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Research paper

The role of neonatal vitamin D in the association of prenatal depression with toddlers ADHD symptoms: A birth cohort study

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ABSTRACT

Background: Vitamin D has been demonstrated a "neuroprotective" effect, but it is unclear whether early-life adequate vitamin D protect adverse neurodevelopment. We aimed to examine the role of neonatal vitamin D in the association of maternal depression (MD) symptoms with toddlers ADHD.

Methods: Participants included 1 125 mother-infant pairs from the China-Anhui Birth Cohort study. MD was assessed by the Center for Epidemiological Studies Depression Scale (CES-D) at 30-34 gestational weeks. Toddlers ADHD was reported by the Conners' Hyperactivity Index (CHI) at 48-54 months postpartum. Multiple logistic regression models were performed to evaluate the association of maternal depressive score and toddlers ADHD while cord blood 25(OH)D levels were stratified.

Results: Toddlers of mothers with higher depression score were at higher risk of ADHD (20.1% vs 11.1%, $P = 0.003$; adjusted RR=1.75, 95% CI: 1.10-2.81). Among toddlers with neonatal vitamin D deficiency (VDD), ADHD risk was significantly increased with maternal MD (adjusted RR=3.74, 95% CI: 1.49-9.41), but the association was not found in toddlers with neonatal vitamin D adequacy (VDA). Compared to toddlers without MD, toddlers with both MD and neonatal VDD had higher risk of ADHD (adjusted RR=3.10, 95% CI: 1.44-6.63). But the risk did not significantly increase in toddlers with MD and neonatal VDA (adjusted RR=1.53, 95% CI: 0.86-2.72).

Limitations: Maternal depressive symptoms in early pregnancy and anxious symptoms were needed to include.

Conclusion: This prospective study indicated that the detrimental effect of maternal prenatal depressive symptoms on offspring's ADHD symptoms strengthened in toddlers with neonatal VDD.

1. Introduction

It has been estimated that as much as 15% of childhood emotional and behavioral problems may be attributable to prenatal exposure to maternal psychopathology (TalgeNeal and Glover, 2007). Maternal depression (MD) is a common mood disorder during pregnancy as 12.7-23.0% of pregnant women will experience a depressive disorder (Gavin et al., 2005). However, most pregnancy-related depression is frequently undetected. Several prospective cohort studies have shown that MD was associated with child's adverse neurodevelopmental

Abbreviations: ADHD, Attention-Deficit-Hyperactivity Disorder; BMI, Body Mass Index; CES-D, Center for Epidemiological Studies Depression Scale; CHI, Conners' Hyperactivity Index; EPDS, Edinburgh Postnatal Depression Scale; GWG, Gestational Weight Gain; MD, Maternal Depression; SGA, Small for Gestational Age; VDA, Vitamin D Adequacy; VDD, Vitamin D Deficiency.

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outcomes such as maladjustment, emotional disorders, behavioral problem and cognitive deficits (Evans et al., 2012, Hay et al., 2008; Cents et al., 2013).

A number of clinical trials suggested that it seems to be unhelpful to treat prenatal depression using antidepressants from a child's neurodevelopmental perspective (Figueroa 2010; Clements et al., 2015; Laugesen et al. 2013). There is a growing body of convergent evidence that vitamin D may have "neuroprotective" properties (Cui et al., 2015; Whitehouse et al., 2012; Morales et al., 2015). A double-blind, placebo-controlled trial of vitamin D supplementation on Parkinson disease suggested that adequate vitamin D status may help recover from brain lesions (Suzuki et al., 2013). However, it is unclear whether low early-life vitamin D may leave the developmental brain vulnerable to any internal and/or external insults, and adequate vitamin D could protect the developmental brain from the progression of a wide range of brain disorders.

We hypothesize that MD during pregnancy increased the risk for the early symptoms of attention-deficit/hyperactivity disorder (ADHD) in toddlers, which may be mitigated by the early-life adequate vitamin D status. In this study, we examined whether newborn adequate vitamin D mitigates the effect of maternal depression on ADHD symptoms in toddlers using data from a prospective birth cohort in China.

2. Subjects and methods

2.1. Subjects

As part of the China-Anhui birth cohort study (Tao et al., 2013), pregnant women in this birth cohort were recruited from the Hefei Maternal and Child Health Hospital between January and September 2008 (**Figure 1**). Briefly, during gestational ages from 30 to 34 weeks, 2 552 pregnant women were recruited by a team of midwives, nurses and health professionals. Participants completed a structured questionnaire including sociodemographic characteristics, life style and depressive symptoms through face to face interviews. After delivery, midwives or research nurses collected the newborn's anthropometric details and cord blood, when available. To remove potential confounding factors, in this study, stillbirth, newborns with birth defect or 5 min Apgar score below 7, women with delivery before 32 gestational weeks or mental disorder with medication, pregnancy with assisted reproductive technology, or multiple gestations were excluded from the study. Due to an overlap in confounders of the excluded samples, 1434 participants with cord blood samples were eligible. And they were interviewed by telephone at 3 months postpartum, information on maternal depressive symptoms and infant feeding was collected. Between 48 to 54 months postpartum, parents were invited to evaluate their children's ADHD symptoms by face-to-face interview at the above antenatal clinic. Finally, full data including prenatal depression, cord blood samples, postnatal depression and ADHD symptoms in toddlers were obtained from 1125 mother-infant pairs. The ethical approval was granted by the Ethics Committee of Anhui Medical University, and informed consent was obtained from each participant.

2.2. Study variables

2.2.1. Maternal depression

Maternal depressive symptoms in late pregnancy were determined using a Chinese version of Center for Epidemiological Studies Depression Scale (CES-D). The CES-D was designed to evaluate the levels of depressive symptomatology within the last week and consists of 20 self-reported items and each item is rated on the scale of 0-3, producing a total score range from 0 to 60 (Radloff, 1977). A higher score correlates with more severe depression. CES-D has been widely used in pregnant women with a common cut-off score of ≥16. The reliability of the Chinese version of the CES-D has been previously validated (Zhang et al., 2010). In this study, the internal consistency (Cronbach's alpha) of the scales was 0.86, and the sensitivity and specificity were 0.92 and 0.87, respectively.

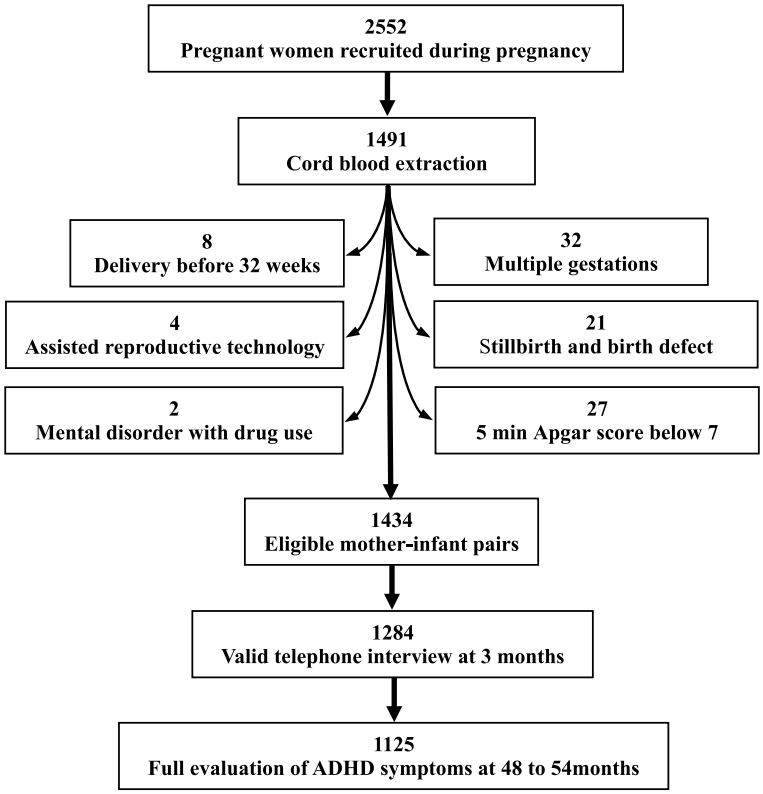

Figure. 1. Flow diagram for the recruitment of mother-infant pairs in the prospective follow-up study.

2.2.2. 25(OH)D measurement in cord blood

Cord blood samples were collected immediately after delivery by research nurses. Plasma samples were centrifuged and promptly refrigerated at -4°C and then transferred, within 12 h, to -80°C freezers for long-term storage. Concentrations of 25(OH)D were measured using the radioimmunoassay (DiaSorin Stillwater) with the detection limit of 3.75 nmol/L. In this study, intra-assay and inter-assay coefficients of variation were 8.8% and 11.1%, respectively. Cord blood 25(OH)D concentrations were analyzed as both continuous and categorical variables. As recommended by Canadian Paediatric Society (Godel and Society, 2007), a cutoff of 25 nmol/L was used for the categorical variables. 25 (OH)D concentrations of <25 nmol/L and ≥25 nmol/L were defined as VDD and vitamin D adequacy (VDA), respectively.

2.2.3. ADHD symptoms in toddlers

The behavioral symptoms of ADHD in toddlers were evaluated using the parent version of the Conners' Hyperactivity Index (CHI) (Conners and Barkley, 1985), which has been widely used in epidemiological and clinical studies and has previously been validated in pre-school children in China (Ou et al., 2001; Zhang et al., 2003). This index consists of 10 items rated on the scale of 0-3 (from 0=not at all to 3=very much). The higher score, the more behavioral symptoms of ADHD. In this study, CHI scores were also treated as both continuous and categorical variables. Toddlers were classified using a clinically significant cut-off of the CHI scores of >15. The internal consistency (Cronbach's alpha) of the scales was 0.84, and the reliability was 0.89. The sensitivity and specificity for CHI cutoff >15 were 0.76 and 0.92, respectively.

2.3. Confounding variables

2.3.1. Demographic characteristics

Information on maternal age, education (≤9 or >9 years of completed schooling) and income (<2000 or ≥2000 RMB Yuan/month) was obtained by face to face interviews at 30-34 gestational weeks.

2.3.2. Perinatal factors

Prepregnancy body mass index (BMI), gestational weight gain (GWG), parity (primipara or multipara) and pregnancy complications (including hypertension, diabetes mellitus, moderate or severe anemia, glandula thyreoidea disease, abnormal heart function and intrahepatic cholestasis of pregnancy) were obtained through medical records. Participants were divided into underweight (<18.5), normal weight (18.5-23.9), and overweight or obese (≥24.0) based on their BMI (Coorperative Meta-analysis Group of China Obesity Task Force, 2002). The GWG was categorized as insufficient, normal and excessive weight gain based on the authoritative recommendation for Chinese women (Wong et al., 2000).

2.3.3. Perinatal lifestyle

Data on maternal alcohol consumption during up to 6 months before pregnancy (none or any), perinatal folic acid supplementation (none or any), vitamin D supplementation during pregnancy (< or ≥ two months), paternal smoking (less or more than 6 cigarettes daily) and alcohol consumption (none or any) during up to 6 months before pregnancy was collected by self-report in the interviews.

2.3.4. Postnatal factors

Information on breastfeeding and maternal postnatal depressive symptoms was obtained through telephone interview at 3 mo postpartum. Maternal postnatal depressive symptoms were assessed using the Edinburgh Postnatal Depression Scale (EPDS) (Chinese version), with a widely used 10-item self-report questionnaire that has been validated (Cox et al., 1987). Maternal postnatal depression was defined as the EPDS score of more than 12. The questionnaire showed good internal consistency (Cronbach's $\alpha = 0.85$-0.91), and the sensitivity and specificity for EPDS cutoff >12 was 0.86 and 0.78, respectively.

2.3.5. Birth outcomes

Gestational age at birth, infant gender, fetal growth and birth season was assessed through medical records. The gestational age at birth was categorized as <37 (preterm birth) or ≥37 gestational weeks. Small for gestational age (SGA) was defined as birth weight <10th percentile of distribution for gestational age (Zhang, 1992). Birth season was categorized as spring (March, April, May), summer (June, July, August), autumn (September, October, November) and winter (December, January, February).

2.4. Statistical analysis

Demographic characteristics and clinic data were compared between mother-infant pairs with and without prenatal depression using Student's t-test for continuous variables and Chi square analysis for categorical variables. Risks of clinically significant ADHD symptoms in toddlers by the characteristics of mother-infant pairs were evaluated using logistic regression models.

We conducted a series of analyses to test our hypotheses. First, we studied the relations between maternal depressive symptoms and ADHD symptoms in toddlers. Multiple linear regression models were performed to evaluate the regression coefficients between MD scores on CES-D and ADHD symptoms scores in toddlers. Multiple logistic regression models were performed to assess the risk of clinically significant ADHD symptoms among the toddlers of depressed mothers, after taking into account the confounding effects of covariates. We adjusted for a range of potential confounders in multiple regression including demographic characteristics such as maternal age, education and income; perinatal factors such as pre-pregnancy BMI, GWG, parity, and pregnancy complication; perinatal lifestyle such as maternal alcohol consumption, vitamin D supplementation, paternal alcohol consumption and smoking; birth outcomes such as delivery model, infant gender, birth season, gestational weeks, SGA and cord blood 25(OH)D; and postnatal factors such as postnatal depression and breastfeeding at 3 months. Sensitivity analyses were conducted by adjusting for propensity scores as a continuous or categorical variable with three levels (Brookhart et al., 2006). Furthermore, stratified by cord blood 25(OH)D levels, we compared the associations of MD with ADHD symptoms between toddlers with VDD at birth and those with VDA at birth. Finally, we investigated whether the association between maternal depression and ADHD symptoms in toddlers was modified by vitamin D status at birth.

Statistical analyses were performed using SPSS version 21.0. All analyses were two-tailed, and the statistical significant level was set at $P < 0.05$.

3. Results

3.1. Characteristics of the study population

Of the 1 434 eligible mother-infant pairs, 1 125 (78.5%) completed the 4-year follow-up and had full data in this study. There was no significant difference in demographic characteristics and clinic data, except for education, between participating mothers and those lost to follow-up. Participating mothers had higher mean values of education years.

In this study, one hundred forty-five (12.9%) pregnant women experienced major depressive symptoms during pregnancy, and they were more likely to suffer from postnatal depression. The prevalence of preterm birth and SGA was 4.1% and 7.6%, respectively. The mean of 25 (OH)D concentrations in newborns was 40.34 nmol/L (SD=20.73) and 25.3% of babies had VDD. Compared to healthy pregnant women, depressed pregnant women were younger and of less GWG, and had more alcohol consumption and husband smoking before pregnancy, but less gestational weeks at birth; they were also more likely to have an infant with cesarean section, less birth weight and lower cord blood 25 (OH)D concentrations, but these differences did not reach statistical significance. There were 138 children (12.3%) above the clinically

significant cutoff for ADHD symptoms (Table 1).

3.2. Maternal depression and ADHD symptoms in toddlers

The prevalence of clinically significant ADHD symptoms in toddlers of MD was significantly higher than those of non-MD (NMD) (20.1% vs 11.1%, p=0.003). In binary analysis, toddlers with SGA, or delivered by cesarean section, or primiparous mothers, or with paternal smoking more than 6 cigarettes daily were in a significantly higher risk for clinically significant ADHD Symptoms (Table 2).

The analyses show no significant collinearity among predictors and covariates as the indexes of tolerance are both more than 0.95 and the indexes of VIF are both less than 1.1. The association between MD and ADHD symptoms in toddlers slightly reduced, but remained significant after adjusted for all potential confounders (Table 3). All following analyses were conducted with adjustment for all these potential confounders.

3.3. Sensitivity analyses and interaction effects

We conducted the sensitivity analyses by adjusting for propensity scores in the multiple regression models. The association between MD and ADHD symptoms remained statistical significant (adjusted β= 0.119, 95% CI: 0.068-0.170, $p<0.001$; adjusted RR= 1.85, 95% CI: 1.17-2.93, p=0.009) when adjusting for propensity scores as a continuous variable or categorical variable with three levels. Additionally, we restricted our analyses to those toddlers (n=997) without preterm birth and SGA, to avoid the confounding by adverse birth outcomes. The associations were still significant (adjusted β= 0.099, 95% CI: 0.046 - 0.152, $p<0.001$; adjusted RR= 1.64, 95% CI: 1.00 - 2.68, p=0.049).

Although there was no significant association between cord blood 25 (OH)D levels and ADHD symptoms in toddlers, significant interactive effects of MD and newborn vitamin D status on ADHD symptoms were found. The association between MD and ADHD symptoms in toddlers was modified by vitamin D levels in newborns.

3.4. The role of early-life vitamin D in the association between MD and ADHD symptoms in toddlers

Stratified by cord blood 25(OH)D levels, the role of early-life vitamin D in the association between MD and ADHD symptoms in toddlers was further examined. Among toddlers with VDD at birth, the association of maternal depressive symptoms scores with ADHD symptoms scores remained significant, and toddlers of depressed mothers had a significantly increased risk for clinically significant ADHD Symptoms. However, among toddlers with VDA at birth, there was no significant association between MD and ADHD symptoms (Figure 2).

The ADHD symptoms scores and proportion of clinically significant ADHD symptoms in toddlers of NMD and VDD at birth were not significantly different from that in toddlers of NMD and VDA at birth. In order to increase the statistical power, these toddlers were pooled as the reference category. Compared with the reference group, toddlers of MD and VDA at birth had a slight increase by 1.36 points (95% CI: 0.47 - 2.25) in the ADHD symptoms scores, and those of MD and VDD at birth had a strong increase by 2.35 points (95% CI: 1.03 - 3.68) (Figure 3A). The adjusted risk of clinically significant ADHD symptoms in toddlers of MD and VDA at birth did not reach statistical significant; however, the adjusted risk in toddlers of MD and VDD at birth significantly increased (**Figure 3B**).

4. Discussion

There was a significant association between MD and the early symptoms of ADHD in toddlers. Toddlers with both MD and neonatal VDD had more significant symptoms of ADHD and were in a higher risk of clinically significant ADHD symptoms than toddlers of non-depressed

Table 1
Characteristics of the study sample by maternal depression.

Characteristics	Total (N = 1125)	Maternal depression score on CES-D		P value
		≥ 16 (n = 145)	< 16 (n = 980)	
Sociodemographic characteristics				
Maternal age [Mean (SD)] (years)	27.73 (3.45)	27.15 (2.87)	27.81 (3.52)	0.031
Maternal education <9 years [*n* (%)]	183 (16.3)	30 (20.7)	153 (15.6)	0.122
Maternal income <2000 yuan/RMB [*n* (%)]	164 (14.6)	26 (17.9)	138 (14.1)	0.220
Perinatal health status				
Prepregnancy BMI [Mean (SD)] (kg/m^2)	20.25 (2.51)	20.18 (2.28)	20.26 (2.54)	0.720
GWG [Mean (SD)] (kg)	16.78 (4.74)	15.73 (4.43)	16.93 (4.77)	0.004
Primipara [*n* (%)]	994 (88.4)	129 (89.0)	865 (88.3)	0.806
Complication of pregnancy[a] [*n* (%)]	166 (14.8)	23 (15.9)	143 (14.6)	0.687
Perinatal lifestyle				
Maternal alcohol consumption[b] [*n* (%)]	170 (15.1)	31 (21.4)	139 (14.2)	0.024
Maternal vitamin D supplementation[c] [*n* (%)]	572 (50.8)	65 (44.8)	507 (51.7)	0.120
Paternal alcohol consumption[b] [*n* (%)]	914 (81.2)	119 (82.1)	795 (81.1)	0.785
Paternal smoking[d] [*n* (%)]	254 (22.6)	49 (33.8)	205 (20.9)	0.001
Folic acid supplementation [*n* (%)]	584 (51.9)	76 (52.4)	508 (51.8)	0.897
Birth outcomes				
Cesarean section [*n* (%)]	633 (56.3)	91 (62.8)	542 (55.3)	0.091
Female infant [*n* (%)]	532 (47.3)	77 (53.1)	455 (46.4)	0.133
Birth during winter or spring [n(%)]	521 (46.3)	70 (48.3)	451 (46.0)	0.611
Gestational weeks [Mean (SD)] (weeks)	38.97 (1.41)	38.48 (1.84)	39.04 (1.32)	<0.001
Birth weight [Mean (SD)] (g)	3415 (424)	3359 (492)	3423 (413)	0.091
SGA [n(%)]	86 (7.6)	16 (11.0)	70 (7.1)	0.100
Cord blood 25(OH)D [Mean (SD)] (nmol/L)	40.34 (20.73)	37.50 (19.09)	40.76 (20.94)	0.076
Cord blood 25(OH)D <25 nmol/L [*n* (%)]	285 (25.3)	44 (30.3)	241 (24.6)	0.137
Postnatal factors				
Breastfeeding at 3 months [*n* (%)]	311 (27.6)	46 (31.6)	265 (27.0)	0.299
Postnatal depression [*n* (%)]	192 (17.1)	34 (23.4)	158 (16.1)	0.029
ADHD symptoms scores [Mean (SD)]	10.68 (4.34)	12.20 (4.88)	10.45 (4.21)	<0.001
Clinically significant ADHD Symptoms [*n* (%)]	138 (12.3)	29 (20.1)	109 (11.1)	0.003

Note: ADHD = attention-deficit/hyperactivity disorder; BMI = body mass index; CES-D = Center for Epidemiological Studies Depression Scale; GWG = gestational weight gain; RMB = renminbi; SD = standard deviation; SGA = small for gestational age.

[a] Pregnancy complications included hypertension, diabetes mellitus, moderate or severe anemia, glandula thyreoidea disease, abnormal heart function and intrahepatic cholestasis of pregnancy.

[b] Alcohol consumption was defined as any alcohol consumption during up to 6 months before pregnancy.

[c] Maternal vitamin D supplementation was identified as supplementation lasted more than two months during pregnancy.

[d] Smoking was defined as more than 6 cigarettes daily during up to 6 months before pregnancy.

Table 2
Relative risk of ADHD symptoms in toddlers by characteristics of mother-infant pairs.

Characteristics	Clinically Significant ADHD Symptoms (n = 138)			
	%	RR	95% CI	*P* value
Sociodemographic characteristics				
Maternal age ≥30 y (Ref: <30 y)	11.6	0.91	0.60, 1.39	0.676
Maternal education <9 y (Ref: ≥9 y)	9.8	0.75	0.44, 1.26	0.275
Family income <2000 yuan/RMB (Ref: ≥2000 yuan/RMB)	10.4	0.80	0.47, 1.37	0.803
Perinatal factors				
Prepregnancy underweight (Ref: normal weight)	10.8	0.85	0.55, 1.32	0.459
Prepregnancy overweight/obesity (Ref: normal weight)	14.5	1.18	0.62, 2.25	0.617
Inadequate GWG (Ref: adequacy)	9.9	0.75	0.47, 1.21	0.244
Excessive GWG (Ref: adequacy)	13.6	1.08	0.71, 1.63	0.721
Primipara (Ref: multipara)	13.2	2.69	1.23, 5.89	0.013
Any of pregnancy complication[a] (Ref: none)	14.5	1.25	0.78, 2.01	0.352
Prenatal depression (Ref: non-depression)	20.1	2.00	1.27, 3.14	0.003
Perinatal lifestyle				
Any of maternal alcohol consumption (Ref: none)	15.9	1.44	0.91, 2.27	0.120
Maternal vitamin D supplementation ≥2 mo (Ref: <2 mo)	11.9	0.93	0.65, 1.33	0.694
Any of paternal alcohol consumption (Ref: none)	12.3	0.99	0.63, 1.57	0.978
Paternal smoking ≥6 cigarettes daily (Ref: <6 cigarettes daily)	16.9	1.67	1.13, 2.46	0.011
Any of folic acid supplementation (Ref: none)	13.5	1.28	0.89, 1.83	0.181
Birth outcomes				
Cesarean section (Ref: vaginal delivery)	14.4	1.59	1.09, 2.31	0.015
Female infant (Ref: male)	12.8	1.09	0.77, 1.56	0.618
Birth during winter or spring (Ref: summer-autumn)	10.6	0.74	0.52, 1.07	0.105
Gestational weeks ≥37 w (Ref: <37 w)	15.2	1.30	0.57, 2.96	0.534
SGA (Ref: non-SGA)	12.9	3.03	1.10, 8.45	0.033
Cord blood 25(OH)D <25 nmol/L(Ref: ≥25nmol/L)	11.9	0.96	0.63, 1.45	0.841
Postnatal factors				
Breastfeeding at 3 mo (Ref: bottle-feeding or mixed-feeding)	6.0	0.38	0.22, 0.66	0.001
Postnatal depression (Ref: non-depression)	13.1	1.11	0.58, 2.12	0.754

Note: ADHD = attention-deficit/hyperactivity disorder; GWG = gestational weight gai; Ref = reference; RMB = renminbi; SGA = small for gestational age.
[a] Pregnancy complications included hypertension, diabetes mellitus, moderate or severe anemia, glandula thyreoidea disease, abnormal heart function and intrahepatic cholestasis of pregnancy.

Table 3
Associations between maternal depression and ADHD symptoms in toddlers.

Model	ADHD Symptoms Scores			Clinically Significant ADHD Symptoms		
	β	95% CI	*P* value	RR	95% CI	*P* value
Model 1	0.132	0.08, 0.18	<0.001	2.13	1.35, 3.38	0.001
Model 2	0.133	0.08, 0.18	<0.001	2.12	1.34, 3.37	0.001
Model 3	0.123	0.07, 0.17	<0.001	1.94	1.21, 3.10	0.006
Model 4	0.122	0.07, 0.17	<0.001	1.87	1.18, 2.98	0.008
Model 5	0.120	0.07, 0.17	<0.001	1.75	1.10, 2.81	0.019

Note: ADHD = attention-deficit/hyperactivity disorder.
Model 1 adjusted for demographic characteristics including maternal age, education and income.
Model 2 further adjusted for perinatal factors including prepregnancy BMI, GWG, parity, and pregnancy complication.
Model 3 further adjusted for perinatal lifestyle including maternal alcohol consumption, vitamin D supplementation, folic acid supplementation, paternal alcohol consumption and smoking.
Model 4 further adjusted for birth outcomes including delivery model, infant gender, birth season, gestational weeks, SGA, cord blood 25(OH)D.
Model 5 further adjusted for postnatal factors including postnatal depression and breastfeeding at 3 months.

mothers. For toddlers of depressed mothers, neonatal VDA appeared to alleviate the detrimental effect induced by MD. Given the limited sample size, we cannot examine the relative impact of MD and neonatal vitamin D status on ADHD symptoms. However, their effects are in opposite directions and suggest that neonatal VDA at least partially mitigates the effect of MD. To the best of our knowledge, this is the first report about the protective role of early-life VDA against adverse effects of prenatal psychological stress on brain development in children.

The relationship between prenatal depression and offspring's ADHD symptoms is consistent with previous studies that attention dysfunction or delayed cognitive development is associated with maternal psychological stress (Evans et al. 2012, Rodriguez and Bohlin 2005, Li et al. 2010). Little is known about the mechanisms that may underlie the link between maternal stress and fetal brain development in humans, but there are several possible pathways. Fetal overexposure to glucocorticoids could occur through increases in maternal cortisol associated with depression or stress, which then crosses the placenta into the fetal environment (Arborelius et al. 1999). Depression or stress may itself increase transplacental transfer of maternal cortisol to the fetal compartment. Some evidence in rat (Mairesse et al. 2007) and human (O'Donnell et al., 2012) shows that prenatal stress during late pregnancy can down-regulate placental 11b-hydroxysteroid dehydrogenase type II (11b-HSD2), the barrier enzyme which converts cortisol to the inactive cortisone. A deleterious direct effect of cortisol on the developing brain is one of the possible explanations. Another possible mechanism is that maternal stress is associated with raised levels of inflammatory cytokines such as interleukin-6 (IL-6) (HaeriBaker and Ruano 2013), which may directly induce fetal injury or insults in neuroendocrine structures (Stolp et al. 2011). Other possibilities include effects of estrogen, serotonin, epigenetic changes, and shared genetic risk of MD and offspring attentional dysfunction (Bonnin et al., 2011; Lahey et al., 2011).

Rodent studies have confirmed that low maternal vitamin D during pregnancy was linked to atypical behavior among adult offspring, which has important ramifications for the developing brain morphology (Eyles et al. 2003). Expression of the vitamin D receptor (VDR) in the hippocampus and prefrontal cortex-brain regions suggested that vitamin D may influence learning, memory, attention and executive control (Eyles et al. 2005). Accruing epidemiological evidence indicates that prenatal lower vitamin D levels may be associated with delayed neuropsychiatric and neurocognitive development in offspring (Whitehouse et al. 2012, Morales et al. 2015). However, randomized controlled trials about the effect of vitamin D supplementation in early life on neurodevelopment outcomes in offspring was limited. Although our analysis was limited to draw firm conclusions due to relatively low power, our results suggest that early-life VDA may be important in protecting the brain from a neurological insult.

The mechanisms about how early-life VDA could mediate the effect of prenatal stress on the fetal brain are unknown. Indeed, vitamin D is a powerful immune modulator and has been shown in animal and in vitro work that vitamin D supplementation inhibits proinflammatory cytokine production (Zhang et al. 2012), which may be involved in the mediating mechanisms. Additionally, the coexistence of glucocorticoid receptors and VDR in hippocampus and prefrontal regions suggest that fetal vitamin D status may regulate the programming effect on brain development induced by excessive glucocorticoids following prenatal

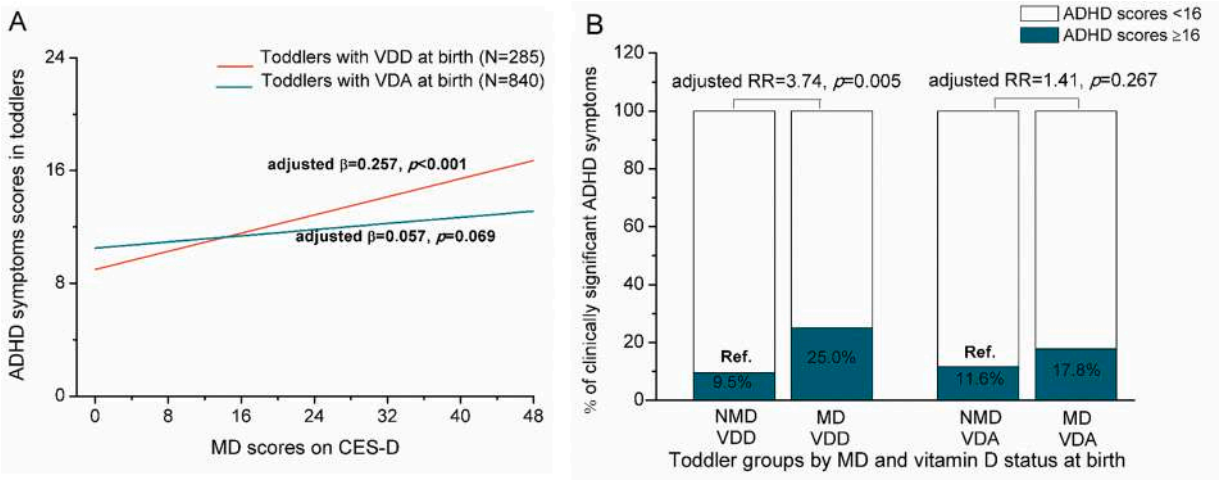

Figure. 2. Associations between MD and ADHD symptoms stratified by vitamin D status at birth. For toddlers with VDD at birth, MD was significantly associated with ADHD symptoms (adjusted β=0.257, 95% CI: 0.163-0.351, $p<0.001$) (**A**), and the risk of clinically significant ADHD symptoms significantly increased (adjusted RR=3.74, 95% CI: 1.49-9.41, $p=0.005$) in toddlers of MD relative to those of NMD (**B**). MD, maternal depression; NMD, non-maternal depression; VDD,vitamin D deficiency; VDA, vitamin D adequacy.

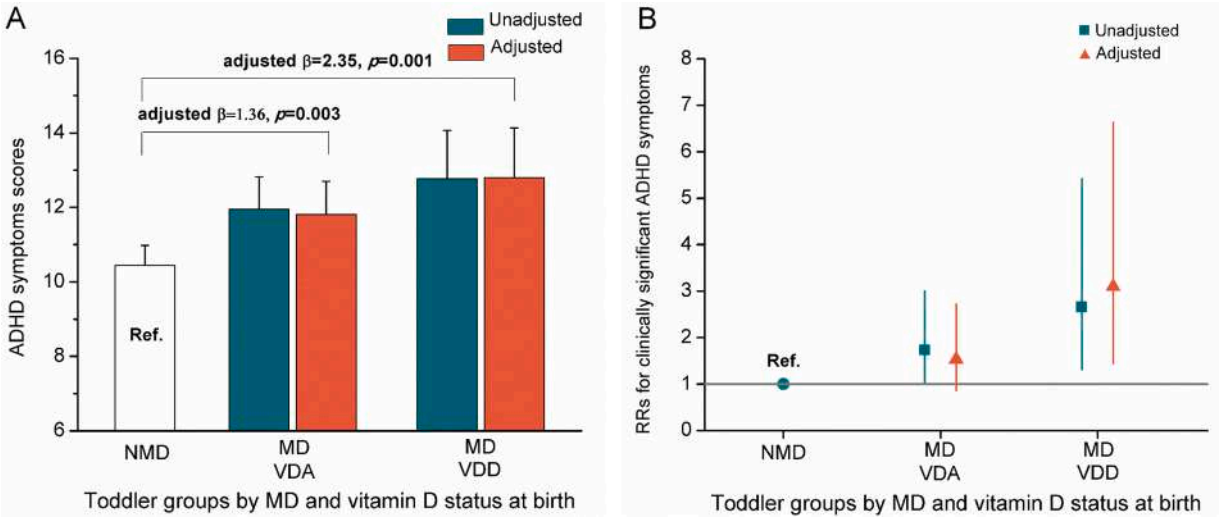

Figure. 3. ADHD symptoms in toddlers by MD and Vitamin D status at birth. Compared to toddlers of NMD, toddlers of MD and VDA at birth had a slight increase by 1.36 points (95% CI: 0.47-2.25, p=0.003 for adjustment) in ADHD symptoms scores, and those of MD and VDD at birth had a strong increase by 2.35 points (95% CI: 1.03-3.68, p=0.001 for adjustment) (A). The risk of clinically significant ADHD symptoms significantly increased (adjusted RR=3.10, 95% CI: 1.44-6.63, p=0.004) in toddlers of MD and VDD at birth, but not significantly increased in toddlers of MD and VDA at birth (adjusted RR=1.53, 95% CI: 0.86-2.72, p=0.144) (B), relative to those of NMD. MD, maternal depression; NMD, non-maternal depression; VDD, vitamin D deficiency; VDA, vitamin D adequacy.

stress (Eyles et al. 2005, Charil et al. 2010).

This study has three major strengths. This is the first study examining the mediating effect of neonatal vitamin D levels on the relationship between MD and later neurodevelopment in offspring. The association between independent and dependent variables were analyzed as both continuous and categorical variables, which strengthen the conclusion. Finally, adjustment for a wide range of sociodemographic characteristics, progestational, prenatal and postnatal covariates reduced the residual confounding in this study to a great extent.

Several limitations should also be acknowledged in this study. A sample size of 1125 is robust enough to detect an effect size of 0.05 with 80% power and 95 % confidence. However, only 145 mothers (12.9% of the sample) experienced depressive symptoms. When combined with vitamin D status at birth, some subgroups were quite small, limiting statistical power. Child behavior was assessed by the parents, and hence reporting bias may have influenced the results of this study. It is possible that depressed mothers reported more attention problems in their children than mothers without depression (Najman et al., 2000), which may result in overestimating the effect of prenatal depression in offspring's attention problems. However, in this study, the association between postnatal depression and ADHD symptoms was not observed. We speculated the reporting bias was relatively small. Although we have adjusted for many potential confounders, the changes of factors over time were not completely controlled. Moreover, the lack of data on sunlight exposure and dietary intake of mothers may result in selection bias, despite prepregnancy BMI, vitamin D supplementation and gestational weight gain were taken into consideration. Finally, data on the MD in early pregnancy, heritable and parenting factors and other risk traits or behaviors were unavailable, which may result in some residual confounding. Additionally, we also could not exclude the effect of other comorbid psychopathology, as prenatal anxiety.

5. Conclusion

This prospective study indicated that toddlers with both MD and neonatal VDD had more significant symptoms of ADHD and were at risk of higher clinically significant ADHD symptoms than toddlers of non-depressed mothers. However, the detrimental effect of MD on offspring's ADHD symptoms attenuated in toddlers with neonatal VDA relative to those with neonatal VDD. In future studies, it is vitally important to confirm the potential protective effects of vitamin D supplementation during pregnancy on impaired neurodevelopment in offspring induced by maternal psychological stress.

Author Contributions

P.Z., R.X.T. and F.B.T. conceived and designed the study. S.S.M., D. M.Z., R.X.T., W.J.Y., K.H. and J.H.H. involved in data collection and statistical analysis. S.S.M., D.M.Z., R.X.T. and P.Z. drafted the manuscript. P.Z., R.X.T. critically reviewed the manuscript for accuracy and intellectual content. All authors read and approved the final draft.

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Declaration of Competing Interest

All authors declare that there is no conflict of interest.

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