Ecotoxicology and Environmental Safety 285 (2024) 117037

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Associating prenatal antibiotics exposure with attention deficit hyperactivity disorder symptoms in preschool children: The role of maternal vitamin D

Menglong Geng [a,b,c,d,e,1], Zhen Yu [f,1], Baolin Wang [c,1], Wanhong Xiong [c], Guanlin Sang [g], Yunfeng Song [g], Juan Tong [a,b,c,d], Hui Gao [a,b], Peng Ding [a,b,c,d,e], Kaiyong Liu [a,b,c], Xiaoyan Wu [a,b,c,d,e], Kun Huang [a,b,c,d,*,2], Fangbiao Tao [a,b,c,d,*]

[a] Key Laboratory of Population Health Across Life Cycle (Anhui Medical University), Ministry of Education of the People's Republic of China, No. 81 Meishan Road, Hefei, Anhui 230032, China
[b] Anhui Provincial Key Laboratory of Environment and Population Health across the Life Course, Anhui Medical University, No. 81 Meishan Road, Hefei , Anhui 230032, China
[c] School of Public Health, Anhui Medical University, No. 81 Meishan Road, Hefei, Anhui 230032, China
[d] NHC Key Laboratory of Study on Abnormal Gametes and Reproductive Tract (Anhui Medical University), No. 81 Meishan Road, Hefei, Anhui 230032, China
[e] Scientific Research Center in Preventive Medicine, School of Public Health, Anhui Medical University, No. 81 Meishan Road, Hefei, Anhui 230032, China
[f] Department of Obstetrics and Gynecology, the First Affiliated Hospital of Anhui Medical University, No.218 Jixi Road, Hefei, Anhui 230022, China
[g] Huaibei People's Hospital, Huaibei, Anhui 235000, China

ARTICLE INFO

Edited by Dr. Renjie Chen

Keywords:
Antibiotics
Biomonitoring
Prenatal exposure
Attention-deficit/hyperactivity disorder
Birth cohort

ABSTRACT

Background: The associations between prenatal antibiotics exposure and attention-deficit/hyperactivity disorder (ADHD) in preschoolers, and the role of maternal vitamin D in these associations, remain to be explored.
Objectives: To evaluate the relationships between multiple maternal urinary antibiotics levels and preschoolers' ADHD symptoms, and to identify the potential modifying effects of maternal vitamin D.
Methods: Based on a prospective birth cohort, the present study included 2033 mother—child pairs. Maternal urine and serum samples were collected during all three trimesters to measure the urinary concentrations of 43 antibiotics (including two metabolites) and the serum vitamin D levels. The ADHD symptoms of preschoolers were assessed using the Diagnostic and Statistical Manual–oriented ADHD problems scale in the Achenbach Child Behavior Checklist. Multiple informant models in the form of logistic regression were conducted to investigate the associations between prenatal antibiotics exposure and preschooler ADHD symptoms, and these associations were stratified by child sex and maternal vitamin D status.
Results: Compared with the lowest tertile concentrations, maternal exposure to the middle tertile concentrations of doxycycline and human antibiotics/preferred as human antibiotics (HAs/PHAs), and the highest tertile concentrations of doxycycline during the first trimester were associated with an increased risk of ADHD symptoms in children. An increased risk of ADHD symptoms was observed in girls exposed to the highest tertile levels of sulfamethazine during the second trimester. Furthermore, pregnant women with vitamin D deficiency have a greater risk of ADHD symptoms in their offspring after exposure to doxycycline in the first trimester.
Conclusions: Maternal exposure to doxycycline and HAs/PHAs during the first trimester increases the risk of ADHD symptoms in preschoolers. Mid-pregnancy sulfamethazine exposure increases the risk of ADHD symptoms in girls. Maternal vitamin D deficiency during pregnancy may exacerbate the adverse effects of doxycycline exposure on ADHD symptoms.

* Corresponding authors at: Key Laboratory of Population Health Across Life Cycle (Anhui Medical University), Ministry of Education of the People's Republic of China, No. 81 Meishan Road, Hefei, Anhui 230032, China.
E-mail addresses: wuweihk8028@163.com (K. Huang), fbtao@ahmu.edu.cn (F. Tao).
[1] These authors contributed equally to this work.
[2] *Valid reason provided for adding multiple corresponding authors*: Professor Kun Huang and her team have contributed greatly to the development and completion of this study, so Professor Kun Huang is listed as the corresponding author

https://doi.org/10.1016/j.ecoenv.2024.117037
Received 5 July 2024; Received in revised form 5 September 2024; Accepted 9 September 2024
Available online 12 September 2024

1. Introduction

Attention deficit hyperactivity disorder (ADHD), whose typical symptoms include hyperactivity, impulsivity and inattentiveness, is one of the most common childhood neurodevelopmental disorders worldwide (Sayal et al., 2018). Currently, the prevalence of ADHD in children is between 2 % and 7 % worldwide (Sayal et al., 2018; Thomas et al., 2015). In China, approximately 5.4 % of children are diagnosed with ADHD (Li et al., 2022). ADHD not only is a risk factor for other mental and behavioral problems (e.g., emotional problems, disruptive behaviors, antisocial behavior, and self-harm) but can also lead to a wider range of negative outcomes (e.g., educational underachievement, difficulties with employment, and criminality), which results in a significant burden for the individual, their family, and wider society (Posner et al., 2020; Sayal et al., 2018). Data from the Global Burden of Disease Study 2019 revealed that ADHD was the 84th leading cause of disability-adjusted life years in 2019 at the disorder level among the 0–14-year age group (GBD 2019 Mental Disorders Collaborators, 2022). Given the relatively high prevalence of ADHD in children and significant adverse health and personal development effects in the short term and subsequent life stages, identifying potential risk factors and developing early prevention and intervention strategies for ADHD have become multidisciplinary priority issues, including in the disciplines of pediatrics and public health.

The full etiology of ADHD is not fully understood. The current mainstream view is that the onset and development of ADHD are the result of complex interactions between multiple environmental and genetic factors (Faraone et al., 2015). Emerging compelling evidence has demonstrated that prenatal antibiotic exposure is associated with ADHD in children. A previous systematic review and meta-analysis revealed that pregnant women who ingested antibiotics during pregnancy delivered children with a 14 % increased risk of developing ADHD in childhood (Ai et al., 2021). A large birth cohort study suggested that pregnant women exposed to either clinically used broad-spectrum or narrow-spectrum antibiotics during pregnancy significantly increased the risk of ADHD in their offspring during childhood (Lavebratt et al., 2019). However, because pregnant women are often exposed to different antibiotics (e.g., human and veterinary antibiotics, or preferred as human and veterinary antibiotics) through multiple pathways (e.g., clinical practice, inhalation ingestion, and dietary intake) in the real world (Hu et al., 2022b; Wang et al., 2017; Zeng et al., 2020), the potential adverse effects of prenatal exposure to multiple types of antibiotics on the development of ADHD in offspring urgently need to be explored.

Fluctuations in maternal vitamin D levels during pregnancy are well known to be closely related to the correct neurological development of the fetus (García-Serna and Morales, 2020). The appropriate vitamin D concentrations during pregnancy promote fetal neurodevelopment, whereas maternal vitamin D deficiency/insufficiency may increase the risk of childhood neurodevelopmental disorders in offspring (Upadhyaya et al., 2022). Evidence from a nationwide epidemiological study indicated that relatively low maternal vitamin D concentrations were related to an elevated risk of ADHD in children (Sucksdorff et al., 2021). A randomized clinical trial revealed that higher maternal serum vitamin D concentrations were associated with a lower risk of ADHD in offspring (Aagaard et al., 2024). Previous population-based birth cohort studies have reported that maternal vitamin D status can moderate the adverse effects of prenatal environmental pollutant exposure on offspring neurodevelopment (Gao et al., 2024b; Lu et al., 2024), suggesting that maternal vitamin D can serve as the main basis for formulating intervention strategies for the toxic effects of environmental pollutants in the early life stage. As a potentially modifiable intervening factor, the ability of maternal vitamin D status to modulate the relationship between prenatal antibiotic exposure and childhood ADHD needs to be further explored.

Using data from the Ma'anshan birth cohort (MABC), we aimed to achieve two objectives: (1) to determine the association between prenatal antibiotics exposure and the risk of ADHD symptoms in preschool children; (2) to explore whether different maternal vitamin D levels during pregnancy could modify these associations.

2. Methods

2.1. Study population

Mother—child pairs were identified in the MABC (No. 20131195). In brief, 3474 pregnant women were recruited from Ma'anshan city, China (2013−2014). Urine and serum samples from pregnant women were collected at three time points (first trimester, 10.39 ± 2.11 weeks; second trimester, 25.99 ± 1.02 weeks; third trimester, 34.39 ± 1.09 weeks), and detailed information is provided in our published articles (Zhou et al., 2023; Zhu et al., 2020). Overall, after excluding 201 pregnant women who did not have normal labor or had multiple pregnancies, 86 pregnant women who did not provide urine samples, 416 pregnant women who lacked data on vitamin D during pregnancy, and 738 preschoolers who lacked information on ADHD symptoms, 2033 mother—child pairs were ultimately included (Fig. 1).

2.2. Instrumental analysis

Maternal urine and serum samples were obtained in all three trimesters and stored in our laboratory (−80 °C). A multiple stable isotope dilution assay was developed to determine the urinary concentrations of forty-three antibiotics (41 antibiotics and 2 metabolites), as previously published (Geng et al., 2020; Liu et al., 2020). Briefly, the intraday and interday precisions ranged from 1.9 % to 10.3 % and from 1.0 % to 12.1 %, respectively. The recoveries of the antibiotics ranged from 76.3 % to 112.5 %. The limit of detection (LOD) for the targeted chemicals was in the range of 0.016–1.481 ng/mL (Table S1), and urinary antibiotic levels greater than or equal to the LOD were used to calculate creatinine-adjusted concentrations (Geng et al., 2024). Antibiotics with positive detection rates ≥ 10 % throughout pregnancy were used for further analysis (Table S2) (Geng et al., 2024; Hu et al., 2022a).

Maternal serum total 25-hydroxyvitamin D [25(OH)D] was determined by well-trained experimenters using a kit from Diasorin (DiaSorin Inc, Stillwater, MN, USA) (Gao et al., 2022). The intra- and inter-coefficients were <10 %, and the 25(OH)D concentrations in all the samples exceeded the limit of detection (1.5 ng/mL). The average serum 25(OH)D concentrations of the three trimesters were calculated to reflect maternal vitamin D levels throughout the entire pregnancy. Maternal vitamin D status was divided into two groups: "deficiency (< 20 ng/mL)" and "non-deficiency (≥ 20 ng/mL)" (Lisi et al., 2020; Lu et al., 2024).

2.3. Preschoolers' ADHD symptoms

The ADHD symptoms of children in the MABC were assessed at the age of 4 years via the Diagnostic and Statistical Manual (DSM)–oriented ADHD problems scale in the Achenbach Child Behavior Checklist (Geng et al., 2024; Hansen et al., 2021). The DSM–oriented ADHD problems scale has been validated as an effective predictor of DSM-diagnosed ADHD problems in children (Bellina et al., 2013; Teng et al., 2022). It contains six items (i.e. concentrate, can't sit still, can't wait, demanding, get into things, shifts quickly), and each item was scored 0 for "never", 1 for "sometimes", and 2 for "often". The normalized T score of the DSM–oriented ADHD problems scale was calculated based on the raw scores of the six items (sum of individual raw scores minus the sum of the total population mean raw score, subsequently divided by the standard deviation). In the present study, children with DSM–oriented ADHD problem scale T scores ≥ 60 were defined as exhibiting ADHD symptoms (Liu et al., 2014).

2.4. Covariates

The demographic characteristics of mothers and their children were collected from structured questionnaires or medical records during the follow-up periods. Potential covariates were identified via a directed acyclic graph (Fig. S1), which included maternal age and education level (less than high school, high school, or more than high school), maternal smoking and drinking during pregnancy (yes or no), delivery mode (vaginal delivery or cesarean section), prepregnancy body mass index (BMI), family monthly income per capita (≤2500 CNY, 2500−4000 CNY, or >4000 CNY), residence (urban or rural), and gestational age. In addition, several factors related to ADHD symptoms in children were also selected as covariates, including child sex (boys, girls), breast-feeding duration (less than 1 month, 1−4 months, or greater than 4 months), antibiotic use in infants and toddlers (used at least once or never), average screen time and outdoor activities time per day when children were 4 years old.

2.5. Statistical analysis

The characteristics of the included and excluded participants are presented via descriptive analyses and were compared via Student's t test, the Mann–Whitney U test, or the chi-square test. The selected percentiles were used to describe the maternal urinary antibiotics

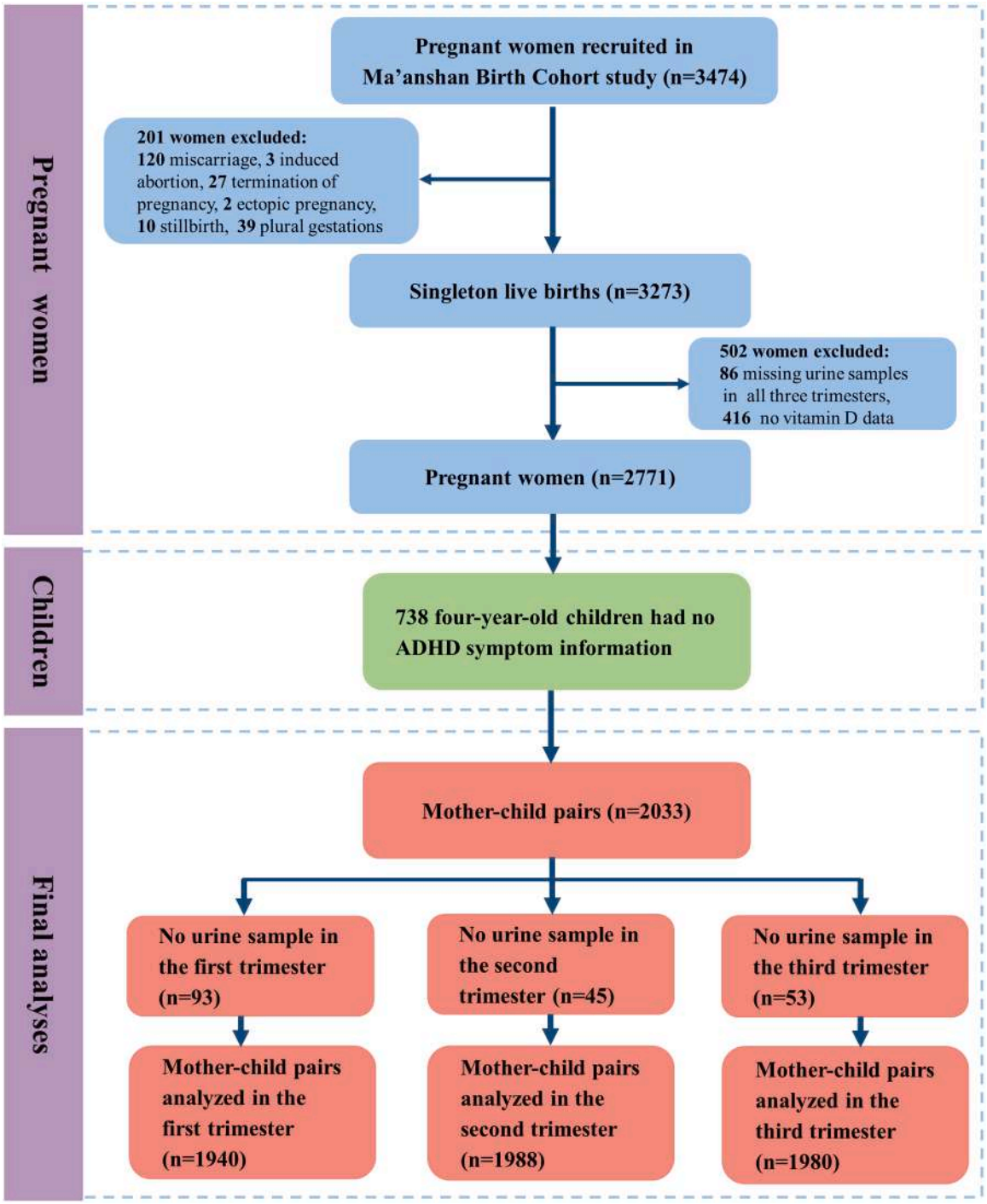

Fig. 1. Flow diagram of study participants.

exposure levels. Urinary antibiotics creatinine-adjusted concentrations below the LOD values were categorized into the "Tertile 1" group, whereas the "Tertile 2" (LODs to median concentrations) and "Tertile 3" (greater than median concentration) groups were divided according to the median concentration of the positive detection fraction (Geng et al., 2024).

Based on the generalized estimating equations, multiple informant models in the form of logistic regression were fitted to investigate the associations between prenatal antibiotics exposure and preschooler ADHD symptoms (Sánchez et al., 2011). This model is applicable to repeated measurement data and can test the differences in estimations across multiple periods via the Type 3 test (Sun et al., 2020). The strength of the association between each exposure variable and the outcome variable is presented as odds ratios (ORs) and 95 % confidence intervals (CIs). Additionally, sex-stratified analyses were conducted to test potential sex-specific associations. To examine the effect modification of maternal vitamin D during pregnancy, stratified analyses were performed to examine the influence of maternal vitamin D status (deficiency and non-deficiency group) on the association between prenatal antibiotics exposure and preschooler ADHD symptoms. P values of the multiplicative interaction term between each individual or category of antibiotics and preschooler sex or maternal vitamin D status were calculated to test differences between groups. The false discovery rate (FDR) was used to correct for multiple testing (Benjamini and Hochberg, 1995).

Three sensitivity analyses were performed. First, because preterm birth is a potential risk factor for ADHD symptoms in preschoolers (Ask et al., 2018), we excluded children with a gestational age of less than 37 weeks. Second, previous studies have reported that screen time (Qu et al., 2023) and outdoor activities (Taylor and Kuo, 2009) may be associated with ADHD symptoms in children. Thus, these variables were included for further adjustment. Third, we calculated E-values to assess potential residual confounding from unmeasured confounders (VanderWeele and Ding, 2017).

We analyzed the data via R (4.3.2) and SPSS (23.0). P values < 0.05, whereas FDR p-values (Lei et al., 2022), Type 3 p-values (Sun et al., 2020) and p-values for interaction < 0.10 (Geng et al., 2024) were considered significant.

3. Results

The demographic characteristics of the mother—child pairs in the included and excluded groups are shown in Table 1. Among the included participants, the mean maternal age, pre-pregnancy BMI, gestational age and child birth weight were 26.68 years, 20.58 kg/m^2, 39.12 weeks and 3379.39 g, respectively. More than half of the pregnant women had a high school education or above (58.3 %), never smoked or drank alcohol during pregnancy (90.9 %), lived in urban areas (79.7 %), and had average serum vitamin D levels less than 20 ng/mL (53.0 %). Forty-two and a half percent of the participants had a family monthly income per capita in the range of 2500–4000 CNY. Half of the children were born vaginally, and half were born by Caesarean section, with a greater number of boys than girls. The proportion of children who had been breastfed for > 4 months and who had used antibiotics at least once during infancy and early childhood exceeded 50 %. The median average screen and outdoor activities time per day were 1.50 and 1.79 hours, respectively. The prevalence of ADHD symptoms in preschool children was 10.6 %. In addition, no difference in characteristics was observed between the source and study populations (Table S3).

Fig. 2 and Table S4 present the exposure levels of multiple antibiotics in maternal urine samples during different trimesters of pregnancy. Fig. 2 shows the composition profiles of antibiotics in maternal urine samples across the three trimesters. The detection frequencies for nine individual antibiotics were greater than 10 % in all three trimesters. The 95th percentile creatinine-adjusted concentration ranges for the nine individual antibiotics were 0.19−2.93 μg/g, 0.23−4.12 μg/g, and 0.16−3.76 μg/g in the first, second, and third trimesters, respectively. PVAs (81.0−88.2 %) had the highest detection frequency in all three trimesters, followed by VAs (56.4−68.5 %) and HAs/PHAs (24.8−50.2 %). The 95th percentile creatinine-adjusted concentration ranges for the PVAs, VAs and HAs/PHAs were 13.48−20.17 μg/g, 3.21−6.05 μg/g, and 12.26−19.64 μg/g, respectively (Table S4).

Fig. 3 and Table S5 show the relationships between maternal antibiotics exposure during each trimester and ADHD symptoms in preschool children. Pregnant women exposure to doxycycline at the middle (OR = 1.66, 95 % CI: 1.14, 2.43, $P\text{-}_{FDR} = 0.059$) and highest (OR = 2.11,

Table 1
Demographic characteristics [mean ± SD or n (%) or median (IQR)] of the participants.

Characteristics	Included (n = 2033)	Excluded (n = 1240)	P value
Maternal and Family characteristics			
Maternal age	26.68 ± 3.57	26.48 ± 3.75	0.125
Pre-pregnancy BMI	20.58 ± 2.78	20.64 ± 2.82	0.564
Maternal education levels			0.043
Less than high school	382 (18.8)	278 (22.4)	
High school	465 (22.9)	270 (21.8)	
More than high school	1186 (58.3)	692 (55.8)	
Smoking and drinking during pregnancy			0.066
Yes	186 (9.1)	138 (11.1)	
No	1847 (90.9)	1102 (88.9)	
Delivery mode[a]			0.391
Vaginal delivery	1017 (50.0)	598 (48.5)	
Cesarean section	1015 (50.0)	635 (51.5)	
Family monthly income per capita (CNY)			0.060
≤ 2500	566 (27.8)	301 (24.3)	
2500−4000	864 (42.5)	538 (43.4)	
> 4000	603 (29.7)	401 (32.3)	
Residence			0.916
Urban	1620 (79.7)	990 (79.8)	
Rural	413 (20.3)	250 (20.2)	
Maternal Vitamin D status			
< 20 ng/mL	1077 (53.0)		
≥ 20 ng/mL	956 (47.0)		
Children's characteristics			
Gestational age (weeks)[b]	39.12 ± 1.24	38.87 ± 1.58	0.001
Birth weight (g)[c]	3379.39 ± 417.66	3342.37 ± 491.74	0.028
Sex[d]			0.270
Boys	1052 (51.7)	614 (49.8)	
Girls	981 (48.3)	620 (50.2)	
Breastfeeding duration (months)			0.009
< 1	583 (28.7)	415 (33.5)	
1−4	310 (15.2)	192 (15.5)	
> 4	1140 (56.1)	633 (51.0)	
Antibiotic use in infants and toddlers			0.070
Used at least once	1153 (56.7)	663 (53.5)	
Never	880 (43.3)	577 (46.5)	
Average screen time per day at 4 years of age (hours)[e]	1.50 (1.76)	1.50 (1.80)	0.531
Average outdoor activities time per day at 4 years of age (hours)[f]	1.79 (1.29)	1.92 (1.57)	0.836
ADHD symptoms T scores	52.76 ± 4.53		
Normal	1818 (89.4)		
ADHD symptoms	215 (10.6)		

Notes: SD, standard deviation; IQR, interquartile range; BMI, body mass index; ADHD, attention deficit hyperactivity disorder.
[a] Delivery mode data for 1 children and 7 children were unavailable in included and excluded group, respectively.
[b] Gestational age data for 3 children were unavailable in excluded group.
[c] Birth weight data for 7 children were unavailable in excluded group.
[d] Sex data for 6 children were unavailable in excluded group.
[e] Screen time data for 715 children were unavailable in excluded group.
[f] Outdoor activity time data for 715 children were unavailable in excluded group.

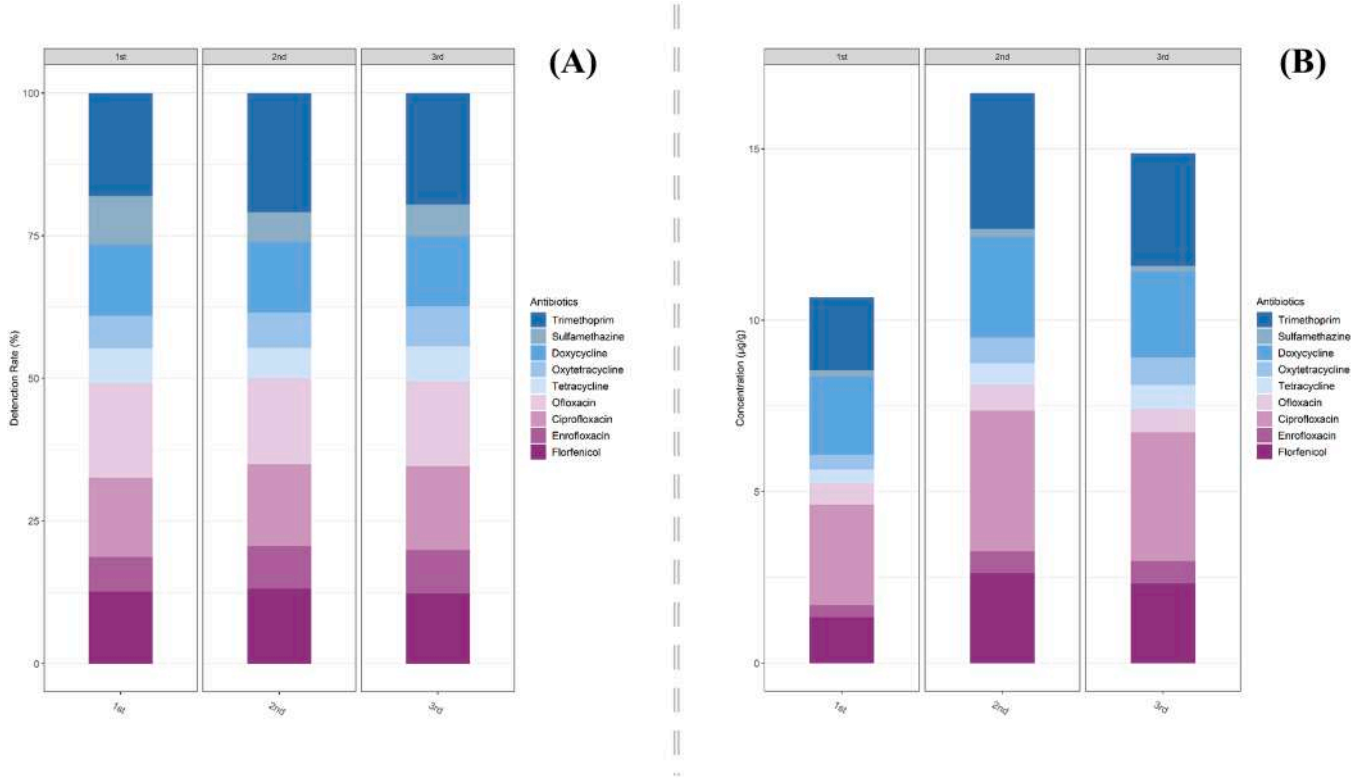

Fig. 2. Composition profiles of urinary antibiotics (Fig. 1A:percentage, %; Fig. 1B: 95th percentile creatinine-adjusted concentrations, μg/g;) in pregnant women from the Ma'anshan birth cohort.

95 % CI: 1.46, 3.04, $P\text{-}_{FDR} < 0.05$) tertile group during the first trimester were significantly elevated the risk of ADHD symptoms in childhood (type 3 p-value = 0.010). In the first trimester, maternal urinary HAs/PHAs (OR = 1.97, 95 % CI: 1.40, 2.78, $P\text{-}_{FDR} < 0.05$) in the middle tertile increased the risk of ADHD symptoms in preschool children (type 3 p-value = 0.011). As shown in Figure S2 and Table S6, the results of sex-stratified analyses indicated that pregnant women with concentrations of sulfamethazine (OR = 2.99, 95 % CI: 1.36, 6.57, $P\text{-}_{FDR} = 0.078$) in the highest tertile levels during the second trimester increased the risk of ADHD symptoms in girls (p-value for interaction = 0.026).

To investigate the modifying role of maternal vitamin D status in the prenatal antibiotics effect on offspring ADHD symptoms, maternal vitamin D status during pregnancy was dichotomized into deficiency and non-deficiency groups. Fig. 4 and Table S7 show the stratified and interaction analyses of prenatal antibiotics exposure and maternal vitamin D status on preschooler ADHD symptoms. After adjusting for covariates, in the vitamin D status deficiency group, maternal exposure to doxycycline at the middle (OR = 1.96, 95 % CI: 1.25, 3.09, $P\text{-}_{FDR} = 0.026$) and highest (OR = 2.89, 95 % CI: 1.83, 4.55, $P\text{-}_{FDR} < 0.05$) tertile concentrations during the first trimester significantly increased the risk of ADHD symptoms in children. Moreover, no significant associations between prenatal doxycycline exposure and ADHD symptoms were found in the non-deficiency groups (p-value for interaction = 0.018).

The results from the sensitivity analyses were generally consistent with those of the primary analyses when children with preterm birth were excluded (Table S8) or when additionally adjusted for preschoolers' average screen time and outdoor activities time per day (Table S9). The E-values suggest that the associations we observed are robust (Table S10).

4. Discussion

The results of the present study indicated that maternal doxycycline and HAs/PHAs exposure during the first trimester was related to an increased risk of ADHD symptoms in offspring. Compared with the non-deficiency group, pregnant women with vitamin D deficiency exposed to doxycycline during the first trimester had a greater risk of preschoolers' ADHD symptoms. In addition, sex-stratified analyses indicated that maternal sulfamethazine exposure during the second trimester was associated with ADHD symptoms in girls.

In the current study, the overall positive detection rate for all antibiotics throughout pregnancy ranged from 90.3 % to 97.2 %. The antibiotic detection rates of pregnant women in Shanghai in 2012 (98.6 %) (Zeng et al., 2020) and 2020 (92.3 %) (Lin et al., 2022) were similar to those reported in the present study, whereas the total antibiotic detection rates in the urine samples of pregnant women in Jiangsu Province (31.0 %) (Zhou et al., 2021), the Xizang Autonomous Region (34.7 %) (Wang et al., 2023) and Eastern China (41.6 %) (Wang et al., 2017) were lower. Numerous potential factors contribute to differences in antibiotics exposure among pregnant women in different regions, such as dietary patterns, the educational level of the population, drug administration policies, regional economic development, the natural climate, the timing of urine sample collection, the type of antibiotic selected, and methods for determining exposure levels. Furthermore, VAs and PVAs were more commonly detected in the urine samples of pregnant women than HAs, whereas overall antibiotic concentrations were relatively low (95th percentile concentration: 51.25–62.32 μg/g), suggesting that dietary intake may be the main route of exposure to multiple antibiotics during pregnancy (Zeng et al., 2020).

In this prospective birth cohort, children born to mothers exposed to doxycycline had a greater risk of ADHD symptoms. Currently, studies on the association between prenatal doxycycline exposure and ADHD in offspring are lacking. A recent biomonitoring-based study reported that concentrations of tetracycline antibiotics (including doxycycline) in placental tissue were positively associated with the risk of offspring neural tube defects (Cheng et al., 2024). Our previous study revealed that maternal doxycycline exposure during pregnancy increased the risk of behavioral and mental problems in children (Geng et al., 2024). These

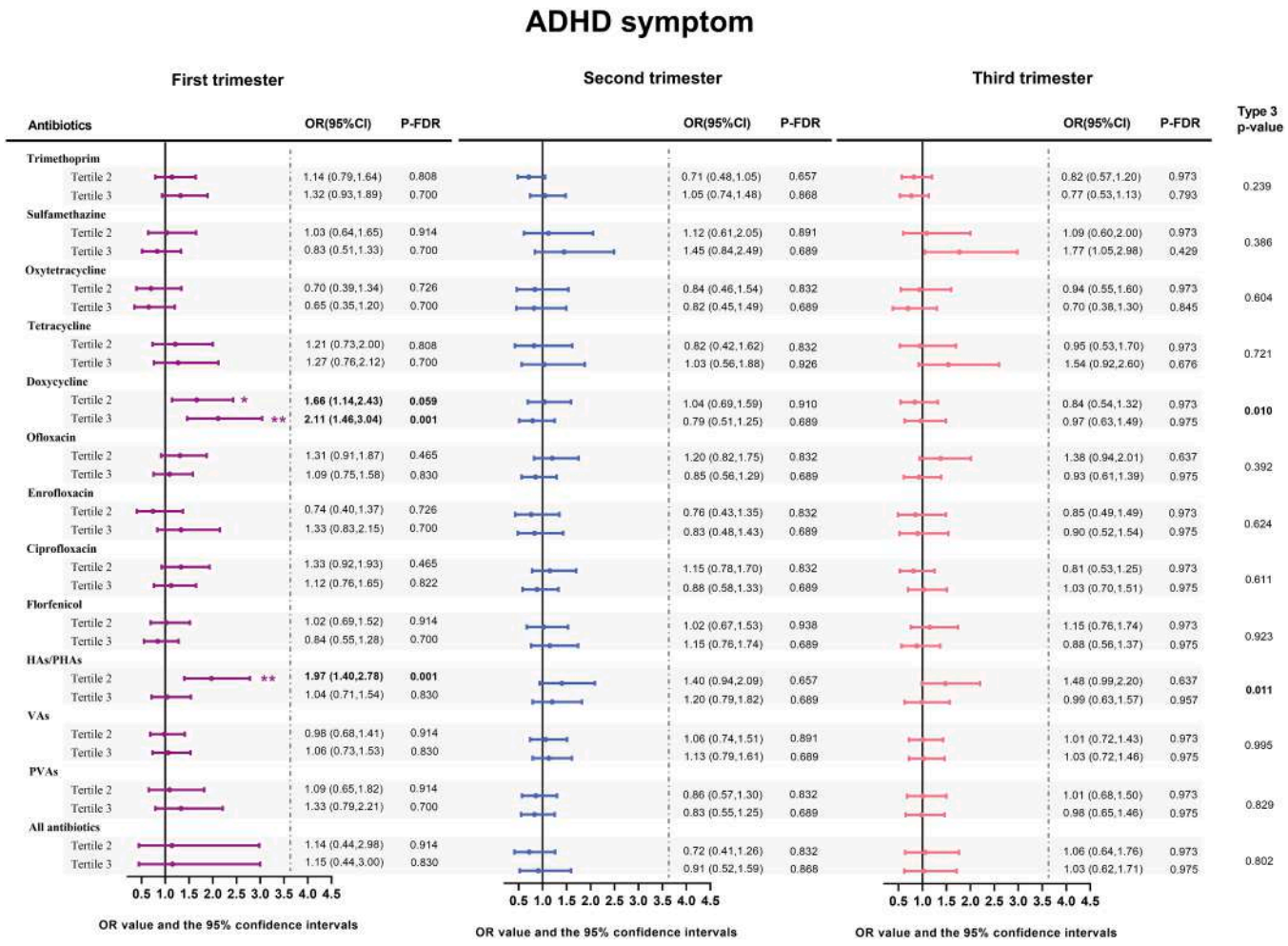

Fig. 3. Association between maternal urinary antibiotics exposure during three trimesters and preschoolers' ADHD symptoms. Analyses were adjusted for maternal age, pre-pregnancy BMI, maternal education levels, smoking and drinking during pregnancy, delivery mode, family monthly income per capita, residence, gestational age, children sex, breastfeeding duration, antibiotic use in infants and toddlers.

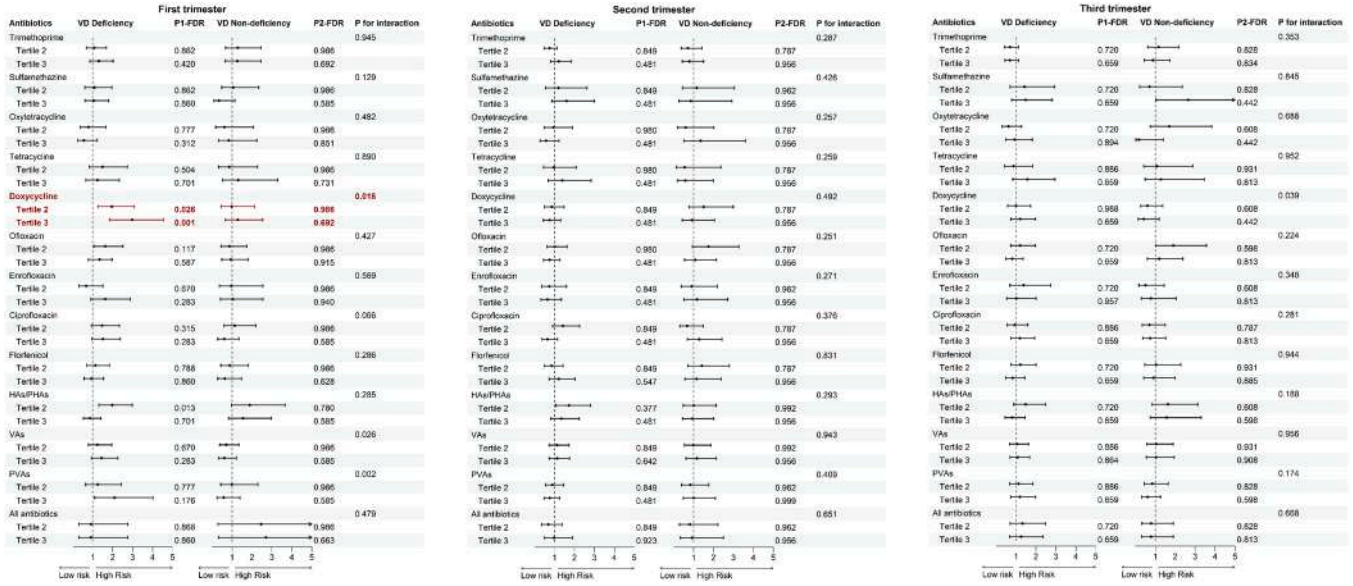

Fig. 4. Association of prenatal antibiotics exposure and preschoolers ADHD symptoms stratified by maternal vitamin D status. Analyses were adjusted for maternal age, pre-pregnancy BMI, maternal education levels, smoking and drinking during pregnancy, delivery mode, family monthly income per capita, residence, gestational age, children sex, breastfeeding duration, antibiotic use in infants and toddlers.

findings suggest that prenatal doxycycline exposure is neurotoxic to offspring development. Although doxycycline has been banned in pregnant women (Smilack, 1999), it can still enter the human body through contaminated food and water (Zeng et al., 2020), suggesting that doxycycline exposure during pregnancy may be considered an environmental risk factor for ADHD development. In addition, epidemiological studies from different studies have reported inconsistent associations between clinically used antibiotics during pregnancy and ADHD in offspring (Hamad et al., 2020; Lavebratt et al., 2019; Njotto et al., 2023). In the present study, prenatal exposure to HAs/PHAs increased the risk of ADHD symptoms in children. The direct determination of antibiotic concentrations in urine samples more accurately reflect real internal exposure levels in humans than accessing antibiotic prescription records.

Identifying the susceptible periods of prenatal environment exposure that affect the health outcomes of offspring can provide epidemiological clues for biological mechanism research while providing sufficient information for environmental policies that minimize negative impacts on public health and vulnerable populations. Based on the results of the Type 3 test in the multiple informant models, we identified the first trimester as a susceptible period of prenatal antibiotics exposure. In the early pregnancy stage, the onset of fetal neurological development (Adibi et al., 2023), affects the typical neurodevelopmental trajectory of the fetus (De Asis-Cruz et al., 2022), which may increase children's susceptibility to ADHD after birth. Similar to our findings, previous convincing results have shown that antibiotics exposure during the first trimester can increase the risk of autism spectrum disorder and neural tube defects in children (Nitschke et al., 2023; Yu et al., 2020). Our prior work confirmed that the first trimester was a critical window for maternal antibiotics exposure to cause children internalizing and externalizing problems (Geng et al., 2024). In addition, sulfamethazine exposure during the second trimester more significantly affected girls than in boys. Our understanding of the potential mechanisms is limited, but maternal gut microbiota may play a pivotal role. Numerous studies have reported that gut microbiota can regulate local and systemic sex steroid hormone levels by producing related enzymes (Pace and Watnick, 2020) and that sex differences in fetal neurodevelopment are related to prenatal sex steroid hormones (Baron-Cohen et al., 2020). Therefore, the altered maternal microbiota composition caused by prenatal antibiotics exposure may be the main driver of the sex dimorphic effect. In summary, we found that prenatal doxycycline, HAs/PHAs and sulfamethazine was associated with an increased risk of developing ADHD in offspring, and these results expand the etiologic horizon of ADHD.

Widespread exposure to antibiotics during pregnancy and the associated potential maternal and child health risks have raised significant concerns. From a public health perspective, any intervention strategy that reduces the adverse health outcomes of antibiotics exposure during pregnancy is important. In our study, vitamin D deficiency during pregnancy exacerbated the adverse effects of doxycycline exposure on children's ADHD symptoms. Consistent with our findings, the results of several previous compelling birth cohort studies have also demonstrated that maternal vitamin D status can modulate the effects of exposure to multiple environmental pollutants (e.g. phthalates, per- and polyfluoroalkyl substances, organophosphate esters) during pregnancy on offspring neurodevelopment (e.g. autistic traits, language, motor, intellectual function) (Gao et al., 2024a, 2024b; Lu et al., 2024). Notably, given the ubiquitous exposure of pregnant populations to multiple antibiotics, even weak effect sizes can result in significant health burdens at the public health dimension. Therefore, developing safe, cost-effective, and acceptable early nutritional screening and intervention strategies, such as vitamin D testing and supplementation, is essential for pregnant populations at high risk of antibiotics exposure.

In addition, maternal vitamin D and prenatal antibiotics exposure may affect offspring neurodevelopment through similar pathways, and these effects may be potential mechanisms for vitamin D modification effects. For instance, prenatal antibiotics exposure can affect maternal thyroid function (Geng et al., 2022), induce maternal inflammatory responses (Ghazanfari et al., 2020), and modulate maternal gut microbiome metabolites (Vuong et al., 2020), all of which are strongly associated with fetal neurodevelopment. Moreover, optimal maternal vitamin D levels also play a crucial role in supporting thyroid function during pregnancy (Wang et al., 2022), attenuating the intensity of the inflammatory response (Ao et al., 2021), and maintaining maternal gut microbiome homeostasis (Aparicio et al., 2023).

The present study is the first biomonitoring-based cohort study to examine the modifying role of maternal vitamin D status in the effects of multiple prenatal antibiotics on childhood ADHD symptoms. The present study utilized a prospective cohort study design, which was able to more accurately elucidate the causal associations between prenatal antibiotics exposure and childhood ADHD symptoms. In addition, determining the positive detection rate and concentration of multiple antibiotics in urine samples can depict the spectrum of antibiotic exposure in pregnant women in detail. Moreover, a repeated measurement design can reduce misclassification of prenatal antibiotic exposure, more fully capture antibiotic exposure levels throughout pregnancy, and identify the susceptible periods of adverse health effects.

The current study is subject to several limitations. First, given the wide variety of antibiotics, an insufficiently comprehensive range of antibiotic classes may underestimate the true levels of antibiotics exposure among pregnant women in the real world. Future studies should expand the spectrum of antibiotic detection. Second, our study did not obtain information on postnatal exposure to multiple antibiotics, including VAs and PVAs. Next, we will determine the urinary antibiotics concentrations in MABC children. Third, although the use of parent-reported screening questionnaires can capture subclinical symptoms and undiagnosed ADHD, it can also introduce reporting bias, which in turn overestimates or underestimates the severity of symptoms. Combining parent-reported ADHD symptoms with clinically diagnosed ADHD can reduce reporting bias and obtain more accurate information about children's ADHD. Fourth, similar to all observational epidemiologic studies, the results of the current study may still be affected by unmeasured confounders, despite adjusting for a large number of demographic characteristics. Nevertheless, the E-values suggest that bias due to unmeasured confounders is unlikely to have contributed significantly to our findings. Fifth, the participants in the MABC all lived in one city, which is regionally limited. Multicenter prospective cohort studies should be conducted in the future to make the findings more representative. Finally, we did not explore the potential effects of other maternal vitamins (e.g., vitamin A or vitamin B) or nutrients (e.g., folic acid or zinc). Future studies should expand the scope of research to develop more practical intervention strategies around the health effects of adverse environmental exposures in the early life stage.

5. Conclusion

Exposure to doxycycline and HAs/PHAs during the first trimester was associated with an increased risk of ADHD symptoms in preschool children. Maternal sulfamethazine exposure during the second trimester was associated with a high risk of ADHD symptoms in girls. The children of pregnant women with vitamin D deficiency exposed to doxycycline in the first trimester are at a greater risk of developing ADHD symptoms. The government and relevant organizations should actively carry out scientific popularization and publicity work around the health effects of antibiotic exposure during pregnancy to increase public health awareness. Moreover, pregnant women should minimize the use of clinical antibiotics, drink filtered water, consume regular marketed food, and ensure that vitamin D is replenished in a timely manner to create a favorable intrauterine environment for fetal neurodevelopment and to improve the quality of births.

CRediT authorship contribution statement

Fangbiao Tao: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Zhen Yu:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Baolin Wang:** Writing – review & editing, Visualization, Supervision, Software, Methodology. **Wanhong Xiong:** Visualization, Data curation. **Guanlin Sang:** Methodology. **Yunfeng Song:** Methodology. **Juan Tong:** Supervision, Investigation, Data curation. **Hui Gao:** Supervision, Investigation, Data curation. **Peng Ding:** Methodology, Investigation. **Kaiyong Liu:** Methodology, Data curation. **Xiaoyan Wu:** Supervision, Methodology, Investigation. **Kun Huang:** Supervision, Project administration, Investigation, Conceptualization. **Menglong Geng:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

Funding

This work was supported by the National Natural Science Foundation of China (NSCF-82204053), the University Natural Foundation of Anhui Educational Committee (KJ2021A0222), and the Discipline Construction Project of School of Public Health, Anhui Medical University (0301001872).

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.

Data availability

The data that has been used is confidential.

Acknowledgements

We appreciate the participants in the Ma'anshan Birth Cohort. We also thank Sheng Wang, Shulong Li, and Guanjun Chen (The Center for Scientific Research of Anhui Medical University) for lab technical support. We are grateful to the Scientific Research Center in Preventive Medicine, School of Public Health, Anhui Medical University for the technical support in our experiment.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117037.

References

Aagaard, K., Møllegaard Jepsen, J.R., Sevelsted, A., Horner, D., Vinding, R., Rosenberg, J.B., Brustad, N., Eliasen, A., Mohammadzadeh, P., Følsgaard, N., Hernández-Lorca, M., Fagerlund, B., Glenthøj, B.Y., Rasmussen, M.A., Bilenberg, N., Stokholm, J., Bønnelykke, K., Ebdrup, B.H., Chawes, B., 2024. High-dose vitamin D3 supplementation in pregnancy and risk of neurodevelopmental disorders in the children at age 10: a randomized clinical trial. Am. J. Clin. Nutr. 119, 362–370. https://doi.org/10.1016/j.ajcnut.2023.12.002.

Adibi, J.J., Zhao, Y., Koistinen, H., Mitchell, R.T., Barrett, E.S., Miller, R., O'Connor, T. G., Xun, X., Liang, H.W., Birru, R., Smith, M., Moog, N.K., 2023. Molecular pathways in placental-fetal development and disruption. Mol. Cell Endocrinol., 112075 https://doi.org/10.1016/j.mce.2023.112075.

Ai, Y., Zhao, J., Shi, J., Zhu, T.T., 2021. Antibiotic exposure and childhood attention-deficit/hyperactivity disorder: systematic review and meta-analysis. Psychopharmacology 238, 3055–3062. https://doi.org/10.1007/s00213-021-05989-3.

Ao, T., Kikuta, J., Ishii, M., 2021. The effects of vitamin D on immune system and inflammatory diseases. Biomolecules 11. https://doi.org/10.3390/biom11111624.

Aparicio, A., Gold, D.R., Weiss, S.T., Litonjua, A.A., Lee-Sarwar, K., Liu, Y.Y., 2023. Association of vitamin D Level and maternal gut microbiome during pregnancy: findings from a randomized controlled trial of antenatal vitamin D supplementation. Nutrients 15. https://doi.org/10.3390/nu15092059.

Ask, H., Gustavson, K., Ystrom, E., Havdahl, K.A., Tesli, M., Askeland, R.B., Reichborn-Kjennerud, T., 2018. Association of gestational age at birth with symptoms of attention-deficit/hyperactivity disorder in children. JAMA Pediatr. 172, 749–756. https://doi.org/10.1001/jamapediatrics.2018.1315.

Baron-Cohen, S., Tsompanidis, A., Auyeung, B., Nørgaard-Pedersen, B., Hougaard, D.M., Abdallah, M., Cohen, A., Pohl, A., 2020. Foetal oestrogens and autism. Mol. Psychiatry 25, 2970–2978. https://doi.org/10.1038/s41380-019-0454-9.

Bellina, M., Brambilla, P., Garzitto, M., Negri, G.A., Molteni, M., Nobile, M., 2013. The ability of CBCL DSM-oriented scales to predict DSM-IV diagnoses in a referred sample of children and adolescents. Eur. Child Adolesc. Psychiatry 22, 235–246. https://doi.org/10.1007/s00787-012-0343-0.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc.: Ser. B (Methodology) 57, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Cheng, Q., Chen, Y., Liu, J., Jin, L., Li, Z., Ren, A., Wang, L., 2024. Inadvertent antibiotic exposure during pregnancy may increase the risk for neural tube defects in offspring. Ecotoxicol. Environ. Saf. 275, 116271 https://doi.org/10.1016/j.ecoenv.2024.116271.

Collaborators, G.M.D., 2022. Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Psychiatry 9, 137–150. https://doi.org/10.1016/s2215-0366(21)00395-3.

De Asis-Cruz, J., Andescavage, N., Limperopoulos, C., 2022. Adverse prenatal exposures and fetal brain development: insights from advanced fetal magnetic resonance imaging. Biol. Psychiatry Cogn. Neurosci. Neuroimaging 7, 480–490. https://doi.org/10.1016/j.bpsc.2021.11.009.

Faraone, S.V., Asherson, P., Banaschewski, T., Biederman, J., Buitelaar, J.K., Ramos-Quiroga, J.A., Rohde, L.A., Sonuga-Barke, E.J., Tannock, R., Franke, B., 2015. Attention-deficit/hyperactivity disorder. Nat. Rev. Dis. Prim. 1, 15020 https://doi.org/10.1038/nrdp.2015.20.

Gao, H., Tong, J., Zhu, B.B., Chen, Y., Ye, A.X., Huang, K., Liang, C.M., Wu, X.Y., Sheng, J., Jin, Z.X., Zhu, P., Hao, J.H., Tao, F.B., 2022. Lag associations of gestational phthalate exposure with maternal serum vitamin D levels: repeated measure analysis. Chemosphere 299, 134319. https://doi.org/10.1016/j.chemosphere.2022.134319.

Gao, Y., Zhang, Y., Luo, J., Mao, D., Lei, X., Liu, C., Zhang, S., Yao, Q., Li, J., Zhang, J., Yu, X., Tian, Y., 2024b. Effect modification by maternal vitamin D status in the association between prenatal exposure to per- and polyfluoroalkyl substances and neurodevelopment in 2-year-old children. Environ. Int. 185, 108563 https://doi.org/10.1016/j.envint.2024.108563.

Gao, H., Zhang, C., Zhu, B., Geng, M., Tong, J., Zhan, Z., Zhang, Y., Wu, D., Huang, K., Tao, F., 2024a. Associating prenatal phthalate exposure with childhood autistic traits: investigating potential adverse outcome pathways and the modifying effects of maternal vitamin D. Eco-Environ. Health. https://doi.org/10.1016/j.eehl.2024.01.007.

García-Serna, A.M., Morales, E., 2020. Neurodevelopmental effects of prenatal vitamin D in humans: systematic review and meta-analysis. Mol. Psychiatry 25, 2468–2481. https://doi.org/10.1038/s41380-019-0357-9.

Geng, M., Ding, P., Wang, S., Wang, B., Tong, J., Gao, H., Yan, S., Liu, K., Wu, X., Zhu, P., Cao, Y., Huang, K., Tao, F., 2024. Prenatal antibiotics exposure and preschoolers' internalizing and externalizing problems: a biomonitoring-based prospective birth cohort study. Sci. Total Environ. 919, 170891 https://doi.org/10.1016/j.scitotenv.2024.170891.

Geng, M., Gao, H., Wang, B., Huang, K., Wu, X., Liang, C., Yan, S., Han, Y., Ding, P., Wang, W., Wang, S., Zhu, P., Liu, K., Cao, Y., Tao, F., 2022. Urinary tetracycline antibiotics exposure during pregnancy and maternal thyroid hormone parameters: a repeated measures study. Sci. Total Environ. 838, 156146 https://doi.org/10.1016/j.scitotenv.2022.156146.

Geng, M., Liu, K., Huang, K., Zhu, Y., Ding, P., Zhang, J., Wang, B., Liu, W., Han, Y., Gao, H., Wang, S., Chen, G., Wu, X., Tao, F., 2020. Urinary antibiotic exposure across pregnancy from Chinese pregnant women and health risk assessment: repeated measures analysis. Environ. Int. 145, 106164 https://doi.org/10.1016/j.envint.2020.106164.

Ghazanfari, T., Norooznezhad, A.H., Javidan, S., Norouz, L., Farzanehdoust, A., Mansouri, K., Ahmadi, M.H., Mostafaei, S., Javadian, P., Sheikh, M., Hantoushzadeh, S., 2020. Indicated and non-indicated antibiotic administration during pregnancy and its effect on pregnancy outcomes: Role of inflammation. Int. Immunopharmacol. 89, 107081 https://doi.org/10.1016/j.intimp.2020.107081.

Hamad, A.F., Alessi-Severini, S., Mahmud, S., Brownell, M., Kuo, I.F., 2020. Prenatal antibiotic exposure and risk of attention-deficit/hyperactivity disorder: a population-based cohort study. Cmaj 192, E527–e535. https://doi.org/10.1503/cmaj.190883.

Hansen, J.B., Bilenberg, N., Timmermann, C.A.G., Jensen, R.C., Frederiksen, H., Andersson, A.M., Kyhl, H.B., Jensen, T.K., 2021. Prenatal exposure to bisphenol A and autistic- and ADHD-related symptoms in children aged 2 and5 years from the Odense Child Cohort. Environ. Health 20, 24. https://doi.org/10.1186/s12940-021-00709-y.

Hu, Y., Wei, X., Zhu, Q., Li, L., Liao, C., Jiang, G., 2022a. COVID-19 pandemic impacts on humans taking antibiotics in China. Environ. Sci. Technol. 56, 8338–8349. https://doi.org/10.1021/acs.est.1c07655.

Hu, Y., Zhu, Q., Wang, Y., Liao, C., Jiang, G., 2022b. A short review of human exposure to antibiotics based on urinary biomonitoring. Sci. Total Environ. 830, 154775 https://doi.org/10.1016/j.scitotenv.2022.154775.

Lavebratt, C., Yang, L.L., Giacobini, M., Forsell, Y., Schalling, M., Partonen, T., Gissler, M., 2019. Early exposure to antibiotic drugs and risk for psychiatric disorders: a population-based study. Transl. Psychiatry 9, 317. https://doi.org/10.1038/s41398-019-0653-9.

Lei, X., Zhang, Y., Wang, Z., Lu, Z., Pan, C., Zhang, S., Chen, Q., Yuan, T., Zhang, J., Gao, Y., Tian, Y., 2022. Effects of prenatal exposure to PM(2.5) and its composition on cognitive and motor functions in children at 12 months of age: The Shanghai Birth Cohort Study. Environ. Int 170, 107597. https://doi.org/10.1016/j.envint.2022.107597.

Li, F., Cui, Y., Li, Y., Guo, L., Ke, X., Liu, J., Luo, X., Zheng, Y., Leckman, J.F., 2022. Prevalence of mental disorders in school children and adolescents in China: diagnostic data from detailed clinical assessments of 17,524 individuals. J. Child Psychol. Psychiatry 63, 34–46. https://doi.org/10.1111/jcpp.13445.

Lin, J., Ding, J., Di, X., Sun, W., Chen, H., Zhang, H., 2022. Association between prenatal antibiotics exposure and measures of fetal growth: A repeated-measure study. Ecotoxicol. Environ. Saf. 244, 114041 https://doi.org/10.1016/j.ecoenv.2022.114041.

Lisi, G., Ribolsi, M., Siracusano, A., Niolu, C., 2020. Maternal vitamin D and its role in determining fetal origins of mental health. Curr. Pharm. Des. 26, 2497–2509. https://doi.org/10.2174/1381612826666200506093858.

Liu, J., Liu, X., Wang, W., McCauley, L., Pinto-Martin, J., Wang, Y., Li, L., Yan, C., Rogan, W.J., 2014. Blood lead concentrations and children's behavioral and emotional problems: a cohort study. JAMA Pediatr. 168, 737–745. https://doi.org/10.1001/jamapediatrics.2014.332.

Liu, K., Zhang, J., Geng, M., Zhu, Y., Liu, X., Ding, P., Wang, B., Liu, W., Liu, Y., Tao, F., 2020. A stable isotope dilution assay for multi-class antibiotics in pregnant urines by LC–MS/MS. Chromatographia 83, 507–521. https://doi.org/10.1007/s10337-020-03866-3.

Lu, M., Gan, H., Zhou, Q., Han, F., Wang, X., Zhang, F., Tong, J., Huang, K., Gao, H., Yan, S., Jin, Z., Wang, Q., Tao, F., 2024. Trimester-specific effect of maternal co-exposure to organophosphate esters and phthalates on preschooler cognitive development: the moderating role of gestational vitamin D status. Environ. Res. 251, 118536 https://doi.org/10.1016/j.envres.2024.118536.

Nitschke, A.S., do Valle, H.A., Vallance, B.A., Bickford, C., Ip, A., Lanphear, N., Lanphear, B., Weikum, W., Oberlander, T.F., Hanley, G.E., 2023. Association between prenatal antibiotic exposure and autism spectrum disorder among term births: a population-based cohort study. Paediatr. Perinat. Epidemiol. 37, 516–526. https://doi.org/10.1111/ppe.12972.

Njotto, L.L., Simin, J., Odsbu, I., Mussche, I., Callens, S., Fornes, R., Engstrand, L., Bruyndonckx, R., Brusselaers, N., 2023. Maternal and early-life exposure to antibiotics and the risk of autism and attention-deficit hyperactivity disorder in childhood: a Swedish population-based cohort study. Drug Saf. 46, 467–478. https://doi.org/10.1007/s40264-023-01297-1.

Pace, F., Watnick, P.I., 2020. The interplay of sex steroids, the immune response, and the intestinal microbiota. Trends Microbiol. https://doi.org/10.1016/j.tim.2020.11.001.

Posner, J., Polanczyk, G.V., Sonuga-Barke, E., 2020. Attention-deficit hyperactivity disorder. Lancet 395, 450–462. https://doi.org/10.1016/s0140-6736(19)33004-1.

Qu, G., Hu, W., Meng, J., Wang, X., Su, W., Liu, H., Ma, S., Sun, C., Huang, C., Lowe, S., Sun, Y., 2023. Association between screen time and developmental and behavioral problems among children in the United States: evidence from 2018 to 2020 NSCH. J. Psychiatr. Res 161, 140–149. https://doi.org/10.1016/j.jpsychires.2023.03.014.

Sánchez, B.N., Hu, H., Litman, H.J., Téllez-Rojo, M.M., 2011. Statistical methods to study timing of vulnerability with sparsely sampled data on environmental toxicants. Environ. Health Perspect. 119, 409–415. https://doi.org/10.1289/ehp.1002453.

Sayal, K., Prasad, V., Daley, D., Ford, T., Coghill, D., 2018. ADHD in children and young people: prevalence, care pathways, and service provision. Lancet Psychiatry 5, 175–186. https://doi.org/10.1016/s2215-0366(17)30167-0.

Smilack, J.D., 1999. The tetracyclines. Mayo Clin. Proc. 74, 727–729. https://doi.org/10.4065/74.7.727.

Sucksdorff, M., Brown, A.S., Chudal, R., Surcel, H.M., Hinkka-Yli-Salomäki, S., Cheslack-Postava, K., Gyllenberg, D., Sourander, A., 2021. Maternal vitamin D levels and the risk of offspring attention-deficit/hyperactivity disorder. J. Am. Acad. Child Adolesc. Psychiatry 60, 142–151.e142. https://doi.org/10.1016/j.jaac.2019.11.021.

Sun, Y., Wang, Y.X., Liu, C., Chen, Y.J., Lu, W.Q., Messerlian, C., 2020. Trimester-specific blood trihalomethane and urinary haloacetic acid concentrations and adverse birth outcomes: identifying windows of vulnerability during pregnancy. Environ. Health Perspect. 128, 107001 https://doi.org/10.1289/ehp7195.

Taylor, A.F., Kuo, F.E., 2009. Children with attention deficits concentrate better after walk in the park. J. Atten. Disord. 12, 402–409. https://doi.org/10.1177/1087054708323000.

Teng, Y., Li, P., Yang, M., Han, Y., Yan, S., Xu, Y., Tao, F., Huang, K., 2022. Sex-specific effect of thyroid peroxidase antibody and thyroglobulin antibody exposure during pregnancy on preschoolers' emotional and behavioral development: a birth cohort study. Thyroid 32, 1229–1242. https://doi.org/10.1089/thy.2022.0044.

Thomas, R., Sanders, S., Doust, J., Beller, E., Glasziou, P., 2015. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Pediatrics 135, e994–e1001. https://doi.org/10.1542/peds.2014-3482.

Upadhyaya, S., Ståhlberg, T., Silwal, S., Arrhenius, B., Sourander, A., 2022. Maternal vitamin D levels during pregnancy and offspring psychiatric outcomes: a systematic review. Int J. Mol. Sci. 24, 63. https://doi.org/10.3390/ijms24010063.

VanderWeele, T.J., Ding, P., 2017. Sensitivity analysis in observational research: introducing the E-value. Ann. Intern Med 167, 268–274. https://doi.org/10.7326/m16-2607.

Vuong, H.E., Pronovost, G.N., Williams, D.W., Coley, E.J.L., Siegler, E.L., Qiu, A., Kazantsev, M., Wilson, C.J., Rendon, T., Hsiao, E.Y., 2020. The maternal microbiome modulates fetal neurodevelopment in mice. Nature 586, 281–286. https://doi.org/10.1038/s41586-020-2745-3.

Wang, Y., Chen, Y., Gesang, Y., Yang, Z., Wang, Y., Zhao, K., Han, M., Li, C., Ouzhu, L., Wang, J., Wang, H., Jiang, Q., 2023. Exposure of Tibetan pregnant women to antibiotics in China: a biomonitoring-based study. Environ. Pollut. 325, 121439 https://doi.org/10.1016/j.envpol.2023.121439.

Wang, H., Wang, H.J., Jiao, M., Han, N., Xu, J., Bao, H., Liu, Z., Ji, Y., 2022. Associations between dynamic vitamin D level and thyroid function during pregnancy. Nutrients 14. https://doi.org/10.3390/nu14183780.

Wang, H., Wang, N., Qian, J., Hu, L., Huang, P., Su, M., Yu, X., Fu, C., Jiang, F., Zhao, Q., Zhou, Y., Lin, H., He, G., Chen, Y., Jiang, Q., 2017. Urinary antibiotics of pregnant women in eastern China and cumulative health risk assessment. Environ. Sci. Technol. 51, 3518–3525. https://doi.org/10.1021/acs.est.6b06474.

Yu, P.A., Tran, E.L., Parker, C.M., Kim, H.J., Yee, E.L., Smith, P.W., Russell, Z., Nelson, C. A., Broussard, C.S., Yu, Y.C., Meaney-Delman, D., 2020. Safety of antimicrobials during pregnancy: a systematic review of antimicrobials considered for treatment and postexposure prophylaxis of plague. Clin. Infect. Dis. 70, S37–s50. https://doi.org/10.1093/cid/ciz1231.

Zeng, X., Zhang, L., Chen, Q., Yu, K., Zhao, S., Zhang, L., Zhang, J., Zhang, W., Huang, L., 2020. Maternal antibiotic concentrations in pregnant women in Shanghai and their determinants: A biomonitoring-based prospective study. Environ. Int. 138, 105638 https://doi.org/10.1016/j.envint.2020.105638.

Zhou, J., Tong, J., Ru, X., Teng, Y., Geng, M., Yan, S., Tao, F., Huang, K., 2023. Placental inflammatory cytokines mRNA expression and preschool children's cognitive performance: a birth cohort study in China. BMC Med. 21, 449. https://doi.org/10.1186/s12916-023-03173-2.

Zhou, Y.J., Zhu, F., Zheng, D.Y., Gao, M.M., Guo, B.F., Zhang, N., Meng, Y., Wu, G.L., Zhou, Y.L., Huo, X., 2021. Detection of antibiotics in the urine of children and pregnant women in Jiangsu, China. Environ. Res 196, 110945. https://doi.org/10.1016/j.envres.2021.110945.

Zhu, Y.D., Wu, X.Y., Yan, S.Q., Huang, K., Tong, J., Gao, H., Xie, Y., Tao, S.M., Ding, P., Zhu, P., Tao, F.B., 2020. Domain- and trimester-specific effect of prenatal phthalate exposure on preschooler cognitive development in the Ma'anshan Birth Cohort (MABC) study. Environ. Int 142, 105882. https://doi.org/10.1016/j.envint.2020.105882.