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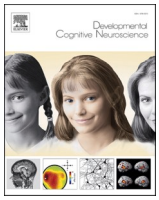

Effects of single and combined exposure to lead and stress during pregnancy on offspring neurodevelopment

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ABSTRACT

Objective: To assess associations of single and combined exposures to lead and stress during different stages of pregnancy with offspring neurodevelopment.
Methods: We measured prenatal lead (maternal blood-lead in early-pregnancy and umbilical-cord-blood-lead) and maternal stress levels in Shanghai-Birth-Cohort from 2013 to 2016. Maternal stress was assessed using Center-for-Epidemiological-Studies-Depression-Scale and Self-Rating-Anxiety-Scale during mid-pregnancy. The Ages-Stages-Questionnaires-3 (at 6/12-months-of-age) and Bayley-III (at 24-months-of-age) were both used to assess neurodevelopment.
Results: A total of 2132 mother-child pairs with both prenatal lead and stress measurements were included. The geometric-means of blood-lead in early-pregnancy and cord-blood-lead were 1.46 μg/dL and 1.33 μg/dL, respectively. Among the study women, 1.89 % and 0.14 % were screened positive for depression and anxiety. Adjusting for related confounders, the combined exposures had stronger adverse associations with offspring social-emotional skills than single exposures; and the combined exposure in early-pregnancy was associated with greater neurodevelopmental differences than combined exposure around-birth, especially in social-emotion at 24 months-of-age [β (95 %CI): − 10.48(−17.42, −3.54) *vs.* − 5.95(−11.53, −0.36)].
Conclusions: Both single and combined prenatal exposures to lead/stress impaired infant neuro-development, and the effects of combined exposure may be more profound than single exposures. Combined exposure in early-pregnancy may be associated with worse neurodevelopmental outcomes than combined exposure around-birth, especially in social-emotional development.

1. Introduction

As a major public health concern, lead exposure may affect multiple organs/systems (WHO, 2016; Herbicides, 2017; Organization, 2017). There is no safe level of lead exposure for human body, even low-level lead exposure is hazardous over time (Li et al., 2009; Sun et al., 2010). During pregnancy, lead is mobilized from maternal bone stores at an accelerated rate and is transferred to the fetus, making the fetus exposed to endogenous lead accumulated in the maternal body before pregnancy and exogenous lead in the environment (Gulson et al., 1997). Due to the immature blood-brain barrier, the central nervous system (CNS) of the fetus is vulnerable to lead exposure (Allen, 2015), resulting in the inhibition of fetal brain plasticity, and potential neurobehavioral dysfunctions in the future (Dorea, 2019). Relevant epidemiological studies found that lead exposure in early development was associated with cognitive deficits and behavioral abnormalities in later life (Eubig et al., 2010). Animal studies found that lead exposure in early life could cause pathological changes of synaptic structures, which were related to

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the changes of key synaptic protein levels, resulting in impairments in neural connection and synaptic transmission (Gąssowska et al., 2016).

On the other hand, with the acceleration of the pace of life, people are increasingly suffering from mental stress. Pregnant women not only have to endure the psychological distress and fluctuating emotions caused by pregnancy-related physiological changes, but also need to bear the mental pressure due to changes of maternal roles in the society or families. Previous prospective studies have shown that depression and anxiety during pregnancy are risk factors for adverse outcomes for mothers and children, inducing emotional, behavioral and cognitive problems in children (Bergh et al., 2005; Talge et al., 2007; Glover, 2011), and other animal and human studies also demonstrated that such adverse effects were mediated by the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Barbazanges et al., 1996; A et al., 1992).

Because the prenatal stage is a critical period of the formation and development of the CNS (Selevan et al., 2000; Rice and Barone, 2000), the embryo or fetus is susceptible to environmental exposure factors (Adams et al., 2000). The complexity of the living environment calls for attention to be paid on the impacts of prenatal multiple exposures on fetal neurodevelopment. Although there is significant evidence of the cognitive deficits induced by prenatal single lead or stress exposure, there has been considerably less evaluation of realistic combinations. Therefore, using a large-scale prospective birth-cohort study, we examined prenatal lead and stress exposure levels in pregnant women participating in the Shanghai birth cohort (SBC) study, and aimed to evaluate the associations of single/combined prenatal exposures to lead and stress at different prenatal stages with offspring neurodevelopment. We hypothesized that, combined prenatal exposure to lead and stress may induce worse neurodevelopmental outcomes compared with single exposures. In addition, since the responses of embryos and fetuses (at different prenatal stages) to external exposures may be different, we further hypothesized that combined exposure to lead and stress at different prenatal stages had different impacts on offspring neurodevelopment.

2. Material and methods

2.1. Study design

Participants of this study were women of the couples in the pregnancy cohort of the SBC (Zhang et al., 2019). In brief, from 2013 to 2016, pregnant women who visited for booking for prenatal care were approached for enrollment at six SBC participating hospitals in the four administrative districts of Shanghai city (including two urban districts, one suburban district, and one semi-rural district). The inclusion criteria included: (1) women $\geq$ 20 years of age; (2) women or their husbands were registered Shanghai residents; (3) their prenatal care and deliveries were planned at the SBC participating hospitals; (4) the families planned to stay in Shanghai for at least 2 years; (5) the mother-child pairs would be followed-up for at least 2 years. Women were invited for the second study visit at 24–28 weeks of gestation and for the third visit at 32–36 weeks of gestation. After delivery, women were contacted by the trained research staff. During the postnatal stage, infants and their mothers were invited to return to the delivery hospitals at 42 days after birth, and at 6, 12 and 24 months of age. Questionnaires were completed at each follow-up visit and medical records were reviewed and abstracted if needed. Women were assessed on their depression and anxiety levels in the second trimester, and children were invited to have neurodevelopmental assessments at 6 and 12 months using the Ages & Stages Questionnaires®, Third Edition (ASQ-3TM) and Ages & Stages Questionnaires®: Social-Emotional (ASQ:SE), and at 24 months using the Bayley Scales of Infant development (BSID) (3rd Edition) (Bayley III). Biological samples (including maternal venous blood in the first trimester, umbilical cord blood at birth) were collected during the research process and blood lead levels were measured using the whole blood. The collection and treatment of biological samples used unified standard operating procedures. The subjects' basic information including information on past medical history, family history, social environment, living environment, nutrition, behavior, physical activity and psychological stress were collected. Finally, A total of 5749 couples who met the inclusion criteria were recruited. Except for 1622 women with high-risk pregnancies, and women who withdrew during pregnancy and experienced miscarriages or stillbirths, a total of 3692 mothers delivered their babies (Fig. 1).

This study was approved by the Institutional Review Boards of Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine. Pregnant women were informed of the purposes and procedures of the study and signed an appropriate informed consent form at the beginning of the study. Details on the inclusion and exclusion criteria were shown in the supplemental Fig. 1.

2.2. Measurements of lead in maternal blood in the first trimester and in umbilical cord blood

Maternal venous blood (gestational week $\leq$16 weeks) and umbilical cord blood were collected using trace metal-free ethylenediaminetetraacetic acid (EDTA)-coated tubes by trained phlebotomists in the first trimester and at delivery, respectively. Whole blood lead levels were determined using inductively coupled plasma mass spectrometry (ICP-MS) (7500ce, Agilent, USA) and using validated, previously described laboratory methods (CDC, 2021).

Quality Control (QC) was conducted using two levels of highly characterized QC materials (Trace elements whole blood L-1, 0.79–1.19 μg/dL, lot. 1406263, serorm, Norway; Trace elements whole blood L-2, 26.9–40.5 μg/dL, lot. 1406264, serorm, Norway) in each analytical run. The analytical method participated in external proficiency testing programs according to Clinical Laboratory Improvement Amendments requirements.

Blood specimens were not used for the blood lead analysis because of the following reasons: some whole-blood samples had micro-clotting; and whole blood volume was too low [because the collection of plasma and serum (*vs.* whole blood) was considered a priority in blood sample collection]. In addition, the last tube of whole blood was stored for future use. This made 1041 whole blood samples in the first trimester and 2140 cord blood samples tested. The comparisons of the mother-child pairs who had blood lead levels in the first trimester or had cord blood lead levels and the pairs who did not have were presented in Supplemental Table 1.

2.3. Assessment of maternal stress

In this study, we measured the levels of maternal anxiety and depression during mid-pregnancy to represent the levels of prenatal maternal stress. Stress is a multi-level concept, originating from the imbalance between environmental needs in the form of acute and chronic stressors and personal resources (such as the ability of coping with socio-economic conditions, personality characteristics and social support) (McEwen, 1998). Compared with the general population, pregnant women may suffer specific stress due to: concerns about their own health and the health of their fetuses, or about transformation of social and family relations (Alderdice et al., 2012). The persistence of prenatal stress at a high level may lead to depression and anxiety in pregnant women (Biaggi et al., 2016). Lobel et al. showed that the use of multidimensional measures (including levels of anxiety and depression in pregnancy) to assess the impact of prenatal maternal stress on birth outcomes could provide the most consistent and convincing evidence (Molfese et al., 1987). Therefore, maternal depression and anxiety levels during pregnancy were used to indicate prenatal maternal stress levels in this study.

The Center for Epidemiological Studies-Depression Scale (CES-D) and Self-rating Anxiety Scale (SAS) were used to evaluate maternal

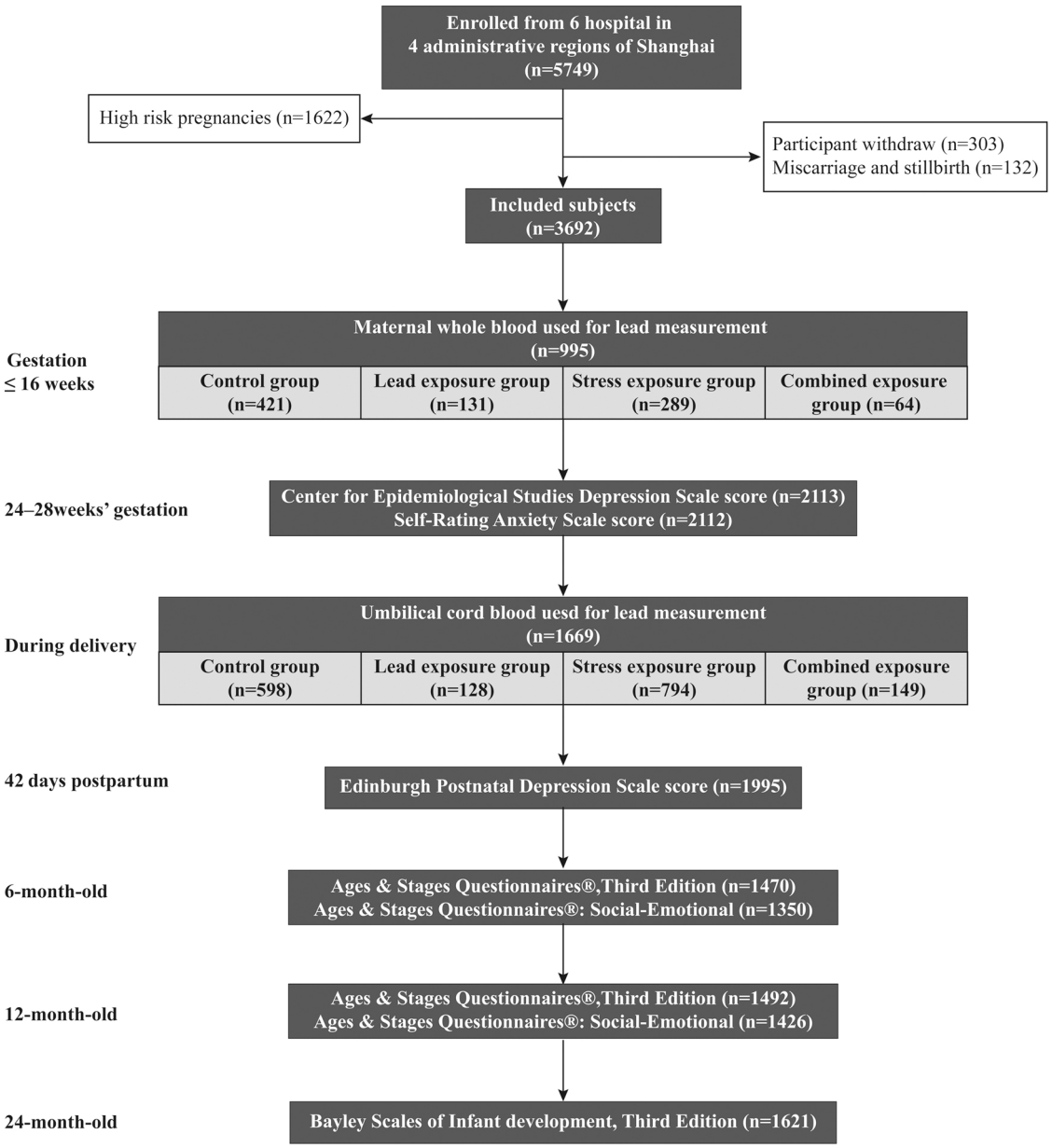

Fig. 1. The study design and the sample size of subjects.

stress levels at 24–28 weeks of gestation. Compared with other self-rating depression scales, CES-D focuses more on individual emotional experience. CES-D includes 20 items with each item focusing on one depression symptom. SAS uses a 4-graded level to evaluate the degree of the anxiety symptoms. Higher CES-D/SAS total scores were associated with higher depression/anxiety levels, respectively.

The Edinburgh Postnatal Depression Scale (EPDS) was used to assess maternal depression levels at 42 days after delivery. A large number of studies showed that the Chinese-version EPDS had good reliability and validity in screening depression during pregnancy and at the postpartum period (Lau et al., 2010; Zhao et al., 2015).

2.4. Assessment on offspring neurodevelopment

The Chinese versions of ASQ-3 and ASQ-SE are the screening tools widely used to assess the cognitive and social-emotional developmental levels in children aged 0–5 ½ years in China (Wei et al., 2015), and were used on infants of 6 and 12 months of age in this study. ASQ-3 is available for 20 age groups, covering the domains of communication, gross movement, fine movement, problem solving and personal social skills. ASQ-SE is available for 8 age groups, and mainly focuses on child social-emotional development. The Chinese versions of ASQ-3 and ASQ-SE are well-validated, and the cutoffs of the Chinese ASQ-3/SE are same as those of the English versions of ASQ-3/SE (Wei et al., 2015; Yue et al., 2019). Higher ASQ-3 scores are associated with higher neuro-developmental levels, and scores above the cut-off values indicate that the neurodevelopmental levels of young children are age-appropriate. However, for ASQ-SE, higher ASQ-SE scores are related with worse social-emotional development, and scores below the cut-off values indicate that the social-emotional development is age-appropriate.

Bayley III was used to evaluate children's cognitive development at the age of 24 months. Bayley III is internationally recognized as one of the most comprehensive developmental assessment instruments. Bayley III achieves good reliability and validity in China (Hua et al., 2019), and contains the five domains including cognition, language, motor, social-emotional and adaptive behavior. Higher developmental-quotient scores in these five domains are associated with higher neuro-developmental levels in each domain.

2.5. Statistical Analysis

Demographic characteristics of the mother-child pairs were analyzed using ANOVA and Chi-square analyses. The intraclass correlation coefficient (ICC) analysis was used for comparisons between groups and for testing inter-observer reliability and test-retest reliability when neurodevelopmental assessments were performed. So far, there is no reliable threshold for the harmful effects of lead. Studies have shown that children with blood lead levels < 5 μg/dL also have insufficient cognitive and academic skills (Binns et al., 2007; Lanphear et al., 2000), and a potential cutoff point of blood lead of 2 μg/dL was suggested due to significant differences observed in the degree of neurodevelopmental deficits induced by lead exposure when child blood lead levels ≥ 2 μg/dL *vs.* < 2 μg/dL (Hui et al., 2015). Considering the number of mother-child pairs with prenatal blood lead levels ≥ 5 μg/dL very limited in this SBC cohort (about 1.3 %), we grouped the mother-child pairs using the maternal/umbilical-cord blood lead of 2 μg/dL as the cutoff point to define the high/low prenatal lead exposure. In addition, sensitivity analyses were conducted to find the cutoff points of maternal anxiety/depression levels to define low/high anxiety/depression levels. We found similar trends in the relationships between maternal prenatal stress (depression/anxiety) and child neurodevelopment, and we finally used the cutoff point of the 70th percentiles of maternal depression/anxiety scores to define prenatal low/high maternal stress in consideration of more sample size in the high stress group and significant differences in neurodevelopmental outcomes induced by high *vs.* low stress groups (maternal depression or anxiety scores ≥P70 and <P70 were included in the high and low prenatal stress groups, respectively). Therefore, the subjects were divided into the following four groups including the control group (blood lead <2 μg/dL and both maternal depression and anxiety scores <P70), prenatal lead exposure group (blood lead ≥2 μg/dL and both maternal depression and anxiety scores <P70), prenatal stress group (blood lead <2 μg/dL and maternal depression or/and anxiety scores ≥P70) and prenatal lead & stress combined group (blood lead ≥2 μg/dL and maternal depression or/and anxiety scores ≥P70). Comparisons on ASQ-3/ASQ-SE scores and Bayley III scores among the four groups were analyzed by univariate and multivariate regression analyses so as to demonstrate the differences in neurodevelopmental scores between each exposure group and the control group. The results were expressed by β (95 % CI) and the *P*-values. The data were analyzed using Empower Stats (version 2.13.9, X&Y solutions, MA, USA) and R (version 3.6.0), and the graphs were generated by Origin Pro 2020b (Learning Edition, Version 9.7.5.184). $P < 0.05$ (two tail) was considered statistically significant.

3. Results

3.1. Prenatal lead exposure in early pregnancy/around birth and prenatal maternal stress levels

In this study, a total of 2132 mother-child pairs who had both prenatal lead and maternal depression/anxiety measurements were finally included in our analyses. The median (P25-P75) of maternal blood lead in early pregnancy was 1.47 (1.11, 1.94) μg/dL, and the blood lead levels of the 206 cases (21.57 %) were higher than 2 μg/dL. The median (P25-P75) of umbilical cord blood lead was 1.34 (1.00, 1.75) μg/dL, and the umbilical cord blood lead levels of the 277 cases (16.60 %) were higher than 2 μg/dL (Supplemental Table 1). The ICC of maternal blood lead in early pregnancy and umbilical cord blood lead was 0.818. Maternal CES-D scores were 10.14 ± 7.00, and a total of 40 mothers (1.89 %) were screened positive for depression. Maternal SAS scores were 30.30 ± 5.32, and a total of 3 mothers (0.14 %) were screened positive for anxiety. Maternal Edinburgh Postnatal Depression Scale scores were 5.45 ± 3.14. The ICC results between the three scales (CES-D, SAS, and Edinburgh Postnatal Depression Scale) were shown in Supplemental Table 2.

3.2. Associations of single/combined prenatal exposure to lead (exposed in early pregnancy) and stress with offspring neurodevelopment

The characteristics of the groups of single/combined prenatal exposure to lead (in early pregnancy) and stress were described in Table 1. In this study, the numbers of the mother-child pairs in the control group, high lead exposure (in early pregnancy) group, high stress group, and the combined exposure group were 421, 131, 289, and 64, respectively. Most of the mothers were Han ethnicity, with more than 2 years of living in Shanghai. More than half of the families had total annual household income between 100,000 to 300,000 RMB. Among the four exposure groups, the mothers of the combined exposure group had relatively low educational levels and family income levels. There were significant differences in blood lead levels, maternal depression and anxiety levels among the four exposure groups ($P < 0.001$). Significant differences in the ASQ-3 social-emotional scores among the four groups at 6-months-old and 12-months-old were observed ($P < 0.001$), and the average social-emotional scores of any exposure group were higher than that of the control group, indicating that average social/emotional developmental levels in the single or combined lead (in early pregnancy) and stress exposure groups were lower than that of the control group. The Bayley-III social-emotional scores at 24-months-old of age were significantly lower in the combined exposure group than those in the control group, indicating social behavior may be impaired in combined exposure groups ($P = 0.003$) (Table 1).

The effects of single/combined prenatal exposure to lead (in early pregnancy) and stress on offspring neurodevelopment were described in Fig. 2 (results from adjusted models) and Supplemental Table 3 (results from unadjusted and adjusted models). After adjusting for related confounders, at 6 months of age, compared with the control group, the social-emotional scores from the ASQ assessment showed an increase of 4.36 (95 % CI: 1.55, 7.18) points ($P = 0.003$) in the high stress group and an increase of 4.62 (95 % CI: −0.22, 9.45) points (at marginal significance, $P = 0.062$) in the combined exposure group. At 24 months of age, compared with the control group, in the adjusted models, the social-emotional scores from the Bayley-III assessment in the high stress group and the combined exposure group had changes of − 4.02 (95 % CI: −8.23, 0.19) points (at marginal significance, $P = 0.062$) and − 10.48 (95 % CI: −17.42, −3.54) points ($P = 0.003$), respectively (Fig. 2, Supplemental Table 3). However, compared with the control group, although the adaptive behavior scores from the Bayley-III assessment in the high lead group had a change of − 4.16 (95 % CI: −8.01, −0.32) points ($P = 0.034$) in the unadjusted models, the changes were observed non-significant in adjusted models in any of the exposure groups. All these results suggested that potential impairments in infant neurodevelopment (especially in social-emotional development) may be induced by single/combined prenatal exposure to stress/lead in early pregnancy, and the effects due to the combined exposure may be stronger than those by the single exposures.

3.3. Effects of single/combined prenatal exposure to lead (exposed around birth) and stress on offspring neurodevelopment

The characteristics of the groups of single/combined prenatal exposure to lead (around birth) and stress were described in Table 2. After grouping, the sample sizes of the control, high lead (around birth), high prenatal stress, and combined exposure groups were 598, 128, 794, and 149, respectively; and the medians of the umbilical cord blood lead levels of these four groups were 11.89 μg/dL, 25.29 μg/dL, 12.35 μg/dL and 23.98 μg/dL, respectively. Among the four exposure groups, the mothers of the combined exposure group had relatively low family income levels and educational levels. The high prenatal stress group and combined exposure group had higher scores of the CES-D and SAS. The ASQ-3 social-emotional scores at 12-months-old were significantly different among the four groups ($P < 0.05$), with the lowest average

Table 1
Basic characteristics of the mother-child pairs in groups of single/ combined prenatal exposure to lead (exposed in early pregnancy)/ stress [(N) Mean±SD or N (%)].

	Control (n=421)	High lead group (n=131)	High stress group (n=289)	Combined exposure group (n=64)	P*
Pre-pregnancy BMI	(279) 20.64 ±2.69	(93) 20.85 ±2.68	(190) 20.85 ±2.94	(47) 20.20 ±2.97	0.484
Maternal age (years)	(392) 28.57 ±3.43	(123) 29.20 ±3.83	(268) 28.19 ±4.05	(61) 28.49 ±3.82	0.099
Ethnicity					0.712
Han	390 (99.49 %)	122 (99.19 %)	265 (98.88 %)	61 (100.00 %)	
Minorities	2 (0.51 %)	1 (0.81 %)	3 (1.12 %)	0 (0.00 %)	
Living years in Shanghai					0.236
< 2 years	8 (2.04 %)	2 (1.63 %)	7 (2.61 %)	3 (4.92 %)	
2–5 years	17 (4.34 %)	4 (3.25 %)	20 (7.46 %)	5 (8.20 %)	
≥ 5 years	367 (93.62 %)	117 (95.12 %)	241 (89.93 %)	53 (86.88 %)	
Maternal education level					0.001
Junior High school or lower	6 (1.54 %)	3 (2.44 %)	18 (6.72 %)	2 (3.28 %)	
Senior high school	30 (7.67 %)	12 (9.76 %)	33 (12.31 %)	10 (16.39 %)	
Junior college	105 (26.85 %)	40 (32.52 %)	90 (33.58 %)	17 (27.87 %)	
College or university	218 (55.76 %)	63 (51.22 %)	109 (40.67 %)	30 (49.18 %)	
Postgraduate and above	32 (8.18 %)	5 (4.06 %)	18 (6.72 %)	2 (3.28 %)	
Birth head circumference (cm)	(343) 34.24 ± 1.10	(100) 34.30 ± 1.34	(237) 34.13 ± 0.98	(48) 34.41 ± 1.08	0.311
Birth length (cm)	(337) 49.90 ± 1.40	(98) 49.93 ± 1.26	(236) 49.92 ± 1.07	(48) 50.01 ± 1.29	0.956
Birth weight (kg)	(407) 3.42 ± 0.45	(129) 3.34 ± 0.48	(285) 3.40 ± 0.43	(60) 3.36 ± 0.43	0.339
Gestational age at birth (weeks)	(355) 39.34 ± 1.43	(93) 39.14 ± 1.32	(251) 39.42 ± 1.12	(48) 38.89 ± 1.34	0.038
Annual household income (RMB)					0.175
< 50,000	13 (6.37 %)	2 (3.85 %)	11 (7.75 %)	3 (9.38 %)	
50,000–100,000	32 (15.69 %)	7 (13.46 %)	35 (24.64 %)	8 (25.00 %)	
100,000–150,000	51 (25.00 %)	15 (28.85 %)	45 (31.69 %)	6 (18.75 %)	
150,000–300,000	92 (45.10 %)	23 (44.23 %)	40 (28.17 %)	14 (43.75 %)	
> 300,000	16 (7.84 %)	5 (9.61 %)	11 (7.75 %)	1 (3.12 %)	
Feeding style before 6 months of age					0.105
Formula feeding	15 (3.69 %)	10 (7.81 %)	21 (7.40 %)	6 (9.83 %)	
Breastfeeding	215 (52.82 %)	71 (55.47 %)	143 (50.35 %)	36 (59.02 %)	
Mixed feeding	177 (43.49 %)	47 (36.72 %)	120 (42.25 %)	19 (31.15 %)	
Maternal blood lead in early pregnancy (μg/dL)	(421) 1.32 ± 0.41	(131) 2.94 ± 1.71	(289) 1.30 ± 0.39	(64) 3.21 ± 2.34	< 0.001
Maternal CES-D scores	(421) 5.68 ± 3.20	(131) 5.62 ± 3.29	(281) 16.33 ± 6.07	(62) 17.18 ± 6.20	< 0.001
Maternal SAS scores	(421) 26.90 ± 3.07	(131) 26.76 ± 2.93	(283) 35.52 ± 4.28	(62) 34.81 ± 5.43	< 0.001
ASQ-3/SE scores at 6 months of age					
Communication	(420) 53.25 ± 6.64	(130) 54.15 ± 6.44	(287) 52.52 ± 7.37	(63) 53.10 ± 6.69	0.152
Gross motor	(420) 42.96 ± 12.78	(130) 44.35 ± 12.06	(286) 43.41 ± 12.12	(63) 45.16 ± 10.89	0.462
Fine motor	(419) 49.71 ± 10.85	(130) 51.06 ± 10.52	(287) 50.26 ± 10.18	(63) 49.87 ± 10.53	0.630
Personal-social	(420) 43.47 ± 13.82	(130) 44.73 ± 12.39	(287) 43.44 ± 13.34	(63) 45.65 ± 13.62	0.519
Problem solving	(420) 49.99 ± 12.09	(129) 52.13 ± 10.28	(287) 50.95 ± 11.03	(63) 51.11 ± 11.62	0.279
Social-emotional	(412) 16.01 ± 12.94	(129) 17.64 ± 14.21	(277) 20.70 ± 14.39	(63) 20.06 ± 12.22	< 0.001
ASQ-3/SE scores at 12 months of age					
Communication	(421) 55.67 ± 6.66	(128) 56.36 ± 6.04	(287) 54.09 ± 8.05	(64) 54.38 ± 8.29	0.019
Gross motor	(419) 46.83 ± 14.26	(129) 47.66 ± 13.49	(286) 46.34 ± 14.18	(64) 50.08 ± 10.56	0.249
Fine motor	(421) 53.48 ± 8.43	(129) 53.63 ± 7.94	(286) 52.75 ± 8.10	(64) 54.41 ± 6.45	0.404
Personal-social	(421) 49.38 ± 11.18	(128) 50.38 ± 9.99	(287) 48.61 ± 10.82	(64) 51.25 ± 10.84	0.220
Problem solving	(421) 50.83 ± 9.36	(128) 51.13 ± 9.05	(287) 50.15 ± 9.44	(64) 52.66 ± 9.88	0.257
Social-emotional	(415) 20.21 ± 14.64	(130) 20.77 ± 16.73	(285) 25.91 ± 16.04	(63) 24.37 ± 15.36	< 0.001
Bayley III DQ scores at 24 months of age					
Cognition	(360) 110.82 ± 21.50	(120) 114.79 ± 21.94	(245) 106.45 ± 22.58	(60) 111.17 ± 21.32	0.005
Language	(360) 94.80 ± 16.05	(120) 96.94 ± 18.02	(245) 91.73 ± 15.02	(60) 96.23 ± 18.38	0.015
Motor	(360) 106.78 ± 15.38	(120) 111.19 ± 17.34	(245) 106.15 ± 16.64	(60) 107.50 ± 17.14	0.037
Social-emotional	(364) 105.71 ± 17.75	(120) 107.67 ± 18.22	(248) 102.74 ± 17.14	(61) 99.18 ± 15.60	0.003
Adaptive behavior	(366) 106.33 ± 18.26	(122) 102.16 ± 22.16	(247) 103.94 ± 17.91	(61) 105.23 ± 17.59	0.147

P * : In the Chi-square test, Fisher exact test was used when more than 20 % expected frequencies < 5 or any expected frequencies < 1.

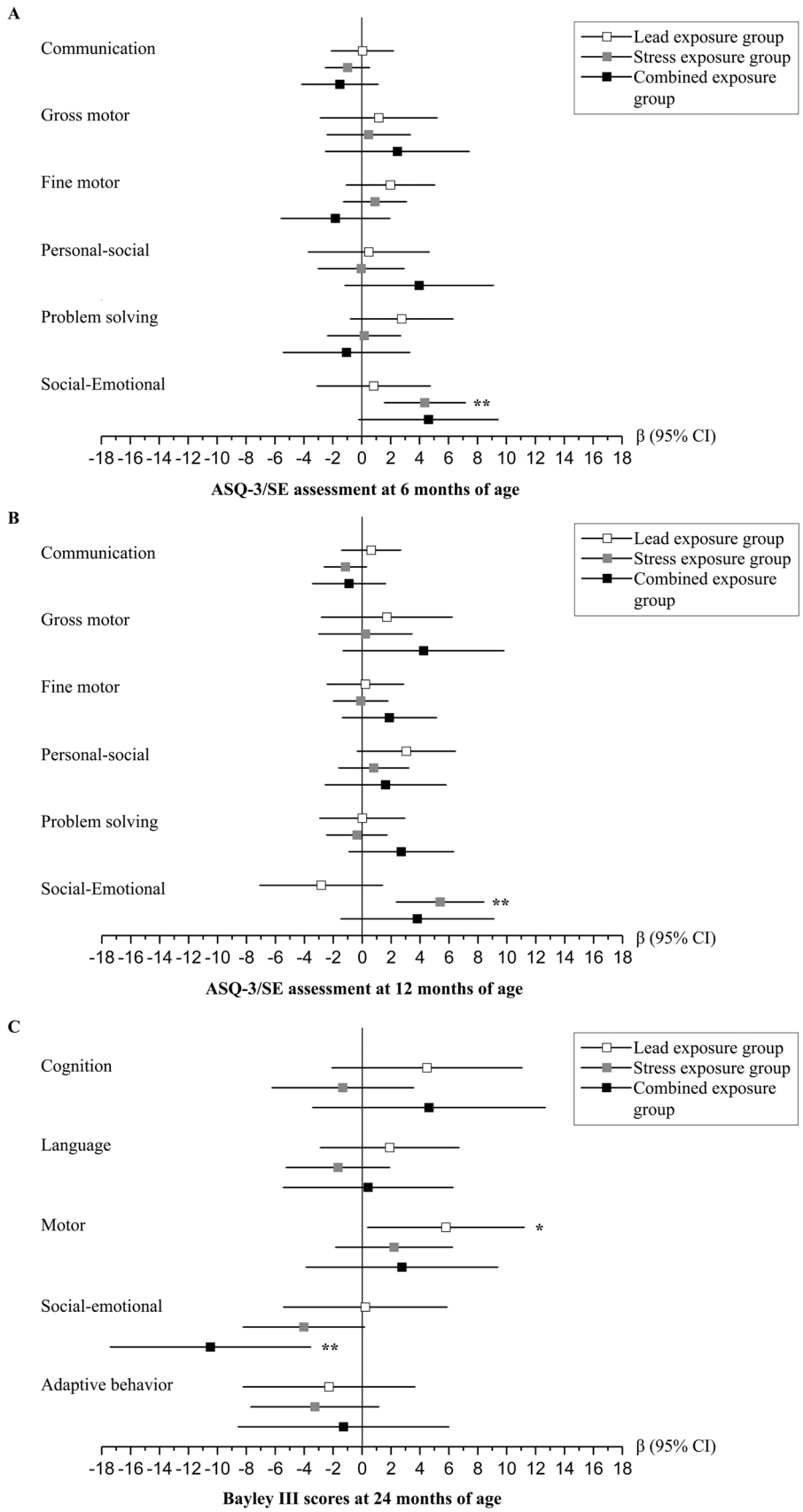

Fig. 2. Effects of single/combined prenatal exposure to lead (exposed in early pregnancy) and stress on offspring neurodevelopment [β (95 % CI)]. Adjusting for birth weight, infant sex, maternal age, maternal education level, and annual household income. (*: $P < 0.05$, **: $P < 0.01$).

Table 2
Basic characteristics of the mother-child pairs in the four groups of single/combined prenatal exposure to lead (around birth) /stress [(N) Mean±SD or N (%)].

	Control (n=598)	High lead group (n=128)	High stress group (n=794)	Combined exposure group (n=149)	P*
Pre-pregnancy BMI	(383) 20.84 ± 2.75	(84) 20.92 ± 2.86	(492) 20.76 ± 2.77	(102) 20.81 ± 2.81	0.949
Maternal age (years)	(486) 29.09 ± 3.71	(111) 28.93 ± 3.13	(668) 28.77 ± 3.60	(129) 28.75 ± 3.58	0.488
Ethnicity					0.950
Han	481 (99.18 %)	112 (100.00 %)	666 (99.25 %)	131 (100.00 %)	
Minorities	4 (0.82 %)	0 (0.00 %)	5 (0.75 %)	0 (0.00 %)	
Living years in Shanghai					0.455
< 2 years	13 (2.66 %)	5 (4.46 %)	26 (3.86 %)	4 (3.05 %)	
2–5 years	26 (5.32 %)	4 (3.57 %)	43 (6.39 %)	12 (9.16 %)	
≥ 5 years	450 (92.02 %)	103 (91.97 %)	604 (89.75 %)	115 (87.79 %)	
Mothers' education level					0.176
Junior High school or lower	9 (1.85 %)	3 (2.73 %)	8 (1.19 %)	3 (2.29 %)	
Senior high school	28 (5.76 %)	8 (7.27 %)	67 (9.99 %)	16 (12.21 %)	
Junior college	125 (25.72 %)	23 (20.91 %)	171 (25.48 %)	37 (28.25 %)	
College or university	255 (52.47 %)	65 (59.09 %)	343 (51.12 %)	57 (43.51 %)	
Postgraduate and above	69 (14.20 %)	11 (10.00 %)	82 (12.22 %)	18 (13.74 %)	
Birth head circumference (cm)	(517) 34.39 ± 1.26	(112) 34.54 ± 1.32	(696) 34.37 ± 1.18	(133) 34.39 ± 1.23	0.617
Birth length (cm)	(522) 49.97 ± 1.27	(112) 49.85 ± 1.76	(704) 49.88 ± 1.15	(133) 49.88 ± 1.19	0.568
Birth weight (kg)	(573) 3.38 ± 0.44	(125) 3.46 ± 0.48	(768) 3.37 ± 0.44	(144) 3.41 ± 0.44	0.204
Gestational age at birth (weeks)	(504) 39.32 ± 1.50	(110) 39.20 ± 1.60	(663) 39.28 ± 1.33	(118) 39.21 ± 1.63	0.818
Annual household income (RMB)					0.016
< 50,000	4 (1.50 %)	2 (3.28 %)	22 (6.23 %)	4 (6.56 %)	
50,000–100,000	37 (13.91 %)	7 (11.47 %)	45 (12.75 %)	10 (16.39 %)	
100,000–150,000	57 (21.43 %)	19 (31.15 %)	95 (26.91 %)	17 (27.87 %)	
150,000–300,000	117 (43.99 %)	31 (50.82 %)	147 (41.64 %)	22 (36.07 %)	
> 300,000	51 (19.17 %)	2(3.28 %)	44 (12.47 %)	8 (13.11 %)	
Feeding style before 6 months of age					0.293
Formula feeding	22 (4.09 %)	7 (5.83 %)	42 (5.79 %)	13 (9.49 %)	
Breastfeeding	283 (52.60 %)	66 (55.00 %)	381 (52.55 %)	65 (47.44 %)	
Mixed feeding	233 (43.31 %)	47 (39.17 %)	302 (41.66 %)	59 (43.07 %)	
Umbilical cord blood lead (μg/dL)	(598) 1.21 ± 0.40	(128) 2.89 ± 1.25	(794) 1.23 ± 0.39	(149) 4.00 ± 8.90	< 0.001
Maternal CES-D scores	(598) 5.90 ± 3.39	(128) 5.89 ± 3.43	(780) 13.35 ± 7.49	(147) 14.31 ± 7.12	< 0.001
Maternal SAS scores	(598) 26.98 ± 3.00	(128) 27.51 ± 2.82	(782) 32.75 ± 5.42	(145) 33.28 ± 4.65	< 0.001
ASQ-3/SE scores at 6 months of age					
Communication	(323) 52.78 ± 7.39	(99) 52.78 ± 7.77	(479) 52.15 ± 7.24	(110) 50.91 ± 7.87	0.121
Gross motor	(322) 42.30 ± 11.23	(99) 45.14 ± 11.54	(479) 41.45 ± 11.72	(110) 42.82 ± 10.78	0.031
Fine motor	(322) 49.34 ± 10.58	(99) 49.29 ± 12.68	(479) 49.20 ± 11.14	(110) 48.48 ± 10.82	0.916
Personal-social	(323) 45.73 ± 12.19	(99) 43.01 ± 14.06	(479) 44.05 ± 13.17	(110) 44.09 ± 11.91	0.171
Problem solving	(323) 50.38 ± 10.80	(98) 48.98 ± 11.91	(479) 48.79 ± 12.19	(110) 49.30 ± 12.14	0.300
Social-emotional	(273) 16.33 ± 12.76	(90) 16.78 ± 16.11	(434) 18.78 ± 10.60	(102) 17.81 ± 13.67	0.101
ASQ-3/SE scores at 12 months of age					
Communication	(329) 55.41 ± 6.77	(96) 55.57 ± 8.61	(499) 54.05 ± 8.04	(108) 53.29 ± 8.65	0.014
Gross motor	(328) 46.72 ± 13.65	(96) 46.04 ± 13.65	(495) 45.69 ± 14.48	(108) 46.32 ± 13.07	0.780
Fine motor	(329) 53.20 ± 8.32	(96) 52.40 ± 9.76	(499) 53.12 ± 7.91	(108) 52.12 ± 8.58	0.573
Personal-social	(329) 49.84 ± 10.69	(96) 46.77 ± 12.81	(499) 48.45 ± 11.18	(108) 50.19 ± 10.87	0.045
Problem solving	(329) 51.04 ± 9.73	(96) 49.53 ± 10.36	(499) 50.25 ± 9.15	(108) 50.86 ± 9.30	0.463
Social-emotional	(296) 20.04 ± 13.68	(92) 19.93 ± 17.31	(480) 22.83 ± 15.65	(103) 23.00 ± 15.52	0.043
Bayley III DQ scores at 24 months of age					
Cognition	(433) 120.14 ± 21.92	(98) 117.45 ± 21.34	(565) 117.08 ± 22.39	(116) 113.62 ± 21.97	0.023
Language	(433) 99.62 ± 16.11	(98) 100.77 ± 17.74	(565) 97.24 ± 15.53	(116) 96.38 ± 15.80	0.024
Motor	(433) 109.66 ± 16.15	(98) 110.38 ± 15.09	(564) 108.41 ± 15.57	(116) 105.49 ± 13.68	0.049
Social-emotional	(435) 107.71 ± 17.36	(99) 104.60 ± 19.57	(559) 103.32 ± 18.36	(114) 102.32 ± 15.59	0.001
Adaptive behavior	(439) 106.66 ± 18.90	(99) 104.83 ± 20.12	(564) 103.59 ± 18.29	(112) 106.71 ± 19.05	0.058

P * : In the Chi-square test, Fisher exact test was used when more than 20 % expected frequencies < 5 or any expected frequencies < 1.

level in the control group, suggesting that the social-emotional skills in the single/combined prenatal lead (around birth) and stress exposure groups may be impaired. Besides, the Bayley-III cognition ($P = 0.023$), language ($P = 0.024$), motor ($P = 0.049$) and social-emotional ($P = 0.001$) scores at 24-months-old of age were lower in the single/combined prenatal lead (around birth) and stress exposure groups than those in the control group (Table 2).

The effects of single/combined prenatal exposure to lead (around birth) and stress on infant neurodevelopment were described in Fig. 3 (results from adjusted models) and Supplemental Table 4 (results from unadjusted and adjusted models). After adjusting for birth weight, infant gender, maternal age, maternal education level and annual family income, compared with the control group, at 6 months of age, the communication scores from the ASQ assessment in the high stress group and the combined exposure group had changes of − 1.34 (95 % CI: −2.91, 0.23) points ($P = 0.094$) and − 3.79 (95 % CI: −6.32, −1.26) points ($P = 0.004$), respectively. The social-emotional scores in the high stress group showed an increase of 2.87 (95 % CI: −0.19, 5.94) points ($P = 0.067$) and an increase of 3.60 (95 % CI: −1.24, 8.45) points ($P = 0.146$) in the combined exposure group. At 24 months of age, compared with the control group, in the adjusted models, the social-emotional scores from the Bayley-III assessment in the high stress group and the combined exposure group had changes of − 5.88 (95 % CI: −9.18, −2.59) points ($P = 0.001$) and − 5.95 points (95 % CI: −11.53, −0.36) points ($P = 0.037$), respectively. However, compared with the control group, although the personal-social scores from the ASQ assessment in the high lead group at 12 months of age had a change of − 3.07 (95 % CI: −5.60, −0.53) points ($P = 0.018$) in the unadjusted models, the changes were non-significant in adjusted models in any of the exposure groups (Fig. 3, Supplemental Table 4). These results also suggested that infant neurodevelopment (especially social-emotional skill) may be impaired by single/combined prenatal exposure to stress/lead (around birth), and the effects induced by the combined exposure may be stronger than those by single exposures.

We also separately analyzed the effects of prenatal single and combined exposure to maternal depression/anxiety and lead on offspring neurodevelopment, and we found that the results of the effects (prenatal single exposure to depression vs. prenatal single exposure to anxiety; prenatal combined exposure to depression and lead vs. prenatal combined exposure to anxiety and lead) were similar (Supplemental Fig. 2–5).

4. Discussion

In this study, we found that both prenatal lead and stress exposure levels were relatively low. This study showed that the geometric mean of the blood lead concentrations in early pregnancy was 1.46 μg/dL, slightly lower than the levels reported by other studies including studies in Norway in 2013 (2.5 μg/dL) (Birgisdottir et al., 2013) and in China in 2013 (3.2 μg/dL) (Xie et al., 2013), similar with the levels in South Africa in 2014 (1.4 μg/dL) (Mason et al., 2014). A total of 98.3 % of maternal blood lead levels in early pregnancy in our study were lower than the guideline recommendation (5 μg/dL) for maternal blood lead during pregnancy released by the American Centers for Disease Control and Prevention in 2010 (Ettinger and Wengrovitz, 2010). The geometric mean of the umbilical cord blood lead in this study was 1.33 μg/dL, which was also slightly lower than the levels reported in China in 2013 (2.52 μg/dL) (Xie et al., 2013), in Bangladesh in 2017 (1.8–3.9 μg/dL) (Valeri et al., 2017). We consider that such differences in blood lead in early pregnancy and umbilical cord blood lead may be due to the differences among countries or regions and due to the differences of the investigation years. This study was mainly performed among urban population, and significant decreases in blood lead levels in China in recent years have been reported probably because of national efforts to decrease lead pollution. In addition, the overall rate of maternal stress (anxiety and depression) in pregnancy was lower than 2 %, lower than the rate reported by previous studies in China in 2016 and 2017 (Hu et al., 2017; Wang et al., 2016). The level of the postnatal maternal depression was also slightly lower than the level reported by previous studies, including the level in northern Portugal in 2017 (EPDS scores: 6.78 ± 4.38) (Pinto et al., 2017). The relatively low levels of prenatal lead and stress exposure levels in this study may be because that all the study women in this study were Shanghai local residents and had relatively good social-economic levels.

This study found that single exposure to maternal prenatal stress had adverse effects on offspring neurodevelopment, especially on the offspring's social-emotional, communication and language development, and the effects of developmental neurotoxicity induced by prenatal stress were confirmed by previous studies (Maccari and Morley-Fletcher, 2007; Bock et al., 2015). The mechanisms underlying such developmental neurotoxicity may be due to the increased transplacental transfer of maternal stress hormones (cortisol) to the fetal compartment and therefore impairments induced in fetal brain development. Consistent with previous studies (Jedrychowski et al., 2009), this study also suggested that prenatal lead exposure had negative associations with offspring neurodevelopment. However, consistent with other studies (Hu et al., 2006; Al-Saleh et al., 2009), the association of prenatal lead exposure with neurodevelopment of infants did not reach statistical significance after adjustment for confounding factors, which may be related to relatively low blood lead levels, the selection of the cutoff values for high blood lead and the choice of confounding factors.

This study found that the adverse effects of prenatal combined exposure to lead and stress on offspring neurodevelopment may be more profound than the effects of single lead or stress exposure, although some β-coefficients of the combined exposure did not reach statistical significance, which may be related to relatively small sample size in the combined exposure group. Using data of different birth-cohort studies and with different neurodevelopmental outcomes, our previous studies also showed that prenatal maternal stress may exacerbate the detrimental impacts of prenatal lead exposure on offspring neurodevelopment, or prenatal lead exposure may attenuate the protective effects of maternal high self-esteem on offspring neurodevelopment (Xu et al., 2015; Zhou et al., 2017; Xu et al., 2019). Therefore, in simultaneous consideration of the findings of this study and our previous studies, we suggest that prenatal lead and stress exposure may have a joint effect in affecting offspring neurodevelopment. Previous animal studies have suggested the potential mechanisms underlying the prenatal combined exposure including affecting dopamine and glutamine systems in brain regions (Rossi-George et al., 2011) and the HPA axis (Barrot et al., 2000), but the exact mechanisms need further studies.

Consistent with previous studies comparing the effects of prenatal lead and stress exposure when the exposures occurred during different developmental stages (Hu et al., 2006; Morreale de Escobar, 2001), this study found that higher levels of impairments may be induced when the combined exposure took place in an earlier stage of pregnancy, which may be due to a higher vulnerability of neural development to the combined exposure at an earlier stage of fetal development. The embryonic stage is the period of neural tube formation, while the fetal stage is the period of neuronal proliferation, differentiation, migration, aggregation, synaptogenesis and myelination in the CNS (Sherman et al., 2001). Exposure factors during pregnancy may affect the embryonic/fetal development and correlate with the fetal neurogenesis (Margot, 2019). In early pregnancy, the basic brain structure starts to form and reacts to the adversity of the environment poorly (Rice and Barone, 2000; Schnaas et al., 2006), while in late pregnancy (around birth), the proliferation and differentiation of neurons are largely completed, therefore, the first-trimester level of the combined exposure may be associated with worse neurobehavioral outcomes than the levels of later trimesters.

This study had some strengths. This study used a large-scale birth cohort study (Shanghai birth cohort study) to compare the effects of single or combined prenatal exposure to lead and stress on offspring

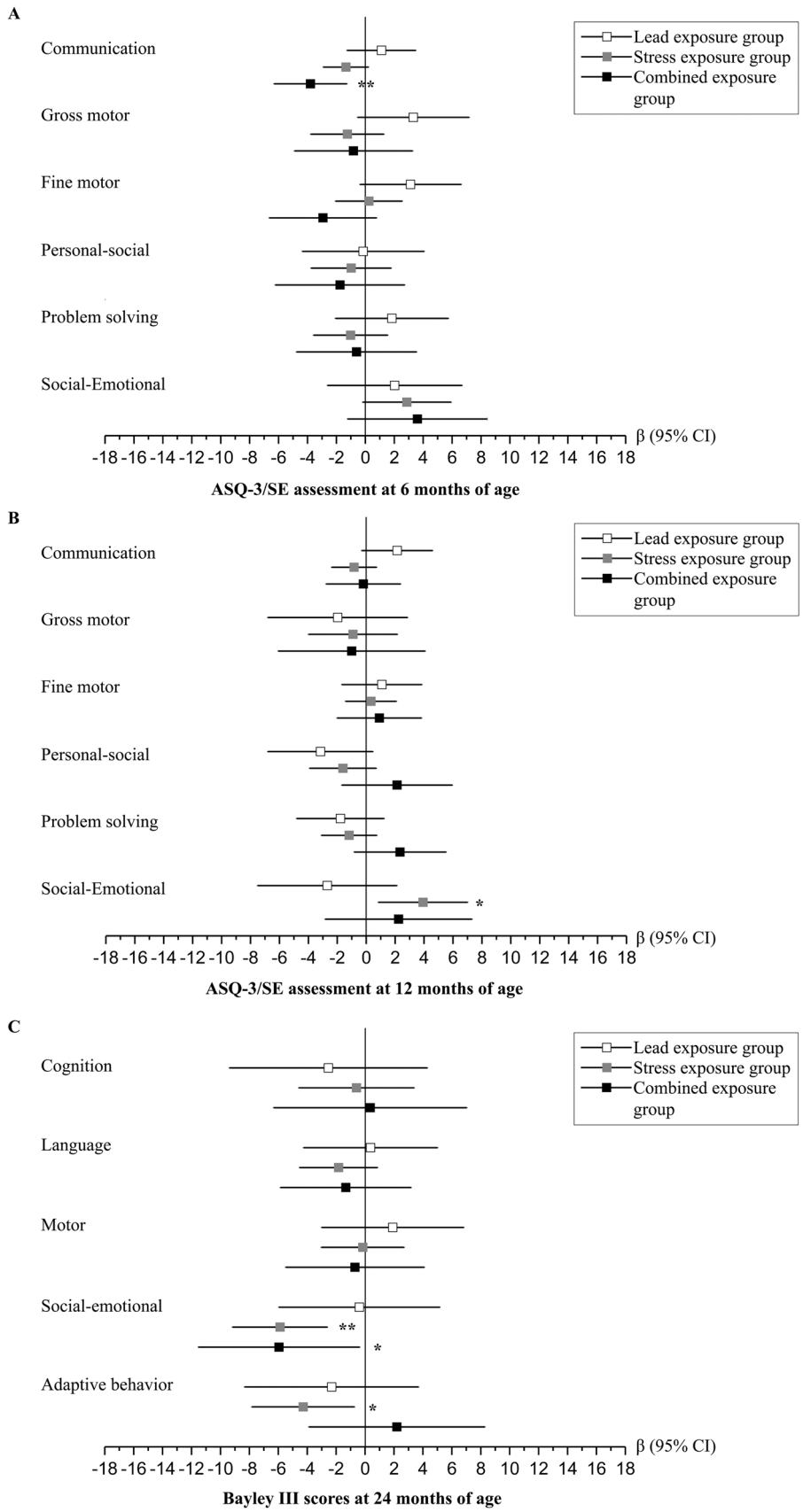

Fig. 3. Single and combined effects of prenatal exposure to lead (around birth) and stress on offspring neurodevelopment. Adjusting for birth weight, infant sex, maternal age, maternal education level, and annual household income. (*: $P < 0.05$, **: $P < 0.01$).

neurodevelopment, with multiple postnatal follow-up time points (6, 12 and 24 months) using different neurodevelopmental assessment tools (ASQ-3, ASQ-SE and Bayley III). We focus on single and combined prenatal exposure to lead and stress because that: 1) lead remains a widespread environmental health hazard; 2) stress is a very common physical and mental reaction to life experiences in pregnancy; 3) lead and mental stress during pregnancy are both demonstrated to have developmental neurotoxicity in animal models and induce cognitive deficits in offspring (Canfield et al., 2003); 4) prenatal lead and stress exposure may share the mechanisms in inducing developmental neurotoxicity including interacting with dopamine and glutamine systems in brain regions such as nucleus accumbens and hippocampus (Barrot et al., 2000; Cory-Slechta et al., 1999), activating the HPA axis (Rossi-George et al., 2011; Cory-Slechta et al., 2004), and inducing epigenetic changes in the fetal brain responsible for "fetal-origin cognitive and behavioral disorders" (Day and Sweatt, 2011; Lester et al., 2011; Sullivan et al., 2008).

However, this study also had some limitations: Firstly, the relatively small sample size in some groups (especially in the combined exposure groups) may induce a relatively high variability of the associations of the prenatal exposures with offspring neurodevelopment (such as relatively wide ranges of confidence intervals of the β-coefficients in the combined exposure groups), which calls for future studies with larger sample size to confirm the findings in this study on the effects of the combined prenatal exposures. Secondly, our study mothers were all Shanghai local residents with an overall low level of prenatal lead or stress exposure, which may raise the issue of the generalizability of our study. Third, although previous studies showed that the levels of depression and anxiety of pregnant women remained stable throughout pregnancy (Baron et al., 2017; Schubert et al., 2017; Da Costa et al., 1999), the possibility of slight differences in maternal depression or anxiety levels among early-, mid- and late-pregnancy cannot be completely excluded, and the differences in the effects of maternal depression or anxiety during different stages of pregnancy on offspring neurodevelopment may be concealed. Fourth, although we controlled for sufficient confounders based on statistical and biological considerations, we may miss some confounding variables such as levels of postnatal exposure to lead or stress in offspring which may affect infant neurodevelopment to a certain degree. However, our previous study found that child cognitive development may be more vulnerable to prenatal stress exposure, whereas child temperamental development may be more impacted by postnatal exposure to maternal stress compared with prenatal exposure. Finally, in this study, maternal "stress" levels were presented by maternal depression and/or anxiety levels. Although stress and depression/anxiety are closely related, they are not exactly same.

5. Conclusions

In conclusion, this study found that prenatal combined exposure to lead and stress had more profound effects than the single lead or stress exposure on child neurodevelopment, especially in the domain of social-emotion, and we found that the adverse effects of the combined exposure may be more significant when occurring in the early pregnancy than around birth.

The combined exposure to lead and stress during pregnancy is of a major public health concern because lead exposure is one of the most common environmental exposure factors and stress is a common reaction to life experiences during pregnancy. Current efforts at primary prevention have often focused on childhood rather than fetal exposure, and the efforts on decreasing fetal exposure need to be emphasized. If future studies confirm our findings, ascertaining women at risk and developing effective strategies for prevention of fetal combined exposure to lead and stress, especially in early pregnancy, may be considered as an important public health priority.

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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

Due to the sensitive nature of the questions asked in this study, survey respondents were assured raw data would remain confidential and would not be shared.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dcn.2022.101124.

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