and offspring neurodevelopment have generally considered diet quality or specific dietary components as independent protective factors (Borge et al., 2021; Markhus et al., 2021). Our study further evaluated whether adherence to the Mediterranean diet modifies the adverse associations between $PM_{2.5}$ constituents and offspring NDD risk and identified potential metabolite-level signals involved in this potential protective role. Therefore, compared with previous studies, our findings offer a potential basis for future targeted nutritional and public health intervention strategies.

Nonetheless, certain limitations warrant careful consideration when interpreting and generalizing our results. First, our evaluation of exposure did not include alternative possible sources of $PM_{2.5}$ constituents, such as indoor or workplace environments. In addition, information on maternal time–activity patterns were unavailable, potentially leading to exposure misclassification. Future research should combine information on indoor pollution sources, personal exposure monitoring, and wearable devices to improve exposure assessment methods and more accurately describe pregnant women's actual exposure to $PM_{2.5}$ constituents. Second, although we adjusted for a wide range of maternal and infant characteristics, residual confounding remains possible. Parental cognitive ability and co-exposures to other neurotoxic environmental factors were not fully measured. Future research should collect more comprehensive family, genetic and exposomic information, and could consider using sibling comparisons or quasi-experimental designs to enhance causal inference. Third, conducting multiple tests and the presence of collinearity among $PM_{2.5}$ constituents could have influenced the accuracy of the single-constituent models, even though the qg-comp and WQS multi-pollutant models produced consistent results. Fourth, although our sample size was sufficient to ensure statistical power, the study population was recruited from a single city, Hefei, China. Therefore, the generalizability of our findings to populations with different sociodemographic characteristics, dietary habits, pollution sources, and $PM_{2.5}$ chemical profiles may be limited. A multicenter cohort from outside Hefei, China, is needed to assess the generalizability of our findings. External validation in independent cohorts would help confirm the robustness of the observed associations. Finally, although our MITM analytical strategy was designed to evaluate a hypothesized temporal pathway among exposure, metabolites, and NDD, causal mediation cannot be definitively established in an observational setting. Future experimental studies, including animal models and neurodevelopmental cell-based experiments, are needed to validate the biological mechanisms underlying our metabolomics findings. In addition, dietary intervention studies or randomized trials targeting Mediterranean diet adherence during pregnancy would help determine whether maternal dietary modification can mitigate air-pollution-related neurodevelopmental risks and whether such effects are mediated by metabolic pathways.

5. Conclusions

In summary, in this prospective cohort study consisting of 6661 mother-infant pairs, we found that maternal exposure to specific $PM_{2.5}$ constituents, was inversely associated with the risk of NDD. This association may be mediated by the dysregulation of several metabolic pathways, including glycine, serine, and threonine metabolism, as well as pathways related to nutrient and energy metabolism. Furthermore, adherence to the Mediterranean diet appeared to modify the association, potentially through the regulation of specific metabolites such as 2-ketobutyric acid, 1-hydroxy−2-naphthoic acid, and 12-hydroxydodecanoic acid. Further research is required to validate these results and clarify the biological processes involved.

Abbreviations

Fine particulate matter ($PM_{2.5}$); neurodevelopmental delay (NDD); sulfate (SO_4^{2-}); nitrate (NO_3^-); ammonium (NH_4^+); organic matter (OM); black carbon (BC); chloride (Cl^-); Mediterranean Diet (Med); Denver Prescreening Developmental Questionnaire (DPDQ); food frequency questionnaire (FFQ), directed acyclic graph (DAG); odds ratios (ORs); confidence intervals (CIs); Weighted quantile sum (WQS); quantile-based g-computation (qg-comp); Least Absolute Shrinkage and Selection Operator (LASSO); false discovery rate (FDR); metabolome wide association study (MWAS); meet-in-the-middle (MITM).

CRediT authorship contribution statement

Xiangdong Wang: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Peng Zhu:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Rubin Pan:** Writing – review & editing, Validation, Conceptualization. **Mengxiang Guo:** Investigation, Data curation, Conceptualization. **Lei Zhang:** Writing – original draft, Methodology, Investigation, Data curation. **Xinrui Peng:** Visualization, Investigation, Formal analysis. **Jing Wei:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation. **Ji Chen:** Writing – review & editing, Methodology, Investigation, Data curation. **Xiaoguang Yin:** Supervision, Investigation. **Haili Hu:** Supervision, Investigation.

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Role of the funder sponsor

The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgments

This study involving human participants was reviewed and approved by the Ethics Committee of Anhui Medical University (No. 20180092). The patients/ participants provided their written informed consent to participate in this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2026.120371.

Data availability

Data will be made available on request.

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