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# Impact of prenatal exposure to mercury and selenium on neurodevelopmental delay in children in the Japan environment and Children's study using the ASQ-3 questionnaire: A prospective birth cohort

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ABSTRACT

Neurodevelopmental delay is associated with neurodevelopmental disorders. Prenatal metal exposure can potentially cause neurodevelopmental delays in children. This study examines whether prenatal exposure to mercury (Hg) and selenium (Se) is associated with the risk of neurodevelopmental delays in children up to 4 years of age. Children enrolled in a prospective birth cohort of the Japan Environment and Children's Study were examined. Hg and Se levels in maternal ($n_{child}$ = 48,731) and cord ($n_{child}$ = 3,083) blood were analyzed by inductively coupled plasma-mass spectrometry. Neurodevelopmental delays were assessed in children between the ages of 0.5 to 4 years using the Ages and Stages Questionnaires, Third Edition. The association between exposure and outcomes was examined using the generalized estimation equation models. In maternal blood, compared to participants with Se levels in the first quartile (83.0 to < 156 ng/g), the odds ratio (95 % confidence intervals) for problem-solving ability in children of mothers in the third (168 to < 181 ng/g) and fourth quartiles (181 to 976 ng/g) were 1.08 (1.01 to 1.14) and 1.10 (1.04 to 1.17), respectively. Furthermore, communication, gross and fine motor skills, and problem-solving delays were also observed. However, prenatal Hg levels in maternal and cord blood and Se levels in the latter were not associated with neurodevelopmental delays in children. Thus, the findings of this study suggest an association between Se levels in maternal blood and slightly increased risks of neurodevelopmental delays in children up to the age of 4 years.

## 1. Introduction

Neurodevelopmental delay refers to the slow development of communication and motor skills, problem solving ability, and personal and social skills. Prolonged neurodevelopmental delay in children can lead to neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD). ASD, which is manifested as deficits in communication and

*Abbreviations:* ADHD, attention-deficit/hyperactivity disorder; APA, American Psychiatric Association; ASD, autism spectrum disorder; ASQ-3, Ages and Stages Questionnaires third edition; DSM, Diagnostic and Statistical Manual of Mental Disorders; GEE, generalized estimation equation; Hg, Mercury; JECS, Japan Environment and Children's Study; MDL, method detection limit; MeHg, Methylmercury; NDD, neurodevelopmental disorder; PUFA, polyunsaturated fatty acid; Se, Selenium.

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social skills along with stereotyped repetitive behavior, is a neurodevelopmental condition that develops within the first 3 years of life and is typically diagnosed at 4 to 5 years of age (McNally Keehn et al., 2020; Xiao et al., 2014). ADHD, characterized by attention difficulty, impulsivity, hyperactivity, and developmentally extreme and impaired levels, is another neurodevelopmental condition (Hinshaw, 2018). The presence of these symptoms before the age of 7 years (as per the Diagnostic and Statistical Manual of Mental Disorders [DSM] fourth edition [DSM-4] of the American Psychiatric Association [APA]) or at 12 years (as per the DSM fifth edition [DSM-5] of the APA) is a criterion for confirmed ADHD diagnosis (Austerman, 2015; Biederman et al., 1997; Doernberg and Hollander, 2016; Maser and Patterson, 2002; Shaffer, 1996; Stein et al., 2010). Since NDDs hinder social life, detecting a child's neurodevelopmental delay during preschooling is an important precautionary measure.

In Japan, many children are enrolled in kindergartens and nursery schools at approximately 4 years of age to their improve social lives. This period is a milestone for assessing neurodevelopmental delays since the social environment of children drastically changes. Therefore, it is important to assess neurodevelopmental delay up to this age.

In a previous study, perinatal events and toxin exposure, including mercury (Hg), were highlighted as two of the most common causes of neurodevelopmental delay in children (Villagomez et al., 2019). Maternal exposure to Hg and selenium (Se) during pregnancy could have significant effects on neurodevelopment.

Hg toxicity is mitigated by the Se's protective effects (Lindh and Johansson, 1987). Hg exists in many forms, of which methylmercury (MeHg) is toxic. These protective effects for Hg (Park and Mozaffarian, 2010) are linked to availability of Se for selenoprotein synthesis.

Se is a cofactor for several antioxidant enzymes, and consequently, there has been much interest in its potential health benefits (Rayman, 2012). Low maternal Se level during pregnancy is associated with impaired child cognitive development (Polanska et al., 2016; Skröder et al., 2015). However, high maternal Se levels during pregnancy have also been associated with increased risks of adverse effects on behavior and social skills (>100 ng/mL in cord serum) (Yang et al., 2013), and ADHD (>59 ng/mL in cord serum) (Ode et al., 2015). These results suggest the importance of examining the adverse health effects on neurodevelopmental delay in children using data from pregnant women with low and high levels of Se.

Since neurodevelopment is associated with thyroid function, a decrease in the availability of free Se can destabilize thyroid hormone homeostasis, thereby leading to neurological damages during susceptible periods such as the second and third trimesters of pregnancy (Raymond et al., 2014; Selevan et al., 2000; Ventura et al., 2017). The Se levels in the maternal blood of pregnant Japanese women are higher than those among pregnant European and American women (Nakayama et al., 2019); hence, it is important to assess the health risks of Se levels in pregnant Japanese women.

The Japan Environmental and Children's Study (JECS) is an ongoing and prospective birth cohort study of pregnant Japanese women in 15 regional centers in Japan (Kobayashi et al., 2019a; Kobayashi et al., 2019b; Michikawa et al., 2018; Nakayama et al., 2019; Tsuji et al., 2018; Tsuji et al., 2019). A previous study by the authors of the JECS showed that the maternal blood Hg levels of Japanese pregnant women were almost one order of magnitude higher than those of pregnant women in North America and Europe, but similar to those among pregnant Chinese women (Nakayama et al., 2019). High levels of Hg (median: 3.7 ng/g) and Se (median: 170 ng/g) in the maternal blood are not associated with risks of placenta previa or placenta accrete (Tsuji et al., 2019), preterm births (Tsuji et al., 2018), or low head circumference at birth, which is one of the indicators of brain development (Kobayashi et al., 2019a). Since neurodevelopmental delay in children during early life is linked to their developmental trajectories in the future, it is necessary to examine the association between maternal Hg and Se exposure during pregnancy and the neurodevelopmental delay during early life in Japan.

We hypothesize that increased maternal Hg and Se levels are associated with a heightened risk of neurodevelopmental delay in children up to 4 years of age. To test this hypothesis, we examined the association between the Hg and Se levels in the maternal blood during the second/third trimesters and cord blood at delivery, with neurodevelopmental delay in children up to 4 years of age using the Ages and Stages Questionnaire, Third Edition (ASQ-3) (Mezawa et al., 2019; Squires et al., 2009) as the follow-up of the previous JECS findings (Kobayashi et al., 2019a).

## 2. Material and methods

### 2.1. Study design and population

JECS is an ongoing prospective birth cohort study. The detailed methodology has been previously reported (Kawamoto et al., 2014). Briefly, pregnant women from 15 regional centers in Japan (Appendix 1) were recruited for 3 years from 2011 to 2014. A total of 103,060 pregnancies (104,062 fetal records) were included in JECS (datasets of jecs-ta-20190930 and jecs-qa-20210401). Fig. 1 illustrates the selection of study participants. Of the 103,060 pregnancies (104,062 fetal records), 48,481 mothers (48,731 children), whereby the data for Hg and Se levels in maternal blood and ASQ-3 measurements were available, were analyzed for the association between Hg and Se levels in maternal blood and neurodevelopmental delays in children up to 4 years of age. To investigate the association between Hg and Se levels in cord blood and neurodevelopmental delays in children up to 4 years, the data from 3,059 mothers (3,083 children) on both the Hg and Se levels in cord blood and one of the ASQ-3 measurements were analyzed.

### 2.2. Assessment of dietary and lifestyle information

Follow-up questions on dietary and lifestyle information were evaluated using self-administered questionnaires including the food frequency questionnaire (FFQ) (Yokoyama et al., 2016) and the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987; Okano et al., 1996). The questionnaires were answered by participants during the first, second, and third trimesters of pregnancy, and at one-month post-delivery. We collected data related to diet and lifestyle, and confounding factors such as maternal smoking during pregnancy, parity, and postpartum depression of mothers when the child was one month of age. n-3 polyunsaturated fatty acid (PUFA) level consumed during pregnancy in the second and third trimesters was calculated using the FFQ (Yokoyama et al., 2016). The mothers were considered positive for postpartum depression if they scored $\geq 9$ in the EPDS at one-month post-delivery (Okano et al., 1996). Medical data at delivery and one month of child age were collected from the transcribed medical records, which included child sex and physical abnormalities (including minor anomalies and congenital anomalies).

### 2.3. Assessment of Hg and Se levels in maternal and cord blood

Maternal and cord whole blood samples were used to analyze the Hg and Se levels. The measurement protocol has been previously described (Nakayama et al., 2019). Briefly, blood samples were collected by the medical staff and stored at − 80 °C in a central biorepository facility. We used one of the 2 mL whole blood aliquots obtained from mothers during the second or third trimester, and one of the 2 mL whole cord blood aliquots obtained from mothers at delivery to analyze Hg and Se levels by inductively coupled plasma-mass spectrometry (ICP-MS) on the Agilent 7700 ICP-MS (Agilent Technologies, Tokyo, Japan) platform. The method detection limits (MDLs) for Hg and Se were 0.0490 and 0.837 ng/g, respectively. The Hg and Se levels in the maternal and cord blood were detected in all samples. A previous study confirms that 1 ng/g is almost equal to 1 ng/mL (Kobayashi et al., 2019a). Hg and Se levels in the maternal and cord blood were transformed using the $\log_{10}$ scale

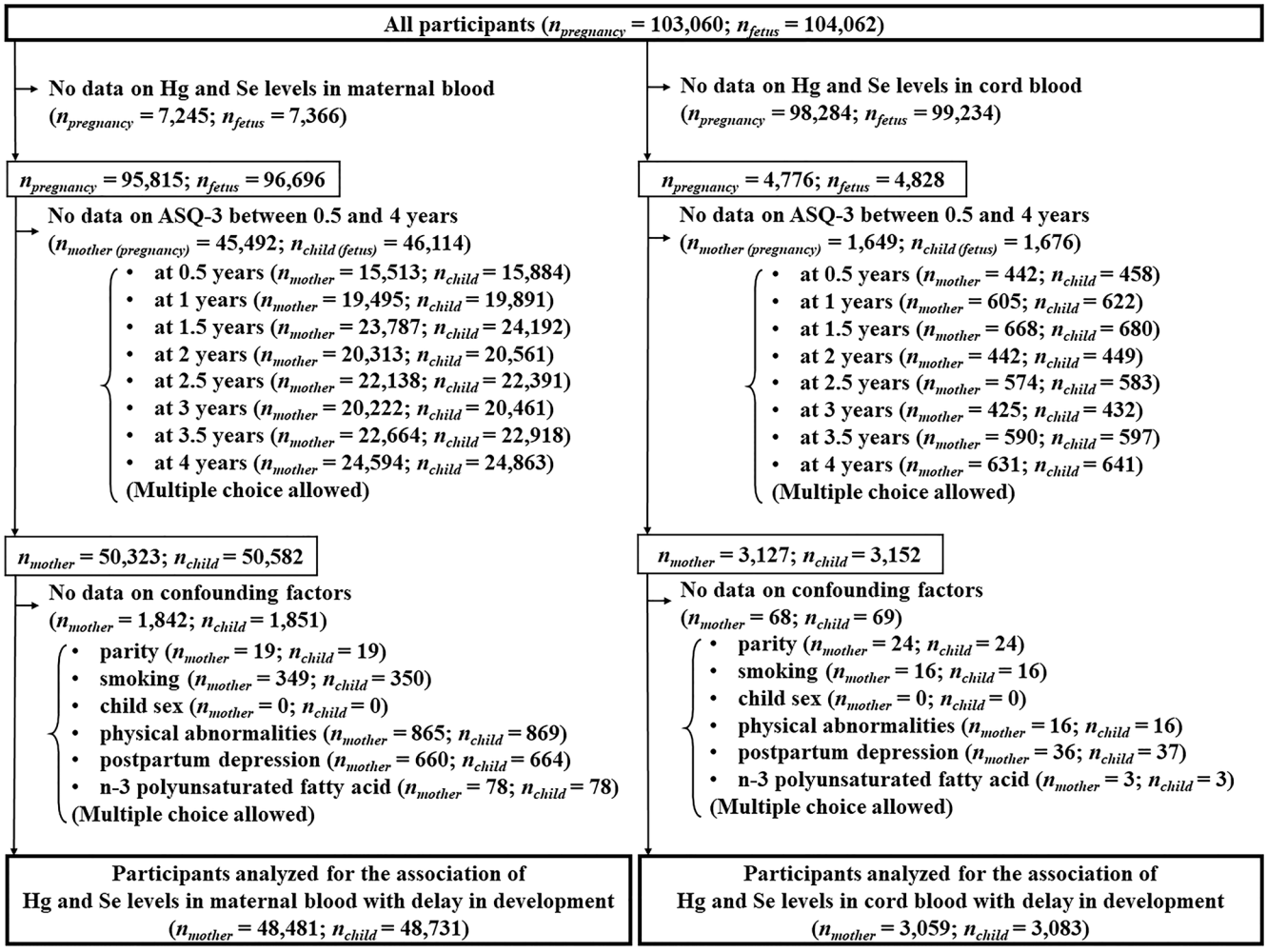


**Fig. 1.** Selection of study participants.

and quartile levels since they constituted a non-normal distribution. Continuous variables ($\log_{10}$ scale) were obtained to examine the association of outcomes with continual level changes. Quartile levels were obtained to examine the association with dose-dependent outcomes. Exposure levels were defined as the $\log_{10}$ scale or quartile levels of Hg or Se levels in the maternal or cord blood.

### 2.4. Assessment of neurodevelopmental delays in children from 0.5 to 4 years

The ASQ-3 is one of the parent-answered screening tools to analyze child neurodevelopment up to 5.5 years of age (Squires et al., 2009). It has a version translated to Japanese (Mezawa et al., 2019) and consists of five domains, which are important milestones, including communication, fine motor, gross motor, problem-solving ability, and personal and social skills, which are assessed based on 30 questions. Communication skills include asking questions, speaking clearly, making friends, and sharing personal information. Gross motor skills include coordination, balance, physical strength, body awareness, and reaction time. Fine motor skills include usage of scissors, keyboard, rulers, and other tools, holding a pen, drawing a picture, and writing neatly. Problem-solving abilities include creativity, decision making, dependability, and team building. Personal skills include dependability, adaptability, and motivation. Social skills include sharing, cooperating, making eye contact, listening, respecting personal space, and displaying manners. The response options "yes," "sometimes," or "not yet," scored 10, 5, and 0, respectively, and the total points for each domain were calculated. Eight ASQ-3 surveys were performed for the children at 0.5 (range: 5–7 months), 1 (range: 11–14 months), 1.5 (range: 17–19 months), 2 (range: 23–25 months), 2.5 (range: 28–31 months), 3 (range: 34–39 months), 3.5 (range: 39–45 months), and 4 (range: 45–51 months) years. For each domain, a cut-off score was determined as previously described by Mezawa et al. (2019). If the score was less than the cut-off for each assessed domain, the child was evaluated as "requiring a referral for further assessment in the assessed domain." Outcomes were defined as the children requiring referrals for further assessment of communication, gross motor, fine motor, problem solving abilities, or personal and social skills from 0.5 to 4 years of age based on ASQ-3.

### 2.5. Statistical analysis

First, we examined the frequencies and distribution of maternal and child characteristics, Hg and Se levels in maternal and cord blood, and the number of children requiring a referral for further assessment based on the ASQ-3. Second, we examined the association between exposure levels and outcomes using generalized estimation equations (GEE), which consider the repeated measurements of outcomes. Adjusted factors included parity (whether mothers were primiparous or multiparous, and if multiparous, the sex of the child/children previously delivered), smoking (no or yes) in the second/third trimesters, child sex (for all, primiparous, or multiparous mothers), physical abnormalities in the child (no or yes), postpartum depression (when the child was 1 month old; no or yes), and n-3 PUFA levels (g/day). We also performed stratified analyses based on the child sex and parity. Moreover, sensitivity

analyses for the association between Hg and Se levels in maternal blood and neurodevelopmental delays in children up to 4 years of age, were also calculated among children without physical abnormalities (including minor and congenital anomalies) using GEE. Adjusted factors used the same variables except for physical abnormalities of the child (including minor and congenital anomalies). All analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA), and statistical significance was set at $p < 0.05$ (two-sided).

### 2.6. Ethics

The JECS protocol was reviewed and approved by the Ministry of the Environment's Institutional Review Board on Epidemiological Studies (No. 100910001) and the Ethics Committees of all participating institutions.

JECS was conducted after obtaining written informed consent from all participants. This study was conducted in accordance with the Declaration of Helsinki and its revisions (World Medical Association), the Guidelines of Ethical Guidelines for Medical and Health Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology, and Ministry of Health, Labour and Welfare, Japan), and Guidelines of the Council for International Organization of Medical Sciences (World Health Organization), and other internationally valid regulations and guidelines for research on human participants.

## 3. Results

Table 1 lists the characteristics of the JECS. For the participants who provided maternal and cord blood samples, the mean (±standard deviation [SD]) n-3 PUFA levels were 1.8 (±1.0) g/day. The mean ages (in months) of children at the time of ASQ-3 measurement at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 years were 5.3, 11.3, 17.3, 23.4, 29.4, 35.5, 42.2, and 48.3 months, respectively. The data for parity, smoking in the second/third trimesters, postpartum depression one month following delivery, child sex, physical abnormalities of the child (including minor and congenital anomalies), and timing of ASQ-3 measurements between participants who provided maternal and cord blood samples, showed no significant difference.

**Table 1**
Maternal and child characteristics in the JECS.

| Characteristics | Participants who provided maternal blood samples ($n_{child}$ = 48,731) | Participants who provided cord blood samples ($n_{child}$ = 3,083) |
|---|---|---|
| **Mother** | | |
| Parity[a] | | |
| Primiparous | 21,718 (44.6) | 1,332 (43.2) |
| Multiparous | 27,013 (55.4) | 1,751 (56.8) |
| Smokers in the second/third trimesters[a] | | |
| No | 41,998 (86.2) | 2,672 (86.7) |
| Yes | 6,733 (13.8) | 411 (13.3) |
| n-3 polyunsaturated fatty acid (PUFA) (g/day)[b] | 1.8 ± 1.0 | 1.8 ± 1.0 |
| Postpartum depression at one-month post-delivery[a] | | |
| No | 42,493 (87.2) | 2,688 (87.2) |
| Yes | 6,238 (12.8) | 395 (12.8) |
| **Child** | | |
| Sex[a] | | |
| Female | 23,879 (49.0) | 1,543 (50.0) |
| Male | 24,852 (51.0) | 1,540 (50.0) |
| Physical abnormalities (including minor and congenital anomalies)[a] | | |
| No | 44,456 (91.2) | 2,825 (91.6) |
| Yes | 4,275 (8.8) | 258 (8.4) |
| **Timing of ASQ-3 measurement**[b] | | |
| 0.5 years (months of age) | 5.3 ± 0.4 | 5.3 ± 0.4 |
| 1.0 years (months of age) | 11.3 ± 0.5 | 11.3 ± 0.5 |
| 1.5 years (months of age) | 17.3 ± 0.5 | 17.3 ± 0.5 |
| 2.0 years (months of age) | 23.4 ± 0.5 | 23.4 ± 0.5 |
| 2.5 years (months of age) | 29.4 ± 0.5 | 29.4 ± 0.5 |
| 3.0 years (months of age) | 35.5 ± 0.6 | 35.5 ± 0.6 |
| 3.5 years (months of age) | 42.2 ± 0.5 | 42.2 ± 0.5 |
| 4.0 years (months of age) | 48.3 ± 0.6 | 48.2 ± 0.5 |

[a] n (%),
[b] mean ± standard deviation.

Table 2 lists the Hg and Se levels in maternal and cord blood. The median (inter-quartile range) of Hg and Se levels in maternal blood was 3.64 (range: 2.56–5.19) ng/g and 168 (range: 156–181) ng/g, respectively. Differences in Hg and Se levels between included and excluded participants in maternal blood were not significant (Supplementary Table 1). Differences in Hg levels between participants with and without ASQ-3 in maternal blood were significant; however, differences in Se levels between participants with and without ASQ-3 in maternal blood were not significant (Supplementary Table 2). The median (inter-quartile range) of Hg and Se levels in the cord blood was 7.56 (range: 5.22–10.7) ng/g and 178 (range: 163–194) ng/g, respectively. Differences in Hg levels between included and excluded participants in cord blood were significant; however, differences in Se levels between included and excluded participants in cord blood were not significant (Table 1). Differences in Hg levels between participants with and without ASQ-3 in cord blood were significant; however, differences in Se levels between participants with and without ASQ-3 in the cord blood were not significant (Supplementary Table 2).

Table 3 shows the frequencies of children suspected of delayed development in the subscales of ASQ-3. When children of participants who provided maternal blood samples were 4 years old, the frequencies of children suspected of having delayed communication, gross motor, fine motor, problem-solving ability, and personal and social skills were 4.5 %, 5.8 %, 6.6 %, 3.5 %, and 5.6 %, respectively. Meanwhile, when children of participants who provided cord blood samples were 4 years old, the frequencies of children suspected of delayed development in communication, gross motor, fine motor, problem-solving ability, and personal and social skills were 5.8 %, 7.7 %, 8.0 %, 4.0 %, and 7.4 %, respectively.

Table 4 shows the association between Hg and Se levels in maternal blood and subscales in ASQ-3, in children up to 4 years old. Hg levels in maternal blood were not associated with developmental delay. For one unit increase in Se level in maternal blood, the odds ratios (OR) [95 % confidence intervals (CI)] of communication skill, gross motor skill, fine motor skill, problem-solving ability, and personal and social skills were 2.65 (range: 1.30–5.40), 2.51 (range: 1.65–3.82), 2.61 (range: 1.68–4.04), 2.39 (range: 1.60–3.64), and 2.08 (range: 1.18–3.65), respectively. Furthermore, Hg levels in the cord blood were not associated with developmental delay. For one unit increase in Se level in cord blood, the OR (95 % CI) of problem-solving ability was 3.85 (range: 1.01–14.61). These results (Table 4) are similar to those obtained from the sensitivity analysis among children without physical abnormalities (Supplementary Table 3).

Table 5 shows the association between Hg and Se levels and subscales in ASQ-3 in children up to 4 years of age, stratified by child sex and parity of the mother. In male children, female children, and children of primiparous mothers, Hg levels in maternal blood were not associated with developmental delay. In children of primiparous mothers, for one unit increase in Hg level in maternal blood, the OR (95 % CI) of personal and social skills was 0.82 (range: 0.68–0.99). In children of multiparous mothers, for one unit increase in Hg level in maternal blood, the OR (95 % CI) of problem-solving ability was 1.15 (range: 1.02–1.30). In male children, for one unit increase in Se levels in maternal blood, the ORs (95 % CI) of gross motor skill, fine motor skill, and problem-solving ability were 2.02 (range: 1.14–3.57), 2.14 (range: 1.23–3.73), and 1.99 (range: 1.14–3.46), respectively. In female children, for one unit increase in Se level in maternal blood, the ORs (95 % CI) for

**Table 2**
Hg and Se levels in maternal and cord blood.

| Blood/participants | Mean | Minimum | 25 percentiles | Median | 75 percentiles | Maximum |
|---|---|---|---|---|---|---|
| Maternal blood ($n_{child}$ = 48,731) | | | | | | |
| Hg (ng/g) | 4.19 | 0.321 | 2.56 | 3.64 | 5.19 | 43.7 |
| Se (ng/g) | 170 | 83.0 | 156 | 168 | 181 | 976 |
| Cord blood ($n_{child}$ = 3,083) | | | | | | |
| Hg (ng/g) | 8.76 | 0.572 | 5.22 | 7.56 | 10.7 | 87.6 |
| Se (ng/g) | 179 | 83.3 | 163 | 178 | 194 | 369 |

From the results of Kobayashi et al. (2019a), ng/g ≈ ng/mL.

**Table 3**
Frequencies of children suspected of delayed development in communication, fine motor, gross motor, problem-solving ability, and personal and social skills in ASQ-3.

| | | Subscales in ASQ-3 | | | | |
|---|---|---|---|---|---|---|
| Blood/participants | ASQ-3 measurement | Communication skill | Gross motor skill | Fine motor skill | Problem-solving ability | Personal and social skills |
| Maternal blood ($n_{child}$ = 48,731) | 0.5 years | 318 (0.7) | 5,384 (11.0) | 2,715 (5.6) | 5,701 (11.7) | 1,950 (4.0) |
| | 1.0 years | 65 (0.1) | 2.892 (5.9) | 2,924 (6.0) | 2,627 (5.4) | 628 (1.3) |
| | 1.5 years | 1,099 (2.3) | 2,367 (4.9) | 2,223 (4.6) | 2,054 (4.2) | 1,275 (2.6) |
| | 2.0 years | 1,864 (3.8) | 2,929 (6.0) | 1,064 (2.2) | 2,060 (4.2) | 1,384 (2.8) |
| | 2.5 years | 2,315 (4.8) | 2,119 (4.3) | 2,832 (5.8) | 2,791 (5.7) | 1,650 (3.4) |
| | 3.0 years | 1,902 (3.9) | 2,172 (4.5) | 3,606 (7.4) | 3,542 (7.3) | 1,589 (3.3) |
| | 3.5 years | 2,039 (4.2) | 2,168 (4.4) | 2,526 (5.2) | 2,807 (5.8) | 2,213 (4.5) |
| | 4.0 years | 2,216 (4.5) | 2,840 (5.8) | 3,234 (6.6) | 1,728 (3.5) | 2,740 (5.6) |
| Cord blood ($n_{child}$ = 3,083) | 0.5 years | 17 (0.6) | 327 (10.6) | 200 (6.5) | 358 (11.6) | 107 (3.5) |
| | 1.0 years | 7 (0.2) | 198 (6.4) | 181 (5.9) | 164 (5.3) | 42 (1.4) |
| | 1.5 years | 65 (2.1) | 153 (5.0) | 146 (4.7) | 131 (4.2) | 87 (2.8) |
| | 2.0 years | 125 (4.1) | 202 (6.6) | 65 (2.1) | 148 (4.8) | 94 (3.0) |
| | 2.5 years | 156 (5.1) | 155 (5.0) | 208 (6.7) | 192 (6.2) | 119 (3.9) |
| | 3.0 years | 122 (4.0) | 167 (5.4) | 257 (8.3) | 257 (8.3) | 109 (3.5) |
| | 3.5 years | 130 (4.2) | 157 (5.1) | 181 (5.9) | 189 (6.1) | 170 (5.5) |
| | 4.0 years | 179 (5.8) | 236 (7.7) | 248 (8.0) | 124 (4.0) | 229 (7.4) |

*n* (%).

communication, gross motor, fine motor, problem-solving abilities, and personal and social skills were 4.81 (range: 1.33–17.36), 3.28 (range: 1.75–6.12), 3.59 (range: 1.71–7.53), 3.11 (range: 1.60–6.04), and 5.34 (range: 1.96–14.54), respectively. In children of primiparous mothers, for one unit increase in Se level in maternal blood, the ORs (95 % CI) of communication, gross motor, fine motor, problem-solving abilities, and personal and social skills were 6.78 (range: 2.10–21.93), 3.29 (range: 1.79–6.04), 3.38 (range: 1.71–6.67), 2.85 (range: 1.48–5.48), and 2.87 (range: 1.20–6.83), respectively. In children of multiparous mothers, for one unit increase in Se level in maternal blood, the ORs (95 % CI) of gross motor skills, fine motor skills, and problem-solving abilities were 2.04 (range: 1.14–3.64), 2.15 (range: 1.21–3.81), and 2.04 (range: 1.17–3.54), respectively. These results (Table 5) are similar to those obtained from the sensitivity analysis among children without physical abnormalities (Supplementary Table 3).

In male children, female children, children of primiparous mothers, and children of multiparous mothers, Hg levels in cord blood were not associated with developmental delay. In male children, for one unit increase in Se levels in cord blood, the ORs (95 % CI) for fine motor skills and problem-solving abilities were 7.28 (range: 1.34–39.45) and 11.37 (range: 1.85–69.84), respectively, while in female children, children of primiparous mothers, and children of multiparous mothers, Se levels in cord blood were not associated with developmental delay. These results (Table 5) differ from those obtained from the sensitivity analysis among children without physical abnormalities (Supplementary Table 3).

Table 6 shows the association between Se quartile levels in maternal blood and subscales in ASQ-3 in children up to 4 years of age. Compared to children with mothers in the first quartile (83.0 to < 156 ng/g), the ORs (95 % CI) for gross and fine motor skills of children with mothers in the fourth quartile (181 to 976 ng/g) were 1.10 (range: 1.04–1.17; $p$ for trend < 0.001) and 1.13 (range: 1.07–1.20; $p$ for trend < 0.001), respectively. The ORs (95 % CI) for the problem-solving abilities in children with mothers in the third (168 to < 181 ng/g) and the fourth quartiles (181 to 976 ng/g) were 1.08 (range: 1.01–1.14) and 1.11 (range: 1.04–1.17; $p$ for trend < 0.001), respectively. The association between Se quartile levels in maternal blood and communication skill was significant in the trend test ($p$ for trend = 0.018), and so was the association between Se quartile levels in maternal blood and personal and social skills ($p$ for trend = 0.039).

## 4. Discussion

This study showed an association between the Hg and Se levels in maternal blood and neurodevelopmental delay in children up to 4 years of age. The levels of these elements in the blood of pregnant Japanese women were higher than in the blood of pregnant women in North America and Europe (Nakayama et al., 2019). Among all participants, Hg levels in maternal and cord blood posed no risks of child neurodevelopmental delay, whereas Se levels in maternal and cord blood were associated with increased risks of child neurodevelopmental delay. However, the accuracy was low, because the association between Se levels in cord blood and the increased risk of child neurodevelopment showed a wide 95 % CI. The association between Se levels in maternal blood and delays in communication, personal, and social skills differed depending on the child's sex and parity, while the association between Hg levels in maternal blood and the delay in problem-solving ability differed depending on the parity.

Although there are limited studies of the borderline levels regarding Se sufficiency, children of Spanish mothers with ≥ 86 ng/mL of Se in the serum in the first trimester showed increased risks of delayed mental and psychomotor development, including performance ability, memory, early language skills, psychomotor skills, and coordination, at 1 year of age (Amorós et al., 2018a), while those of mothers with ≥ 84 ng/mL of Se in the maternal serum were associated with an increased risk of

**Table 4**
Association between Hg and Se levels and subscales in ASQ-3 in children up to 4 years of age.

| Exposure | Outcome: Subscales in ASQ-3 | OR (95 % CI) | *p* value |
|---|---|---|---|
| **Maternal blood ($n_{child}$ = 48,731)** | | | |
| Hg (ng/g) | Communication skill | 0.87 (0.74, 1.01) | 0.072 |
| | Gross motor skill | 0.96 (0.88, 1.06) | 0.419 |
| | Fine motor skill | 1.05 (0.96, 1.15) | 0.316 |
| | Problem-solving ability | 1.05 (0.96, 1.15) | 0.274 |
| | Personal and social skills | 0.92 (0.81, 1.03) | 0.158 |
| Se (ng/g) | Communication skill | 2.65 (1.30, 5.40) | 0.007 |
| | Gross motor skill | 2.51 (1.65, 3.82) | <0.001 |
| | Fine motor skill | 2.61 (1.68, 4.04) | <0.001 |
| | Problem-solving ability | 2.39 (1.60, 3.64) | <0.001 |
| | Personal and social skills | 2.08 (1.18, 3.65) | 0.011 |
| **Cord blood ($n_{child}$ = 3,083)** | | | |
| Hg (ng/g) | Communication skill | 0.70 (0.41, 1.19) | 0.186 |
| | Gross motor skill | 0.93 (0.67, 1.30) | 0.684 |
| | Fine motor skill | 1.26 (0.90, 1.76) | 0.178 |
| | Problem-solving ability | 1.04 (0.75, 1.44) | 0.819 |
| | Personal and social skills | 0.76 (0.51, 1.12) | 0.167 |
| Se (ng/g) | Communication skill | 2.31 (0.32, 16.92) | 0.409 |
| | Gross motor skill | 1.16 (0.32, 4.17) | 0.816 |
| | Fine motor skill | 3.48 (0.96, 12.62) | 0.057 |
| | Problem-solving ability | 3.85 (1.01, 14.61) | 0.048 |
| | Personal and social skills | 2.74 (0.42, 18.01) | 0.295 |

Hg and Se levels in maternal and cord blood transformed using $\log_{10}$ scale.
Generalized estimation equation (GEE) adjusted for parity, smoking in the second/third trimesters, child sex, physical abnormalities of the child (including minor and congenital anomalies), postpartum depression at one-month post-delivery, and n-3 PUFA levels.
Odds ratio (OR) (95 % confidence interval [CI]) represents the OR for one unit increase in exposure levels ($\log_{10}$ scale).

verbal and global memory skills at 5 years of age (Amorós et al., 2018b). Although the exact level of Se in maternal blood that causes a neurodevelopmental delay in children remains unknown, our results suggest that Se levels of ≥ 168 ng/g in maternal blood could produce a slightly increased risk of perinatal neurodevelopmental delay in children between 0.5 and 4 years of age.

Children of Swedish mothers with > 59 ng/mL of Se in cord serum were associated with 2.5-fold increased risks of ADHD at 8–12 years of age (Ode et al., 2015), while those of Chinese mothers with > 100 ng/mL of Se in cord serum were associated with increased risks of impaired function and behavioral problems at 3 days of age (Yang et al., 2013). In this study, we observed that Se levels in cord blood ranged from 83.3 to 369 ng/g. Increased Se levels in cord blood were associated with an increased risk of delayed development in problem-solving ability; however, the wide range of 95 % CI suggested low accuracy. Therefore, the association between Se levels in cord blood and increased risk of delayed neurodevelopment must be verified using a larger-scale cohort study.

We did not observe an association between Hg and Se levels in maternal and cord blood and neurodevelopmental delay in children between 0.5 and 4 years of age (data not shown). Al-Saleh et al. (2019) studied children from Saudi Arabia and reported no protective role for high Se levels (median: 178 ng/mL) in maternal blood against MeHg in child neurodevelopmental delay at 3–12 months of age. Additionally, Castriotta et al. (2020) did not observe an association between total Hg and Se levels in maternal blood on cognitive neurodevelopment in children at 40 months of age. These results are in line with this study.

The association between Se levels and neurodevelopmental delay in children up to 4 years is not influenced by sex or parity, except for communication, personal, and social skills. A previous study highlighted the sex-specific association between Se levels in erythrocyte fraction of maternal blood (median: 0.46 μg/g) and psychomotor development in Bangladeshi children at 1.5 years of age (Skröder et al., 2015). The observed differences between females and males may involve complex interactions between Se levels in female plasma (median: 63 ng/mL), sex, and thyroid hormone secretion in female serum, as was demonstrated in a previous study (Zagrodzki and Ratajczak, 2008). However, the precise mechanisms underlying these findings remain to be clarified. Although there is limited data on the association between Se levels in maternal and cord blood, parity, and child neurodevelopmental delay, studies have reported an association between parity and child neurodevelopmental disorders. The association between parity and child ASD and delay of cognitive function appeared to be more pronounced in primiparous than multiparous mothers (Bilder et al., 2009; Croen et al., 2007; Durkin et al., 2008; Glasson et al., 2004). Differences in patterns of association between maternal parity and Se levels during pregnancy suggest the involvement of environmental factors affecting neurodevelopmental delay.

Ingested Se is absorbed by the intestines and transported to the liver, where it is predominantly metabolized to selenoproteins (Ha et al., 2019). Selenoproteins include glutathione peroxidases, iodothyronine deiodinases (DIOs), and thioredoxin reductases, which play a role in the biosynthesis of thyroid hormone during central nervous system differentiation and development in the fetal brain (Prezioso et al., 2018; Schomburg, 2011). Furthermore, the activity of glutathione peroxidases, which protect against oxidative stress, decreases in the cortex, hippocampus, and cerebellum of mice fed a Se-deficient diet, while glutathione peroxidase activity decreases in the cortex of mice fed a Se-excess diet (Sharma et al., 2019). The mechanisms by which Se affects neurodevelopmental delay remain to be elucidated.

We observed that Hg levels in maternal blood are not associated with a risk of child neurodevelopmental delay, except for problem-solving ability in the children of multiparous mothers, while Hg levels in cord blood are not associated with a risk of child neurodevelopmental delay. Although fish consumption is the primary pathway (≥80 %) of Hg exposure in Japanese individuals (Ministry of Health, Labour and Welfare, Japan, 2010), it also provides nutrients that mitigate the adverse health effects of maternal Hg exposure on a child neurodevelopmental delay. Indeed, several studies suggested that failure to adjust fish consumption results in an underestimation of the adverse health effects of maternal Hg exposure (Budtz-Jørgensen et al., 2007; Davidson et al., 2008; Oken et al., 2005; Strain et al., 2008). The results of this study were similar to those of the previous studies. We speculated that nutrients from fish consumption, such as n-3 PUFA, may explain why maternal Hg levels did not increase the risk of child neurodevelopment.

The JECS is a nationwide prospective birth cohort study with a large sample size of approximately 100,000 pregnant Japanese women of which the results of ASQ-3 with longitudinal follow-up from children between 0.5 and 4 years of age were used. Therefore, it was possible to clarify the causal relationship between Hg and Se levels during pregnancy and the risks of neurodevelopmental delay in children up to 4 years of age. However, our study has some limitations. First, the

**Table 5**
Association between Hg and Se levels and subscales in ASQ-3 in children up to 4 years of age, stratified by sex of child and parity of mothers.

| Exposure | Outcome | Male children | | Female children | | Children of primiparous mothers | | Children of multiparous mothers | |
|---|---|---|---|---|---|---|---|---|---|
| | Subscales in ASQ-3 | OR (95 % CI) | *p* value | OR (95 % CI) | *p* value | OR (95 % CI) | *p* value | OR (95 % CI) | *p* value |
| **Maternal blood** | | | | | | | | | |
| Hg (ng/g) | Communication skill | 0.98 (0.71, 1.37) | 0.923 | 0.86 (0.65, 1.14) | 0.299 | 0.89 (0.69, 1.14) | 0.347 | 0.88 (0.72, 1.08) | 0.209 |
| | Gross motor skill | 0.99 (0.87, 1.12) | 0.844 | 0.94 (0.82, 1.08) | 0.381 | 0.95 (0.83, 1.09) | 0.463 | 0.98 (0.86, 1.11) | 0.766 |
| | Fine motor skill | 1.09 (0.96, 1.23) | 0.173 | 0.98 (0.83, 1.15) | 0.761 | 0.96 (0.83, 1.11) | 0.590 | 1.13 (1.00, 1.28) | 0.057 |
| | Problem-solving ability | 1.11 (0.99, 1.25) | 0.079 | 0.98 (0.84, 1.13) | 0.746 | 0.94 (0.82, 1.08) | 0.412 | 1.15 (1.02, 1.30) | 0.022 |
| | Personal and social skills | 0.91 (0.79, 1.05) | 0.180 | 0.94 (0.76, 1.16) | 0.550 | 0.82 (0.68, 0.99) | 0.036 | 0.99 (0.84, 1.15) | 0.870 |
| Se (ng/g) | Communication skill | 2.57 (0.62, 10.60) | 0.191 | 4.81 (1.33, 17.36) | 0.016 | 6.78 (2.10, 21.93) | 0.001 | 1.50 (0.60, 3.78) | 0.385 |
| | Gross motor skill | 2.02 (1.14, 3.57) | 0.015 | 3.28 (1.75, 6.12) | <0.001 | 3.29 (1.79, 6.04) | <0.001 | 2.04 (1.14, 3.64) | 0.016 |
| | Fine motor skill | 2.14 (1.23, 3.73) | 0.007 | 3.59 (1.71, 7.53) | <0.001 | 3.38 (1.71, 6.67) | <0.001 | 2.15 (1.21, 3.81) | 0.009 |
| | Problem-solving ability | 1.99 (1.14, 3.46) | 0.015 | 3.11 (1.60, 6.04) | <0.001 | 2.85 (1.48, 5.48) | 0.002 | 2.04 (1.17, 3.54) | 0.011 |
| | Personal and social skills | 1.28 (0.65, 2.55) | 0.474 | 5.34 (1.96, 14.54) | 0.001 | 2.87 (1.20, 6.83) | 0.017 | 1.63 (0.78, 3.42) | 0.195 |
| **Cord blood** | | | | | | | | | |
| Hg (ng/g) | Communication skill | 0.94 (0.42, 2.10) | 0.876 | 0.82 (0.26, 2.55) | 0.730 | 0.75 (0.26, 2.15) | 0.591 | 0.84 (0.45, 1.57) | 0.593 |
| | Gross motor skill | 0.78 (0.50, 1.22) | 0.279 | 1.14 (0.69, 1.89) | 0.604 | 1.04 (0.64, 1.68) | 0.876 | 0.88 (0.56, 1.40) | 0.592 |
| | Fine motor skill | 1.38 (0.89, 2.13) | 0.153 | 1.12 (0.66, 1.91) | 0.666 | 1.31 (0.77, 2.22) | 0.320 | 1.25 (0.81, 1.93) | 0.315 |
| | Problem-solving ability | 1.08 (0.70, 1.67) | 0.721 | 0.99 (0.58, 1.67) | 0.965 | 1.06 (0.65, 1.73) | 0.810 | 1.05 (0.68, 1.63) | 0.826 |
| | Personal and social skills | 0.68 (0.42, 1.11) | 0.127 | 0.97 (0.50, 1.91) | 0.937 | 0.62 (0.34, 1.12) | 0.115 | 0.91 (0.55, 1.52) | 0.725 |
| Se (ng/g) | Communication skill | 4.26 (0.17, 105.56) | 0.376 | 0.35 (0.01, 13.08) | 0.572 | 0.72 (0.01, 37.48) | 0.870 | 4.51 (0.33, 61.32) | 0.258 |
| | Gross motor skill | 0.97 (0.18, 5.41) | 0.976 | 1.13 (0.16, 7.87) | 0.902 | 1.11 (0.18, 7.02) | 0.885 | 1.34 (0.23, 7.72) | 0.743 |
| | Fine motor skill | 7.28 (1.34, 39.45) | 0.021 | 0.92 (0.13, 6.39) | 0.934 | 1.96 (0.25, 15.31) | 0.519 | 5.15 (0.99, 26.78) | 0.051 |
| | Problem-solving ability | 11.37 (1.85, 69.84) | 0.009 | 0.65 (0.10, 4.14) | 0.649 | 3.78 (0.45, 31.85) | 0.221 | 3.89 (0.69, 21.84) | 0.122 |
| | Personal and social skills | 5.32 (0.53, 53.22) | 0.155 | 0.43 (0.03, 7.20) | 0.554 | 2.58 (0.13, 50.62) | 0.533 | 3.79 (0.33, 43.03) | 0.283 |

From the results of Kobayashi et al. (2019a), ng/g ≈ ng/mL.
Hg and Se levels in maternal and cord blood transformed using $\log_{10}$ scale.
Generalized estimation equation (GEE) adjusted for parity (males and females only), smoking in the second/third trimesters, child sex (primiparous and multiparous only), physical abnormalities of the child (including minor and congenital anomalies), postpartum depression at one-month post-delivery, and n-3 PUFA levels.
Odds ratio (OR) (95 % confidence interval [CI]) represents the OR for one unit increase in exposure levels ($\log_{10}$ scale).

exposure sources of Se and Hg in this study were unknown. Although diet is generally the primary source of Se and Hg exposure, there are also other sources; for example, Se is used in electronic devices. We had no data on the frequency of individual contact with these appliances; hence, we could not accurately correlate Se and Hg exposure to their sources. Second, maternal blood samples were collected intravenously only once. Due to the lack of repeated sampling from the same participants, the levels of Hg and Se in maternal and cord blood might not have been accurately represented. However, since the biological half-lives of Se and Hg range from several days to several tens of days (Thomson and Stewart, 1974; Yaginuma-Sakurai et al., 2012), Se and Hg can be reflected even in a single sampling. Third, the ASQ-3 subscale scores regarding child neurodevelopmental delay were collected via self-reported questionnaires filled by participants, not trained professionals; since self-reported questionnaires are subjective, the survey results may not necessarily be accurate. Fourth, ASQ-3 is a screening, not diagnostic, a tool for child neurodevelopmental delay. Since ASQ-3 has been validated, it is therefore reliable (Agarwal et al., 2020; Chong et al., 2017; Mezawa et al., 2019). Fifth, all measurement age points in ASQ-3 were treated as one using the GEE model. Sixth, we repeated many statistical analyses; therefore, a type I error, the rejection of an actual true null hypothesis, might have occurred. Seventh, the results of this study must be carefully interpreted, since we did not consider potential confounding factors, such as other chemicals, including dioxins, polychlorinated biphenyls, and perfluoroalkyl substances, and maternal and child genetic factors. Finally, in this study, we measured Se levels in whole blood, not serum or plasma.

Despite these limitations, to the best of our knowledge, this is the first study to have identified an association between the Se levels in the whole blood of pregnant women and delays in child neurodevelopment. Future studies should measure Se levels in maternal serum and plasma to investigate the association between Se levels in maternal serum and plasma and delays in child neurodevelopment. Such work is needed to determine the threshold value for Se levels in pregnant women. The results of this study can be adapted to mothers with Se sufficiency or excess during pregnancy.

## 5. Conclusion

The study findings suggest that associations between Se levels in maternal blood and a slightly increased risk of neurodevelopmental delay in children up to 4 years of age. However, no such association was observed for Se levels in cord blood or Hg levels in maternal and cord blood. The neurodevelopmental delay was observed among children whose mothers had Se levels of ≥ 168 ng/g in their blood. Moreover, our findings show associations between Se levels in maternal blood and sex-specific and parity-specific differences in slightly delayed child communication, personal, and social skills, and associations between Hg levels in maternal blood with parity-specific differences in slightly delayed child problem-solving ability. These results suggest that Se and Hg exposure from maternal blood during early development can cause child neurodevelopmental delay in early life.

## Data statement

*Regarding data of the paper publication*

https://www.env.go.jp/chemi/ceh/en/index.html.

*Data availability*

Data are unsuitable for public deposition due to ethical restrictions and the legal framework of Japan. It is prohibited by the Act on the Protection of Personal Information (Act No. 57 of 30 May 2003, amendment on 9 September 2015) to publicly deposit the data containing personal information. Ethical Guidelines for Medical and Health Research Involving Human Subjects enforced by the Japan Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare also restrict the open sharing of epidemiologic data. All inquiries about access to data should be sent to: jecs-en@nies.go.jp. The person responsible for handling inquiries sent to this e-mail address is Dr. Shoji F. Nakayama, JECS Programme Office, National Institute for Environmental Studies.

**Table 6**
Association between maternal Se quartile levels and subscales in ASQ-3 in children between 0.5 and 4 years of age.

| Subscales in ASQ-3 | Se levels in maternal blood (ng/g) | OR (95 % CI) | *p* value |
|---|---|---|---|
| Communication skill | First quartile (83.0 to ⟨156) | Reference | |
| | Second quartile (156 to ⟨168) | 0.93 (0.83, 1.03) | 0.145 |
| | Third quartile (168 to ⟨181) | 1.10 (1.00, 1.22) | 0.055 |
| | Fourth quartile (181 to 976) | 1.07 (0.97, 1.18) | 0.182 |
| | *p* for trend | | 0.018 |
| Gross motor skill | First quartile (83.0 to ⟨156) | Reference | |
| | Second quartile (156 to ⟨168) | 0.95 (0.90, 1.01) | 0.132 |
| | Third quartile (168 to ⟨181) | 1.06 (0.99, 1.12) | 0.073 |
| | Fourth quartile (181 to 976) | 1.10 (1.04, 1.17) | 0.001 |
| | *p* for trend | | <0.001 |
| Fine motor skill | First quartile (83.0 to ⟨156) | Reference | |
| | Second quartile (156 to ⟨168) | 1.00 (0.94, 1.07) | 0.990 |
| | Third quartile (168 to ⟨181) | 1.06 (1.00, 1.13) | 0.055 |
| | Fourth quartile (181 to 976) | 1.13 (1.07, 1.20) | <0.001 |
| | *p* for trend | | <0.001 |
| Problem-solving ability | First quartile (83.0 to ⟨156) | Reference | |
| | Second quartile (156 to ⟨168) | 1.00 (0.94, 1.06) | 0.967 |
| | Third quartile (168 to ⟨181) | 1.08 (1.01, 1.14) | 0.016 |
| | Fourth quartile (181 to 976) | 1.10 (1.04, 1.17) | 0.001 |
| | *p* for trend | | <0.001 |
| Personal and social skills | First quartile (83.0 to ⟨156) | Reference | |
| | Second quartile (156 to ⟨168) | 0.97 (0.89, 1.05) | 0.402 |
| | Third quartile (168 to ⟨181) | 1.06 (0.98, 1.15) | 0.161 |
| | Fourth quartile (181 to 976) | 1.06 (0.98, 1.14) | 0.165 |
| | *p* for trend | | 0.039 |

From the results of Kobayashi et al. (2019a), ng/g ≈ ng/mL.
Generalized estimation equation (GEE) adjusted for parity, smoking in the second/third trimesters, child sex, physical abnormalities of the child (including minor and congenital anomalies), postpartum depression at one-month postdelivery, and n-3 PUFA levels.
Odds ratio (OR) (95 % confidence interval [CI]) represents the OR compared to the reference group.

### *CRediT authorship contribution statement*

**Sumitaka Kobayashi:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Sachiko Itoh:** Investigation, Data curation, Writing – review & editing. **Chihiro Miyashita:** Investigation, Data curation, Writing – review & editing. **Yu Ait Bamai:** Investigation, Data curation, Writing – review & editing. **Takeshi Yamaguchi:** Investigation, Writing – review & editing. **Hideyuki Masuda:** Investigation, Writing – review & editing. **Mariko Itoh:** Investigation, Writing – review & editing. **Keiko Yamazaki:** Investigation, Data curation, Writing – review & editing. **Naomi Tamura:** Investigation, Writing – review & editing. **Sharon J.B. Hanley:** Investigation, Writing – review & editing. **Atsuko Ikeda-Araki:** Investigation, Data curation, Writing – review & editing. **Yasuaki Saijo:** Methodology, Investigation, Validation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Yoshiya Ito:** Investigation, Validation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Miyuki Iwai-Shimada:** Investigation, Validation, Resources, Writing – review & editing, Funding acquisition. **Shin Yamazaki:** Investigation, Validation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Michihiro Kamijima:** Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Reiko Kishi:** Methodology, Investigation, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

### 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 authors do not have permission to share data.

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### Appendix 1. . Members of the Japan Environment and Children's study (JECS) group 2022

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### Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.

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