ス1999gであった。NQO1遺伝子の多型は身長と頭囲にも影響がみられ、より大きな低下を示した。妊娠初期に禁煙した場合は非喫煙の母親と変わらなかった。

北海道コーホートの今後の予定

以上まとめると、北海道コーホートでは、世界ではじめて同属異性体レベルでPCB・ダイオキシン類の胎児期曝露影響が詳細に検討され、生下時体重に負の影響が示された。ダイオキシン類では生後発達への影響や感染症罹患リスクを上げる可能性も示唆された。総PCDDs濃度、総PCDFs／TEQ濃度と出生体重との関連には性差が認められ男児のほうが感受性は高かった。性差の原因については、Ah受容体とエストロゲン受容体の両者の作用で引き起こされる可能性が示唆されている。しかし、詳細なメカニズムは不明であるので、今後、種々の角度から検討を進める。

PFOS・PFOAについては既にPOPS条約に入れられたが、我が国ではまだ規制が弱い。北海道(小規模)コーホートでは出生体重への影響が認められ、甲状腺機能に対しても影響が認められた。今後、大規模コーホートでさらに検討を進める予定で準備をしている。特に、今後、生後発達への影響についても解析する必要がある。

胎児期に参加した子どもが学童期を迎えた今、1)アレルギーやADHDなどがより明確になる学童期以降までの追跡、軽度発達障害の研究を始めている。今後は、2)複合曝露による影響の評価や3)生後曝露の測定と評価、4)エピジェネテック作用の検討が重要になる。受動喫煙をコチニン量で評価し、また、葉酸値を全員について測定しているので、受動喫煙、葉酸サプリメントや葉酸値そのものと児の胎内成長や先天異常なども解析を進めている。

さらに、「社会的要因」の重要性を指摘し、地域における子どもたちの健康と安全の問題に、これまで以上に積極的な関与と情報発信が不可欠であると考えている。2006年にはOECD統計によれば我が国が先進国の中ではアメリカについで第2位で貧困層の率が高く、13人に1人が貧困と言われている。北海道スタディでは、今後、それら社会的環境要因を含めた調査や解析によって健康障害や疾病のリスク要因を明らかにしていくことになる。

せっかくの疫学研究の成果を環境リスクマネジメントにどう活用するのか

日本の場合、農薬等のみならずダイオキシン類はその取り込み源は9割が食品、特に、魚介類である。また、フッ素系化学物質では6割から8割が食事由来で、そのほか飲用水からも比率が高い。従って一般のお母さんたちへの影響は非常に大きい。しかし、残念なのは、せっかく上述の新しい知見が日本の国民を対象にしたデータから過去10年、数多く出てきており、海外のトップジャーナルに発表

されているのにもかかわらず、それらを利活用し、リスクマネジメントや環境化学物質対策に利用することをまだ十分できていないことである。我が国では研究データの発見や知見の蓄積と、それらへの予防的な諸対策の間の距離が非常に大きいように感じられる。

一方、環境省エコチル研究は、日本で初めての出生コーホートのように伝えられているが、そうではなく、我々の北海道スタディや東北コーホートは既に過去10年の蓄積がある。出生コーホート研究は甚大なエネルギーと費用、時間がかかるが、国家財政が今後、非常に厳しいことを考えると、国（環境省）の政策としては、たとえ他の省庁（厚生労働省と文部科学省）の補助金で実施されていたとしても、環境リスク評価として既に科学的に何がわかってまだ何がわかっていないのかを十分整理しながら、課題を絞って実施するような方向性が国としては本来、大事なのではないだろうか？

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Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood☆,☆☆

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ABSTRACT

Perfluoroalkyl acids (PFAAs) are persistent organic pollutants that are detected in humans worldwide. Laboratory animal studies have shown that PFAAs are associated with immunotoxic effects. However, epidemiological studies investigating the role of PFAAs, in particular PFAAs with longer chains than perfluorooctanoic acid, are scarce. We investigated associations between prenatal exposure to PFAAs, including long-chain compounds, and infant allergic diseases at 12 and 24 months in a large study population. The participants included mothers and their infants who enrolled in the Hokkaido Study on Environment and Children's Health 2003–2009. Eleven PFAAs were measured in maternal plasma taken at 28–32 weeks of gestation using ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. Characteristics of participants and information on infant allergic diseases were obtained from self-administered questionnaires and medical records. At 24 months, the adjusted odds ratio (OR) (first vs. fourth quartiles) for eczema in association with higher maternal perfluorotridecanoic acid (PFTrDA) levels was 0.62 (95% confidence interval (CI) 0.45, 0.86). After stratification by gender, the adjusted ORs in female infants from mothers with higher maternal perfluoroundecanoic acid (PFUnDA) and PFTrDA levels were also statistically significant (PFUnDA: OR = 0.50; 95% CI, 0.30, 0.81; PFTrDA: OR = 0.39; 95% CI, 0.23, 0.64). Our findings suggest that lower prenatal exposure to PFTrDA may decrease the risk of developing eczema in early childhood, only in female infants.

1. Introduction

Perfluoroalkyl acids (PFAAs) are used in a broad range of consumer products because of their surface properties, which include insulation and water resistance. These compounds are persistent organic pollutants that are widespread within the environment, wildlife, and humans (Lau et al., 2007). Contamination of drinking water and foodstuffs such as seafood, leaching from food packaging and non-stick cookware, and household dust are major known routes of human exposure (Fromme et al., 2009). Potential health effects associated with PFAA exposure in humans are worsened by both bioaccumulation and persistence.

PFAA exposure has been suggested to have immunotoxic effects in laboratory animals including altered inflammatory responses, production of cytokines, and adaptive and innate immune responses (Dewitt et al., 2009). Cytokine expression and signaling related to inflammation and T-helper cell responses are altered in PFAA-exposed animals (Dewitt et al., 2012). PFAAs cross the placental barrier and are transferred to the fetus in humans (Midasch et al., 2007; Monroy et al., 2008). Previous epidemiological studies have shown a positive or negative association between perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and levels of cord blood immunoglobulin (Ig) E (Okada et al., 2012; Wang et al., 2011a). Moreover, these studies have reported no association between prenatal PFOS, PFOA, or perfluorononanoic acid (PFNA) exposure and allergic and infectious diseases as health outcomes in children (Fei et al., 2010; Okada et al., 2012; Wang et al., 2011a). In the C8 Health Project, which was a cross-

Abbreviations: PFAAs, perfluoroalkyl acids; PFCAs, perfluorinated carboxylic acids; PFHxA, perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; PFDoDA, perfluorododecanoic acid; PFTrDA, perfluorotridecanoic acid; PFTeDA, perfluorotetradecanoic acid; PFHxS, perfluorohexane sulfonate; PFOS, perfluorooctane sulfonate; MDL, method detection limits; CI, confidence interval; OR, odds ratio; Ig, immunoglobulin; ETS, environmental tobacco smoke; ISAAC, International Study of Asthma and Allergies in Childhood.

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☆☆ Ethics approval: This study was conducted with written informed consent from all participants and was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

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sectional, immune biomarker study that investigated residents in the vicinity of a PFOA plant, IgA, IgE, and C-reactive protein levels significantly decreased with increasing PFOA levels in blood samples (Fletcher et al., 2009).

In 2002, after 50 years of production, the 3M Company phased out the manufacture and distribution of PFOS (Renner, 2001). PFOS was also included in Annex B of the 2009 Stockholm Convention on Persistent Organic Pollutants (UNEP, 2007; Wang et al., 2009). The Environmental Protection Agency of the United States (2006) launched a 2010/2015 PFOA Stewardship Program to voluntarily reduce PFOA emissions. Recent studies indicate that concentrations of PFOS and PFOA are declining in the general human population (Olsen et al., 2012; Sundström et al., 2011; Wang et al., 2011b). In contrast, concentrations of PFNA and perfluorodecanoic acid (PFDA), which are long-chain perfluorinated carboxylic acids (PFCAs), are increasing in the general human population (Wang et al., 2011b). However, the effects of prenatal exposure to other PFAAs, particularly PFCAs, which generally have longer chains than PFOA with a carbon chain length of eight (e.g., PFDA, perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), and perfluorotridecanoic acid (PFTrDA)), have not been characterized. PFCAs with chains longer than those of PFOA have high bioconcentration factors, suggesting that they are environmentally persistent (Martin et al., 2003). Furthermore, between 2003 and 2011, we reported increased PFNA and PFDA in maternal plasma levels in Japanese, whereas levels of PFOS and PFOA decreased (Okada et al., 2013). Epidemiological determination of whether exposure to long-chain PFCAs affects immunity and allergic responses in humans is critical.

In this study, we explored associations between maternal PFAA levels, including long-chain compounds, and allergic diseases in early childhood using a prospective birth cohort study.

2. Methods

2.1. Study population

This prospective ongoing birth cohort study (Hokkaido Study on Environment and Children's Health) includes mothers who gave birth at hospitals in Hokkaido, Japan and their infants. The study was initiated in February 2003, and details have been described elsewhere (Kishi et al., 2011; Kishi et al., 2013). Briefly, participants were considered eligible if they were indigenous Japanese women who had received antenatal care at one of 37 participating hospitals within Hokkaido during their first trimester of pregnancy. Of the 33,500 eligible women invited to participate in the study from 2003 to 2009, 17,869 agreed to join (participation rate 53.3%). These participants signed informed consent forms, completed a baseline questionnaire, and also mailed follow-up questionnaires. From all participants ($n = 17,869$), we selected 12,847 who had submitted a baseline questionnaire and from whom we had obtained a third trimester blood sample and hospital birth records. From these, we excluded cases of miscarriage and stillbirth ($n = 19$), congenital malformation ($n = 143$), and multiple births ($n = 162$), because these are common exclusion criteria for studies investigating allergies, infectious diseases, mental development, and endocrine metabolic disorders. From the selected 12,523 participants, we then extracted 6335 participants who had completed all three self-administered questionnaires (at 4, 12, and 24 months after birth) for long-term follow-up of child development. Finally, from these 6335 participants, we randomly extracted 300 participants per year from 2003 to 2008 and 295 participants in 2009 to give a total of 2095 participants selected for the PFAA analysis of maternal plasma. Of these participants, we excluded cases of congenital malformations that became apparent from the follow-up questionnaire at 12 months ($n = 17$) and those whose maternal blood samples were taken before 26 weeks of gestation ($n = 15$) because the time of blood sampling during pregnancy may have affected the concentrations due to increased maternal blood volume during gestation. Thus, a final total of 2063 study participants met the specific exclusion and inclusion criteria for this study (Fig. 1). The protocol used in this study was approved by the institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2.2. Data collection

Participants completed a self-administered baseline questionnaire during the first trimester of pregnancy. The baseline questionnaire included maternal and paternal information related to age, pre-pregnancy height and weight, previous medical history, educational level, household income, alcohol intake during pregnancy, and parity. Medical birth records from hospitals included the gestational age, infant gender, and birth weight, as well as miscarriage and stillbirth, multiple births, and congenital malformations. At 4 months post-delivery, participants completed a self-administered questionnaire including information about birth size, maternal complications during pregnancy, and maternal smoking status in the third trimester. At 12 and 24 months post-delivery, participants completed another self-administered questionnaire, which included information related to breast feeding, infant weight, length, head and chest circumferences, smoking status of parents, environmental tobacco smoke (ETS) exposure, pets in the home, day care attendance, infant vaccination, and previous or current medical history of infant allergic diseases (eczema, wheezing, and allergic rhinoconjunctivitis symptoms), infectious diseases, and other diseases. ETS exposure was defined as a self-reported positive response of whether a smoker was in the place where children lived their daily life at both 12 and 24 months of age.

2.3. Assessment of infant allergic diseases

Infant allergies that developed during the first 12 months of life and from months 12–24 were assessed based on the mothers' self-administered questionnaires that were obtained twice, at 12 and 24 months post-delivery. Allergic diseases were defined using a modified part of the Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three questionnaire. In this study, we estimated eczema based on positive answers to all three of these questions: "Have you (has your child) had this itchy rash at any time in the past 12 months?", "Have you (has your child) ever had a skin rash which was coming and going for at least 6 months?", and "Has this itchy rash at any time affected any of the following places: the folds of the elbows; behind the knees; in front of the ankles; under the buttocks; or around the neck, ears, or eyes?" Wheezing was based on a positive answer to the question: "Have you (has your child) had wheezing or whistling in the chest in the past 12 months?" Current allergic rhinoconjunctivitis symptoms were based on all positive answers to both of these questions: "In the past 12 months, have you (has your child) had a problem with sneezing or a runny or blocked nose when you (he/she) did not have a cold or the flu?" and if yes, "In the past 12 months, has this nose problem been accompanied by itchy watery eyes?" (Asher et al., 2006). We also defined total allergic diseases as cases with at least one of the following symptoms: eczema, wheezing, allergic rhinoconjunctivitis symptoms.

2.4. Measurement of PFAA concentrations in maternal plasma

Detailed sampling and laboratory methods for analysis of PFAAs have been previously described (Okada et al., 2013). In brief, a 10-mL blood sample was taken from the maternal peripheral vein between 28 and 32 weeks of pregnancy. Maternal plasma was analyzed using ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry instrumentation (Waters, Tokyo, Japan).

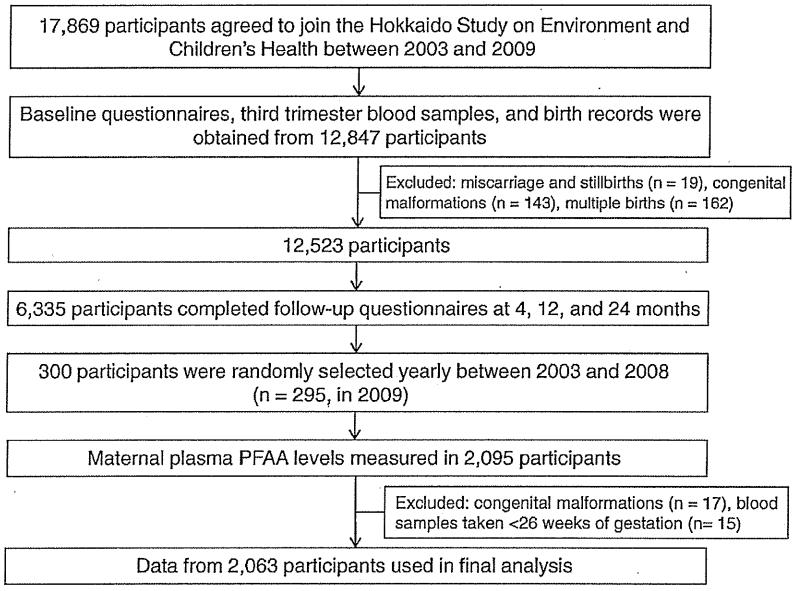

Fig. 1. Flow chart of study participant selection.

The concentrations of 11 PFAAs (perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, perfluorotetradecanoic acid (PFTeDA), perfluorohexane sulfonate (PFHxS), and PFOS) were measured in 2095 maternal plasma samples. The method detection limits (MDLs) were: PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA (0.1 ng/mL), PFOA and PFHxS (0.2 ng/mL), and PFNA and PFOS (0.3 ng/mL).

2.5. Statistical analysis

For participants with PFAA concentrations below the MDL, a value equal to half of the MDL was assigned for statistical analyses. Participants were divided into four categories based on quartiles of maternal PFAA concentrations. Crude and adjusted logistic regression analyses were performed to evaluate associations between maternal PFAA concentrations and the risk of allergic diseases. In logistic models, odds ratios (ORs) for the risk of allergic diseases were evaluated with PFAA concentrations in the second through fourth quartiles and compared to those in the first quartiles. First, to see the risk of developing at least one of the symptoms (eczema, wheezing, and allergic rhinoconjunctivitis symptoms), we examined the relationship with total allergic diseases. Second, we examined the effects on each allergic disease. Potential confounding variables considered in the analysis were: maternal age, educational levels, parental allergic history, infant gender, gestational age, birth season, breast feeding, siblings, ETS exposure, pets in the home, and day care attendance. Covariates in analysis were selected based on a review of the literature and on the change in estimate criteria, which were set to more than 10%. The fully adjusted model used logistic regression analysis of total allergic diseases and was adjusted for maternal age, maternal educational level (≤9 years, 10–12 years, 13–16 years, and ≥17 years), parental allergic history (yes/no), infant gender, breast-feeding period (<6 months or ≥6 months), number of older siblings, day care attendance (yes/no), and ETS exposure (yes/no). The number of older siblings was obtained from the parity information. Logistic regression analysis of eczema was adjusted for maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, and ETS exposure. Logistic regression analysis of wheezing was adjusted for maternal age, maternal educational level, parental allergic history, infant gender, number of older siblings, day care attendance, and ETS exposure. In previous studies, gender differences were observed between prenatal exposure to PFAAs and birth weight or cord blood IgE levels (Okada et al., 2012; Washino et al., 2009), and therefore, we further analyzed the models including the multiplicative interaction term and stratified models to assess potential effect modification by gender. All statistical analyses were performed using JMP 10 Statistical Discovery Software for Windows (S.A.S. Institute Inc., Cary, NC). Differences were considered statistically significant at $p < 0.05$.

3. Results

Demographic characteristics of the parents and infants are shown in Table 1. The mean maternal age was 30.4 ± 4.5 years. The proportion

Table 1
Characteristics of 2062 parents and infants of the Hokkaido Study on Environment and Children's Health, Japan, 2003–2009.

		No.	(%)
Parental characteristics			
Maternal age (years) (mean ± SD)		30.4 ± 4.5	
Annual household income (million yen)[a]	<5	1155	(61.8)
	≥5	640	(34.5)
Maternal educational level (years)	<13	911	(44.2)
	≥13	1151	(55.8)
Parity (times)	0	944	(45.8)
	≥1	1118	(54.2)
Maternal smoking status during pregnancy	Nonsmoker	1912	(92.7)
	Smoker	150	(7.3)
Maternal allergic history	Yes	652	(31.6)
Paternal allergic history	Yes	385	(18.7)
Infant characteristics			
Gender	Male	1044	(50.6)
	Female	1018	(49.4)
Older siblings (number)	0	944	(45.8)
	≥1	1118	(54.2)
Breast-feeding period (months)[a]	<6	420	(20.4)
	≥6	1640	(79.6)
Day care attendance at 24 months	Yes	583	(28.3)
ETS exposure at 24 months[b]	Yes	947	(45.9)

[a] Missing data: annual household income (267), breast-feeding period (2).
[b] ETS: environmental tobacco smoke.

Table 2
Concentrations of 11 PFAAs in 2062 maternal plasma samples from the Hokkaido Study on Environment and Children's Health, Japan, 2003–2009.

Compound (carbon chain length)	Detection			Concentration (ng/mL)						
	MDL[a]	No.	(%)	Geometric mean	Mean	Minimum	25th	50th	75th	Maximum
PFHxS (C6)	0.2	1688	(81.9)	0.275	0.324	<0.2	0.222	0.296	0.395	3.39
PFHxA (C6)	0.1	970	(47.0)	<0.1	0.104	<0.1	<0.1	<0.1	0.146	0.694
PFHpA (C7)	0.1	719	(34.9)	<0.1	0.096	<0.1	<0.1	<0.1	0.125	1.02
PFOS (C8)	0.3	2062	(100)	5.01	5.56	1.00	3.71	5.02	6.83	30.3
PFOA (C8)	0.2	2061	(100.0)	2.08	2.67	<0.2	1.31	2.01	3.26	24.9
PFNA (C9)	0.3	2059	(99.9)	1.19	1.36	<0.3	0.873	1.15	1.57	13.2
PFDA (C10)	0.1	2049	(99.4)	0.501	0.563	<0.1	0.382	0.510	0.684	2.43
PFUnDA (C11)	0.1	2055	(99.7)	1.34	1.50	<0.1	1.02	1.40	1.87	5.89
PFDoDA (C12)	0.1	1857	(90.1)	0.168	0.188	<0.1	0.138	0.182	0.230	0.729
PFTrDA (C13)	0.1	2012	(97.6)	0.312	0.347	<0.1	0.244	0.329	0.424	1.33
PFTeDA (C14)	0.1	271	(13.1)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.303

[a] MDL: method detection limit.

with a maternal allergic history was 31.6%. Our population consisted of 1044 (50.6%) male infants and 1018 (49.4%) female infants.

Concentrations of maternal plasma PFAAs were measured (Table 2). Nearly all study participants had detectable plasma concentrations of PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA (>90%), whereas PFHxS was detected in 81.9% of samples. PFHxA, PFHpA, and PFTeDA were detected in <50% of samples, and therefore, we did not include these compounds in the statistical analysis. PFOS was found at the highest median concentration (5.02 ng/mL), followed by PFOA (2.01 ng/mL), PFUnDA (1.40 ng/mL), and PFNA (1.15 ng/mL). We excluded a participant with an exceptionally high maternal PFOS concentration (464.8 ng/mL) as an outlier; this value was 93 times higher than the median concentration in our study and 51 times higher than the median concentration in the highest PFOS concentration area in Japan (Harada et al., 2010). Therefore, data from a final total of 2062 participants were included in this study.

The incidences of infant allergic diseases during the first 24 months were determined for our study group (Table 3). The numbers of infants who developed allergic diseases up to age 24 months were as follows: eczema, 367 (17.8%); wheezing, 397 (19.3%); allergic rhinoconjunctivitis symptoms, 91 (4.4%). The number of cases with at least one of these diseases was 714 (34.6%). We found significant gender differences in eczema and total allergic diseases, but no differences in wheezing or allergic rhinoconjunctivitis symptoms were observed between male and female infants.

Table 4 shows the results of logistic regression analyses between maternal PFAA concentrations in quartiles and total infant allergic diseases during the first 24 months. Crude and adjusted ORs ranged from 0.74 (95% confidence interval (CI): 0.57, 0.95) to 0.73 (95% CI: 0.56, 0.94) and from 0.71 (95% CI: 0.55, 0.92) to 0.73 (95% CI: 0.56, 0.94) for the three highest quartiles of PFTrDA compared with the lowest. After stratified analysis by infant gender, crude and adjusted ORs of the highest quartiles of PFAAs decreased compared with the lowest except for PFHxS, PFDA, and PFOS among female infants. The adjusted OR of PFTrDA for the highest quartiles versus the lowest quartile was 0.51 (95% CI: 0.35, 0.75). However, among male infants, the risk of allergic disease was not associated with maternal levels of the eight PFAAs. In interaction models based on quartiles of maternal PFAA levels, the adjusted ORs (95% CI) of total allergic diseases at 24 months for female infants compared to male infants were: 0.92 (0.85, 1.00) times lower for PFNA ($p = 0.050$), 0.90 (0.83, 0.98) times lower for PFUnDA ($p = 0.018$), and 0.90 (0.83, 0.98) times lower for PFTrDA ($p = 0.014$).

Crude and adjusted ORs for eczema and PFTrDA significantly decreased for the three highest quartiles compared with the lowest and ranged from 0.71 (95% CI: 0.52, 0.97) to 0.64 (95% CI: 0.47, 0.88) and from 0.69 (95% CI: 0.50, 0.94) to 0.62 (95% CI: 0.45, 0.86), with a dose–response relationship (p for trend = 0.008 and 0.005, respectively; Table 5). Among female infants, adjusted ORs of PFTrDA decreased for the three highest quartiles compared with the lowest and ranged from 0.60 (95% CI: 0.37, 0.95) to 0.39 (95% CI: 0.23, 0.64), with a dose–response relationship (p for trend <0.001). Also, the adjusted OR of PFUnDA for the highest quartiles versus lowest quartile was 0.50 (95% CI: 0.30, 0.81) among female infants (p for trend = 0.016). However, the risk of eczema was not associated with maternal levels of the eight PFAAs in male infants. In interaction models, the adjusted ORs (95% CI) of eczema at 24 months for female infants compared to male infants were: 0.89 (0.80, 0.99) times lower for PFUnDA ($p = 0.033$) and 0.88 (0.79, 0.97) times lower for PFTrDA ($p = 0.014$). Fig. 2 shows the adjusted ORs for eczema stratified by gender in association with the three highest quartiles for PFTrDA compared with the lowest.

At 12 months, no significant association was observed between eczema and PFAAs, although similar patterns of a decreased risk of eczema were seen (data not shown). Regarding wheezing, no significant associations were observed with any maternal PFAA levels at 12 or 24 months (data not shown). We did not analyze allergic rhinoconjunctivitis symptoms because the number of cases with this type of allergic disease was very low, and sufficient statistical power could not be ensured in the multivariate analysis.

4. Discussion

Only in female infants during the first 24 months did we observe an association between high maternal PFAA levels (except for PFHxS, PFDA, and PFOS) and a decline in the risk of developing total allergic diseases as seen in cases with at least one of the following: eczema, wheezing, and allergic rhinoconjunctivitis symptoms. In the Sapporo study, which was a cohort study conducted on mothers and their infants who attended one local maternity hospital from 2002 to 2005 in Sapporo, Hokkaido, Japan, we previously reported that cord blood IgE levels decrease significantly with high maternal PFOA concentrations among female infants (Okada et al., 2012). The results of the C8 Health Project, which was a cross-sectional study that investigated residents exposed

Table 3
Number and proportion of infants who developed allergic diseases during the first 24 months in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2009 (n = 2062).

Symptoms	Total		Male infants		Female infants		p[a]
	(n = 2062)		(n = 1044)		(n = 1018)		
	n	(%)	n	(%)	n	(%)	
Allergic diseases[b]	714	(34.6)	391	(37.5)	323	(31.7)	0.006
Eczema	367	(17.8)	212	(20.3)	155	(15.2)	0.003
Wheezing	397	(19.3)	212	(20.3)	185	(18.2)	0.241
Allergic rhinoconjunctivitis symptoms	91	(4.4)	51	(4.9)	40	(3.9)	0.335

[a] Fisher's exact test.
[b] "Allergic diseases" indicates cases with at least one of the listed symptoms.

Table 4
Odds ratio (95% CI) between PFAA concentrations in maternal plasma and total allergic diseases during the first 24 months in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2009 (n = 2062).

Compound (carbon chain length)	Total (n = 2062)					Male infants (n = 1044)					Female infants (n = 1018)				
	n	Crude		Adjusted[a]		n	Crude		Adjusted[b]		n	Crude		Adjusted[b]	
		OR[c]	(95% CI)[d]	OR[c]	(95% CI)[d]		OR[c]	(95% CI)[d]	OR[c]	(95% CI)[d]		OR[c]	(95% CI)[d]	OR[c]	(95% CI)[d]
PFHxS (C6)															
Quartile 1	189	1.00		1.00		112	1.00		1.00		77	1.00		1.00	
Quartile 2	193	1.05	(0.81, 1.35)	1.04	(0.80, 1.35)	104	0.95	(0.67, 1.34)	0.93	(0.65, 1.33)	89	1.19	(0.82, 1.73)	1.18	(0.80, 1.74)
Quartile 3	163	0.80	(0.62, 1.04)	0.82	(0.63, 1.07)	83	0.66	(0.46, 0.94)	0.65	(0.45, 0.94)	80	1.01	(0.69, 1.47)	1.10	(0.75, 1.64)
Quartile 4	169	0.86	(0.66, 1.11)	0.93	(0.71, 1.21)	92	0.78	(0.55, 1.11)	0.81	(0.56, 1.16)	77	0.96	(0.66, 1.40)	1.13	(0.75, 1.69)
p for trend		0.078		0.281			0.052		0.085			0.002		0.657	
PFOS (C8)															
Quartile 1	195	1.00		1.00		102	1.00		1.00		93	1.00		1.00	
Quartile 2	185	0.91	(0.71, 1.17)	0.97	(0.75, 1.26)	96	1.03	(0.72, 1.46)	1.12	(0.78, 1.61)	89	0.81	(0.56, 1.17)	0.81	(0.55, 1.18)
Quartile 3	171	0.76	(0.59, 0.98)	0.80	(0.61, 1.04)	106	0.89	(0.63, 1.26)	0.91	(0.64, 1.30)	65	0.63	(0.43, 0.93)	0.68	(0.46, 1.01)
Quartile 4	163	0.77	(0.59, 0.99)	0.86	(0.66, 1.13)	87	0.90	(0.63, 1.29)	0.95	(0.65, 1.37)	76	0.66	(0.45, 0.95)	0.79	(0.53, 1.17)
p for trend		0.018		0.139			0.432		0.535			0.629		0.183	
PFOA (C8)															
Quartile 1	197	1.00		1.00		102	1.00		1.00		95	1.00		1.00	
Quartile 2	199	1.01	(0.79, 1.30)	1.05	(0.81, 1.37)	110	1.09	(0.77, 1.54)	1.11	(0.77, 1.60)	89	0.94	(0.65, 1.35)	1.01	(0.69, 1.47)
Quartile 3	162	0.74	(0.57, 0.95)	0.80	(0.61, 1.06)	87	0.77	(0.54, 1.10)	0.82	(0.56, 1.20)	75	0.71	(0.49, 1.02)	0.77	(0.52, 1.15)
Quartile 4	156	0.70	(0.54, 0.91)	0.79	(0.59, 1.04)	92	0.85	(0.60, 1.22)	0.93	(0.63, 1.37)	64	0.56	(0.38, 0.82)	0.64	(0.42, 0.97)
p for trend		0.001		0.030			0.151		0.402			0.001		0.017	
PFNA (C9)															
Quartile 1	203	1.00		1.00			99	1.00		1.00		104	1.00		1.00
Quartile 2	152	0.79	(0.61, 1.03)	0.81	(0.62, 1.06)	89	1.10	(0.76, 1.59)	1.10	(0.75, 1.60)	63	0.55	(0.38, 0.81)	0.59	(0.40, 0.88)
Quartile 3	201	0.81	(0.63, 1.03)	0.82	(0.63, 1.05)	107	0.94	(0.67, 1.33)	0.96	(0.67, 1.37)	94	0.69	(0.48, 0.98)	0.71	(0.49, 1.02)
Quartile 4	158	0.68	(0.53, 0.88)	0.73	(0.55, 0.95)	96	0.93	(0.65, 1.32)	0.95	(0.66, 1.38)	62	0.48	(0.32, 0.69)	0.55	(0.36, 0.82)
p for trend		0.006		0.028			0.522		0.658			0.001		0.010	
PFDA (C10)															
Quartile 1	187	1.00		1.00		96	1.00		1.00		91	1.00		1.00	
Quartile 2	197	1.10	(0.85, 1.42)	1.12	(0.86, 1.44)	107	1.30	(0.91, 1.85)	1.31	(0.92, 1.89)	90	0.92	(0.64, 1.33)	0.94	(0.65, 1.37)
Quartile 3	166	0.84	(0.65, 1.09)	0.84	(0.64, 1.09)	92	1.01	(0.71, 1.44)	0.98	(0.68, 1.41)	74	0.69	(0.48, 1.01)	0.73	(0.50, 1.08)
Quartile 4	164	0.83	(0.64, 1.07)	0.89	(0.69, 1.17)	96	1.11	(0.78, 1.58)	1.13	(0.78, 1.64)	68	0.60	(0.41, 0.88)	0.70	(0.47, 1.04)
p for trend		0.044		0.149			0.915		0.894			0.003		0.039	
PFUnDA (C11)															
Quartile 1	190	1.00		1.00		93	1.00		1.00		97	1.00		1.00	
Quartile 2	184	0.95	(0.74, 1.22)	0.92	(0.71, 1.19)	108	1.23	(0.87, 1.75)	1.22	(0.85, 1.74)	76	0.70	(0.48, 1.02)	0.65	(0.44, 0.96)
Quartile 3	169	0.83	(0.64, 1.08)	0.80	(0.62, 1.04)	90	1.11	(0.77, 1.59)	1.11	(0.76, 1.62)	79	0.63	(0.44, 0.91)	0.57	(0.39, 0.83)
Quartile 4	171	0.85	(0.66, 1.10)	0.82	(0.63, 1.07)	100	1.15	(0.81, 1.64)	1.13	(0.79, 1.63)	71	0.61	(0.42, 0.89)	0.58	(0.39, 0.86)
p for trend		0.136		0.092			0.594		0.642			0.008		0.004	
PFDoDA (C12)															
Quartile 1	202	1.00		1.00		100	1.00		1.00			102	1.00		1.00
Quartile 2	158	0.69	(0.53, 0.89)	0.66	(0.51, 0.86)	99	0.88	(0.62, 1.25)	0.89	(0.62, 1.27)	59	0.51	(0.34, 0.74)	0.45	(0.30, 0.67)
Quartile 3	191	0.91	(0.71, 1.17)	0.87	(0.67, 1.13)	100	1.08	(0.76, 1.54)	1.10	(0.76, 1.58)	91	0.77	(0.54, 1.10)	0.67	(0.46, 0.97)
Quartile 4	163	0.73	(0.56, 0.94)	0.74	(0.57, 0.96)	92	0.89	(0.62, 1.27)	0.93	(0.65, 1.34)	71	0.59	(0.41, 0.85)	0.58	(0.39, 0.85)
p for trend		0.101		0.132			0.818		0.996			0.038		0.030	
PFTrDA (C13)															
Quartile 1	205	1.00		1.00		98	1.00		1.00		107	1.00		1.00	
Quartile 2	169	0.74	(0.57, 0.95)	0.71	(0.55, 0.92)	98	0.91	(0.64, 1.29)	0.93	(0.64, 1.33)	71	0.58	(0.40, 0.84)	0.51	(0.34, 0.75)
Quartile 3	174	0.77	(0.60, 0.99)	0.75	(0.58, 0.98)	100	0.95	(0.67, 1.35)	1.01	(0.70, 1.45)	74	0.61	(0.42, 0.88)	0.54	(0.37, 0.79)
Quartile 4	166	0.73	(0.56, 0.94)	0.73	(0.56, 0.94)	95	0.99	(0.69, 1.42)	1.01	(0.70, 1.46)	71	0.54	(0.37, 0.77)	0.51	(0.35, 0.75)
p for trend		0.026		0.032			0.989		0.834			0.002		0.001	

[a] Adjusted for maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, number of siblings, day care attendance, and ETS exposure in infancy at 24 months.
[b] Adjusted for all the covariates except infant gender.
[c] OR: odds ratio.
[d] CI: confidence interval.

to PFOA-contaminated drinking water, showed a significant decreasing trend in IgE levels with increasing PFOA levels in blood samples among females (Fletcher et al., 2009). Two cohort studies showed that maternal PFOS, PFOA, PFNA, and PFHxS levels negatively correlate with antibody concentrations in children (Grandjean et al., 2012; Granum et al., 2013). These studies are supported by experimental studies showing adverse effects of PFOS and PFOA exposure on humoral immune function. Our results are consistent with laboratory animal experiments in which immunosuppression and reduced IgM antibody production were observed following PFAA exposure (Keil et al., 2008; Peden-Adams et al., 2007). However, the immunotoxic effect varies depending on the type of PFAA and the endpoint being evaluated (Dewitt et al., 2012). Reduced antibody concentrations in children exposed to PFAAs may lead to immunosuppression in childhood (Granum et al., 2013). In this study, therefore, prenatal PFCA exposure may have suppressed the developing immune system in infants and thereby indirectly reduced the risk of developing immune hyperactivity/hypersensitivity diseases, such as eczema, wheezing, and allergic rhinoconjunctivitis. However, despite the reduction in allergic diseases, general immune suppression is not necessarily beneficial because this decrease may be linked to an immune system deficit.

Our findings showed gender differences in the association of allergic diseases with prenatal PFAA exposure. In the same study population (the Hokkaido Study on Environment and Children's Health), we found a negative association between maternal PFUnDA and PFTrDA levels and birth weight only in female infants (Kashino et al., 2013). A

Table 5
Odds ratio (95% CI) between PFAA concentrations in maternal plasma and eczema during the first 24 months in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2009 (n = 2062).

Compound (carbon chain length)	Total (n = 2062)					Male infants (n = 1044)					Female infants (n = 1018)				
	n	Crude		Adjusted[a]		n	Crude		Adjusted[a]		n	Crude		Adjusted[b]	
		OR[c]	(95% CI)[d]	OR[c]	(95% CI)[d]		OR[c]	(95% CI)[d]	OR[c]	(95% CI)[d]		OR[c]	(95% CI)[d]	OR[c]	(95% CI)[d]
PFHxS (C6)															
Quartile 1	107	1.00		1.00		57	1.00		1.00		40	1.00		1.00	
Quartile 2	92	0.84	(0.61, 1.14)	0.82	(0.60, 1.13)	53	0.76	(0.50, 1.15)	0.75	(0.49, 1.13)	40	0.97	(0.60, 1.56)	0.90	(0.55, 1.47)
Quartile 3	80	0.70	(0.51, 0.97)	0.69	(0.50, 0.95)	50	0.55	(0.35, 0.84)	0.55	(0.35, 0.85)	40	0.96	(0.60, 1.55)	0.93	(0.57, 1.52)
Quartile 4	88	0.80	(0.58, 1.09)	0.79	(0.57, 1.08)	52	0.78	(0.52, 1.18)	0.78	(0.51, 1.19)	35	0.83	(0.50, 1.35)	0.82	(0.49, 1.36)
p for trend		0.085		0.080			0.107		0.118			0.473		0.497	
PFOS (C8)															
Quartile 1	94	1.00		1.00		59	1.00		1.00		41	1.00		1.00	
Quartile 2	99	1.06	(0.77, 1.45)	1.06	(0.77, 1.46)	53	0.96	(0.62, 1.48)	1.00	(0.64, 1.55)	50	1.18	(0.75, 1.88)	1.13	(0.71, 1.81)
Quartile 3	90	0.94	(0.69, 1.30)	0.93	(0.67, 1.29)	56	1.32	(0.87, 1.99)	1.33	(0.87, 2.04)	27	0.58	(0.34, 0.97)	0.56	(0.32, 0.94)
Quartile 4	84	0.87	(0.63, 1.20)	0.89	(0.64, 1.24)	44	0.93	(0.60, 1.44)	0.98	(0.63, 1.53)	37	0.81	(0.50, 1.32)	0.84	(0.51, 1.38)
p for trend		0.311		0.372			0.833		0.706			0.093		0.124	
PFOA (C8)															
Quartile 1	100	1.00		1.00		50	1.00		1.00		42	1.00		1.00	
Quartile 2	102	1.02	(0.75, 1.39)	1.03	(0.75, 1.41)	58	1.05	(0.70, 1.59)	1.11	(0.73, 1.69)	40	0.97	(0.60, 1.56)	0.98	(0.60, 1.58)
Quartile 3	90	0.88	(0.64, 1.20)	0.86	(0.62, 1.19)	50	0.76	(0.49, 1.17)	0.76	(0.49, 1.19)	43	1.03	(0.65, 1.65)	0.99	(0.61, 1.61)
Quartile 4	75	0.71	(0.51, 0.98)	0.72	(0.51, 1.00)	54	0.73	(0.47, 1.13)	0.75	(0.48, 1.18)	30	0.67	(0.40, 1.11)	0.65	(0.39, 1.09)
p for trend		0.025		0.032			0.071		0.092			0.184		0.144	
PFNA (C9)															
Quartile 1	97	1.00		1.00		54	1.00		1.00		50	1.00		1.00	
Quartile 2	82	0.97	(0.70, 1.34)	0.97	(0.70, 1.35)	44	1.54	(1.00, 2.40)	1.56	(1.00, 2.44)	26	0.53	(0.31, 0.87)	0.55	(0.32, 0.91)
Quartile 3	107	0.97	(0.71, 1.31)	0.94	(0.69, 1.29)	56	1.20	(0.79, 1.83)	1.24	(0.81, 1.93)	46	0.77	(0.49, 1.19)	0.74	(0.47, 1.16)
Quartile 4	81	0.80	(0.58, 1.11)	0.77	(0.55, 1.08)	58	1.01	(0.65, 1.57)	0.96	(0.61, 1.52)	33	0.62	(0.38, 1.00)	0.63	(0.38, 1.02)
p for trend		0.224		0.145			0.756		0.643			0.127		0.122	
PFDA (C10)															
Quartile 1	104	1.00		1.00		43	1.00		1.00		47	1.00		1.00	
Quartile 2	86	0.80	(0.58, 1.10)	0.80	(0.58, 1.10)	63	1.00	(0.66, 1.52)	1.03	(0.67, 1.59)	32	0.60	(0.37, 0.98)	0.60	(0.36, 0.97)
Quartile 3	86	0.80	(0.58, 1.09)	0.78	(0.57, 1.08)	49	0.90	(0.59, 1.38)	0.92	(0.59, 1.43)	36	0.69	(0.43, 1.11)	0.68	(0.41, 1.09)
Quartile 4	91	0.85	(0.62, 1.17)	0.85	(0.62, 1.17)	57	0.94	(0.62, 1.44)	0.93	(0.60, 1.44)	40	0.77	(0.48, 1.22)	0.78	(0.49, 1.25)
p for trend		0.334		0.331			0.685		0.640			0.360		0.398	
PFUnDA (C11)															
Quartile 1	100	1.00		1.00		47	1.00		1.00		51	1.00		1.00	
Quartile 2	88	0.85	(0.62, 1.17)	0.82	(0.60, 1.14)	57	1.21	(0.79, 1.85)	1.20	(0.78, 1.86)	30	0.54	(0.33, 0.88)	0.52	(0.31, 0.85)
Quartile 3	93	0.91	(0.67, 1.25)	0.87	(0.63, 1.19)	51	1.16	(0.75, 1.80)	1.12	(0.71, 1.77)	43	0.72	(0.46, 1.12)	0.67	(0.42, 1.06)
Quartile 4	86	0.83	(0.60, 1.14)	0.78	(0.56, 1.08)	57	1.18	(0.77, 1.81)	1.16	(0.75, 1.81)	31	0.54	(0.33, 0.88)	0.50	(0.30, 0.81)
p for trend		0.341		0.185			0.526		0.607			0.036		0.016	
PFDoDA (C12)															
Quartile 1	100	1.00		1.00		53	1.00		1.00		47	1.00		1.00	
Quartile 2	83	0.80	(0.58, 1.10)	0.77	(0.56, 1.07)	58	0.92	(0.60, 1.40)	0.91	(0.59, 1.41)	30	0.65	(0.39, 1.06)	0.62	(0.37, 1.02)
Quartile 3	95	0.93	(0.68, 1.28)	0.90	(0.65, 1.23)	52	1.09	(0.71, 1.67)	1.07	(0.69, 1.66)	41	0.80	(0.50, 1.26)	0.71	(0.44, 1.14)
Quartile 4	89	0.88	(0.64, 1.21)	0.87	(0.63, 1.19)	49	0.99	(0.64, 1.52)	1.00	(0.64, 1.55)	37	0.76	(0.47, 1.22)	0.73	(0.45, 1.18)
p for trend		0.633		0.567			0.833		0.830			0.371		0.260	
PFTrDA (C13)															
Quartile 1	114	1.00		1.00		44	1.00		1.00		56	1.00		1.00	
Quartile 2	87	0.71	(0.52, 0.97)	0.69	(0.50, 0.94)	57	0.77	(0.50, 1.17)	0.77	(0.49, 1.18)	37	0.65	(0.41, 1.02)	0.60	(0.37, 0.95)
Quartile 3	87	0.71	(0.52, 0.97)	0.68	(0.50, 0.94)	60	0.83	(0.55, 1.27)	0.86	(0.56, 1.32)	34	0.58	(0.36, 0.92)	0.51	(0.32, 0.83)
Quartile 4	79	0.64	(0.47, 0.88)	0.62	(0.45, 0.86)	51	0.87	(0.57, 1.34)	0.89	(0.58, 1.38)	28	0.44	(0.27, 0.71)	0.39	(0.23, 0.64)
p for trend		0.008		0.005			0.627		0.744			0.001		<0.001	

[a] Adjusted for maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, and ETS exposure in infancy at 24 months.
[b] Adjusted for all the covariates except infant gender.
[c] OR: odds ratio.
[d] CI: confidence interval.

previous study in China showed that compared to other PFAAs, PFTrDA in cord blood is higher than in maternal blood, especially among female infants (Liu et al., 2011). In a Korean study, levels of PFTrDA were negatively correlated with total thyroxine and positively correlated with thyroid stimulating hormone levels, especially among females (Ji et al., 2012). The transport of PFTrDA across the placental barrier from mothers to infants suggests a gender difference. Therefore, prenatal PFTrDA exposure may have a potential impact predominantly on female infants. Our finding also suggests that prenatal PFAAs may differentially affect the development of allergic diseases in female infants. However, the reason for the gender-specific association with PFTrDA is not clear. Very few studies have reported the effects of long-chain PFCAs, particularly PFCAs that have longer chains than PFDA such as PFTrDA. Further investigations into the effects of long-chain PFCAs in different human populations are needed.

Levels of long-chain PFCAs such as PFUnDA, PFDoDA, and PFTrDA in plasma in the present study were higher than those seen in many countries but lower than levels reported in other areas of Japan (Harada et al., 2011). PFNA, PFUnDA, and PFTrDA are manufactured primarily in Japan via the oxidation of a mixture of linear fluorotelomer olefins (Prevedouros et al., 2006). Industrial application of these PFCAs may have contributed to the observed accumulation of longer-chain PFCAs in East Asian populations. Because longer-chain PFCAs have higher environmental persistence (Martin et al., 2003) and longer half-lives (Ohmori et al., 2003), prenatal PFTrDA exposure may have led to a reduction in the risk of developing eczema symptoms in infants in the

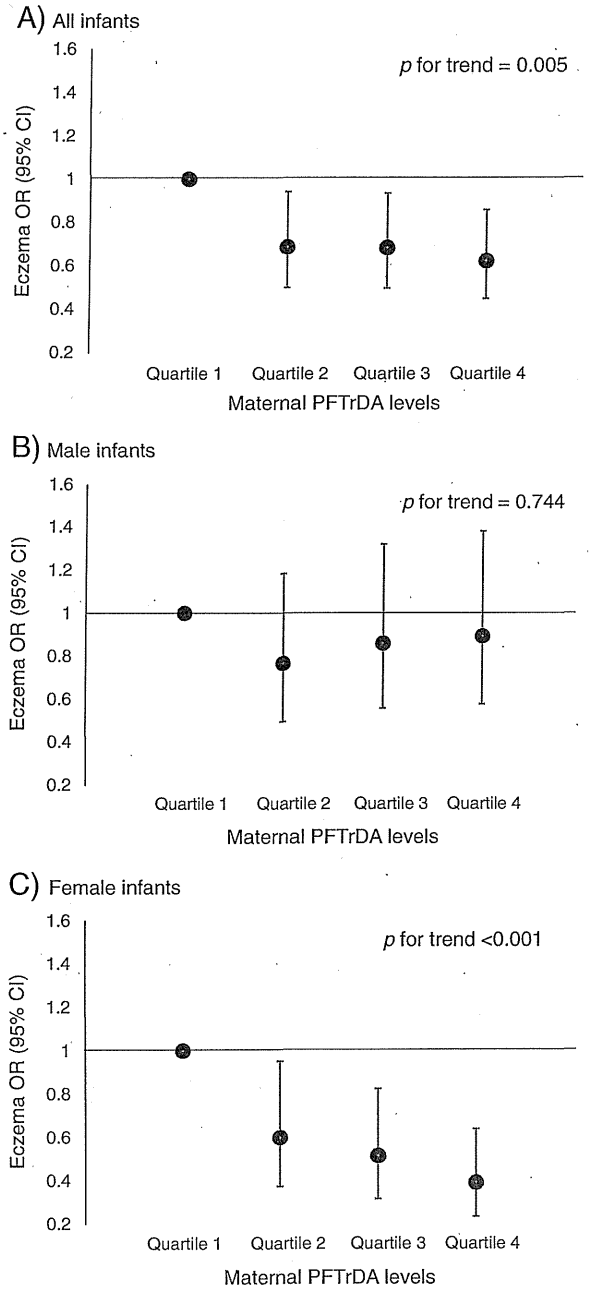

Fig. 2. ORs for eczema in association with the three highest quartiles for PFTrDA compared with the lowest. The data were adjusted for maternal age, maternal educational level, parental allergic history, infant gender, breast-feeding period, and ETS exposure in infancy at 24 months. (A) Among all infants. (B) Among male infants. (C) Female infants.

current study. The toxicity of PFCAs is correlated with the length of the carbon chain and the nature of the functional group (Liao et al., 2009; Wolf et al., 2008).

We found no association between maternal PFOS and PFOA levels and eczema and wheezing during the first 12 and 24 months. Our results are consistent with a previous cohort study that examined prenatal exposure to PFOS and PFOA and the relationship with atopic dermatitis, eczema, and wheezing (Granum et al., 2013; Okada et al., 2012; Wang et al., 2011a). The case–control Genetic and Biomarkers study for Childhood Asthma reported positive associations between serum PFAAs and asthma and positive associations between PFAAs and IgE, absolute eosinophil counts, eosinophilic cationic protein levels, and (to a lesser extent) asthma severity scores in asthmatic children (Dong et al., 2013). They investigated 10 types of PFAAs, but did not include PFTrDA and PFUnDA. The difference in the results between the present study and the Genetic and Biomarkers study for Childhood Asthma may be due to the differences in a prospective cohort study and a case–control study.

The present study has some limitations. First, we assessed allergic diseases in infants based on self-administered questionnaires by the mother. We did not investigate any biomarkers that indicate immunotoxicity. However, because we defined the development of allergic diseases with ISAAC questionnaires, which are internationally standardized procedures, these facts provided validity for the criteria for developing illness. Second, postnatal exposure to PFAAs from intake of food and drinking water or from indoor dust from birth to 24 months of age was not investigated in our study. Sources of postnatal exposure in infants also include breast milk and products in which PFAAs are used. Therefore, postnatal exposure to PFAAs may very well have affected the results. A strength of the present study is that it examined a large cohort of the general population from a relatively wide area (the entire Hokkaido region of Japan).

In conclusion, prenatal exposure to PFTrDA was associated with a decrease in the risk of developing eczema in early childhood in female infants. Prenatal PFCA exposure may have gender-specific effects on allergic diseases in infants. The immunotoxic potential of long-chain PFCAs, including PFTrDA, and the mechanisms of the gender-specific differences warrant further studies.

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REGULAR ARTICLE

Association between maternal antenatal depression and infant development: a hospital-based prospective cohort study

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Abstract
Objective To examine the association between antenatal depression and infant development after controlling for confounding factors.
Methods A hospital-based prospective cohort study (Hokkaido Study on Environment and Children's Health) was conducted between July 2002 and October 2005 in Sapporo, Japan. Of 309 mothers who delivered at Sapporo Toho Hospital during the study period and who agreed with the clinical assessment of depression, 154 mother–infant pairs were eligible for analysis. Antenatal depression was assessed between the second and third trimesters using the Edinburgh Postnatal Depression Scale (EPDS), and infant development was assessed at 6 months by the Bayley Scales of Infant Development II (BSID-II). Data on potential confounders, including socioeconomic status, birth complications, postnatal depression and child care environment, were obtained from medical records and self-administered questionnaires. Univariable and multivariable analyses were conducted in which the EPDS score was entered as an independent variable and the BSID-II scores as a dependent variable, adjusting for confounders.
Results Although the antenatal EPDS score tended to be related to the BSID-II score in the univariable analysis, this correlation was lost in the multivariable analysis. However, based on a series of linear regression analyses, antenatal depression was found to be significantly related to shorter gestational age ($\beta = -0.25$, 95 % confidence interval (CI) [−1.20, −0.17]), and shorter gestational age was significantly related to a lower BSID-II (mental development) score ($\beta = 0.23$, 95 % CI [0.00, 0.00]).
Conclusions Gestational age is an important confounder in the association between maternal antenatal depression and infant development. A delay in infant development may be related to a shorter gestational period caused by maternal depression during pregnancy.

Keywords Maternal depression · Pregnancy · Infant development · Gestational age · Cohort study

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Introduction

There is increasing recognition for the relationship between maternal psychological distress during pregnancy, such as maternal antenatal stress, anxiety, and depression and infant development, and various studies have been conducted using animal models, human physiology and epidemiology. Results from animal experiments suggest that maternal stress during pregnancy is associated with alterations in brain function and behaviour in infants. The fetuses of mothers who experience stress show alterations

in activation of the hypothalamic–pituitary–adrenal (HPA) axis and in brain function compared to fetuses of non-stressed mothers [1, 2]. According to a review of animal experiments, infants born to rodent mothers exposed to antenatal stress demonstrate more problems in learning behaviour than infants of non-stressed mothers [3].

Physiological mechanisms in humans have been proposed by several researchers [2–4]. Antenatal anxiety appears to raise uterine artery resistance, which can influence fetal development and infant birth weight [3]. The psychological status of pregnant women is known to alter the intrauterine environment and function of the fetal HPA axis, which in turn influences longitudinal behavioural and psychological development of infants after birth [2, 4].

Given these observations, epidemiological studies on human populations have been carried out in recent years [5–7]. For example, one report from a large cohort study, the Avon longitudinal study of parents and children (ALSPAC) [6], showed that antenatal depression influences child development independently of postnatal depression. The ALSPAC study also found that anxiety during pregnancy continues to affect child development 4 years after birth [7]. Another study exploring mothers who were pregnant at the time of a tornado disaster in Canada revealed the impact of strong objective stress during pregnancy on the IQ and language capability of infants [5]. Even though exposures to maternal antenatal depression, stress and anxiety are believed to be correlated to one another, there has been less investigation into the effects of antenatal depression than other maternal psychological factors [7–9]. Our study therefore focused on the relation of antenatal depression and infant development.

Previous studies examining antenatal depression and infant development are characterised by two important limitations: contradictory findings and the omission of confounding factors related to child rearing. Although some studies have insisted that antenatal depression is related to lower infant development scores that indicate a developmental delay [6, 10], others have related antenatal depression to higher performance in infant development tests [11] or have shown no correlation with infant development [12]. The ALSPAC study [6] and the study by DiPietro et al. [11] demonstrated contradictory effects of antenatal depression, despite the fact that both studies were conducted using prospective birth cohorts and applied globally standardised measures, including the Edinburgh Postnatal Depression Scale (EPDS) or the Center for Epidemiologic Studies Depression Scale for assessing maternal depression, and the Denver Developmental Screening Test (DDST) or the Bayley Scales of Infant Development II (BSID-II) for assessing infant development. Although the study population of the ALSPAC was large (9,244 women), the study had a number of limitations, such as the small number of depressed mothers and the use of maternal self-reporting to assess infant development [6]. DiPietro et al. [11] used structured assessment to avoid the problem of self-reporting; however, the number of participants was much smaller (94), which limited the study's statistical power.

In addition, confounding factors were not sufficiently controlled for in these earlier studies. In these studies, researchers controlled for diverse maternal and infant factors, including antenatal and postnatal maternal psychological distress, maternal smoking during pregnancy, maternal age, maternal educational level, infant birth weight and gender and infant age at the time of developmental assessment [6, 10–12]. However, they did not consider differences in the rearing attitude of the parents or in the home environment. Infant development is strongly influenced by interactions between the infant and the stimuli surrounding them. For example, mother–child interactions and maltreatment are well-known factors directly affecting infant development [13–15]. When infants do not obtain appropriate stimulation from their caregivers, developmental problems typically result. Therefore, examination of child rearing factors as confounders during the postnatal period is necessary.

Given these two principal limitations to previous studies, the purpose of our study was to examine the association between antenatal depression and infant development while controlling for child care factors in addition to other confounders considered in previous studies.

Methods

Study design and population

A prospective cohort study was carried out between July 2002 and October 2005 at the Sapporo Toho Hospital in Hokkaido, Japan (Hokkaido Study on Environment and Children's Health). Pregnant women who were at 23–35 weeks of gestation during a routine gynaecological check-up in this study period were recruited to the study. All participants were native Japanese and residents of Sapporo or the surrounding area. Of 1,796 potentially eligible women, 514 agreed to participate (Fig. 1; 30 % participation rate). Of all the potential participants we approached, women who had registered for the Japanese Cord Blood Bank (22 % of those who were approached) and those who delivered at another hospital (3 % of those who were approached) were excluded from the study cohort. Some of the women we approached were not interested in our study, and some were unable or unwilling to participate.

Assessment of depression during pregnancy was conducted between October 2002 and April 2004 as a nested

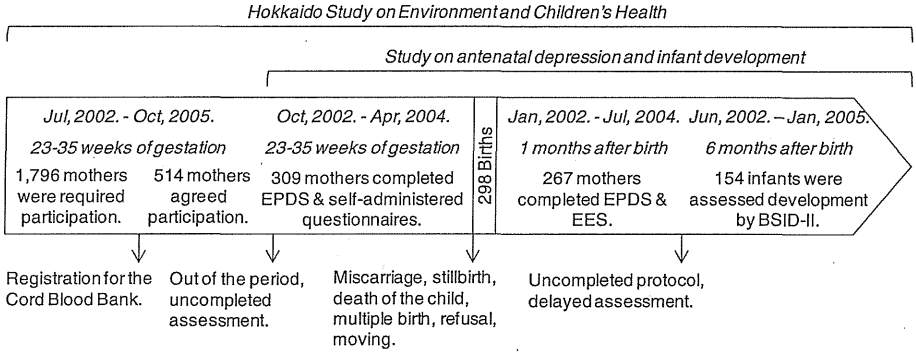

Fig. 1 Selection process for participant eligibility in Hokkaido Study. The prospective cohort study was performed between July 2002 and October 2005 at Toho Hospital (Hokkaido Study on Environment and Children's Health). A total of 1,796 pregnant women were invited to participate in the study during a routine gynaecological checkup and 514 women agreed. Women who were registered for the Cord Blood Bank were not eligible to participate. Assessment of depression during pregnancy was conducted between October 2002 and April 2004; 309 women were involved in the assessment and completed questionnaires (60 % of the initial cohort). Pregnant women who were outside of the study period or who failed to complete the assessment were eliminated from the cohort. Postnatal depression was assessed in 267 mothers between 1 and 4 months post-delivery, and infant development was assessed in 154 mother–infant pairs during the period from 5 months and 16 days to 6 months and 15 days after birth (50 % follow-up rate). Excluded participants were those who did not complete the protocol due to miscarriage, stillbirth, multiple birth, relocation, death of the infant or voluntary withdrawal from the study. Statistical analysis was conducted for 154 mother–infant pairs. *EPDS* Edinburgh Postnatal Depression Scale, *EES* evaluation of Environmental Stimulation, *BSID-II* Bayley Scales of Infant Development II

cohort study within the Hokkaido Study. Pregnant women who were recruited to the Hokkaido Study during this period were assessed for antenatal depression, and 309 women completed questionnaires (60 % of initial cohort). Postnatal depression was assessed in 267 mothers between 1 and 4 months after delivery, and infant development was evaluated in 154 mother–infant pairs during the period from 5 months and 16 days up to 6 months and 15 days after birth (50 % follow-up rate). Excluded participants were those who did not complete the protocol due to miscarriage, stillbirth, multiple birth, relocation, death of the infant, or voluntary withdrawal from the study. Statistical analysis was conducted for 154 mother-infant pairs.

Exposure measure

The EPDS was used to evaluate the incidence of antenatal depression, and pregnant women at 23–35 weeks of gestation were required to complete the EPDS questionnaire at recruitment. We assessed maternal depression between the second and third trimesters since this is the period of fetal development, and previous studies also assessed maternal psychological distress during this period [6, 11, 12]. The EPDS is a widely used self-rating questionnaire [18] and has been used during the antenatal period even though originally developed as a screening tool for maternal depression following childbirth [6]. Because the validity and reliability of the EPDS in Japanese women has been established [19], it has been commonly used for the screening of postnatal depression in Japanese community settings. The EPDS comprises ten questions evaluating depressive symptoms. Women rate their feelings over the previous 7 days using a score from 0 to 30. The standardised cut-off of 8/9 was applied for Japanese women (with a score of $\geq$9 considered to indicate depression [19]) because Japanese women tend to score lower than English-speaking women for whom the suggested cut-off is 12/13 [18].

Outcome measure

Infant development was assessed at 6 months after birth using the BSID-II [20], one of the most widely used and validated assessment tools for preschool children. Because the BSID-II is not standardised in Japan, we translated a BSID-II manual in consultation with a manual for BSID which used in the Hokkaido Study [21]. The validity of the BSID-II for Japanese infants was previously evaluated by referring to the DDST [22], and it was used in one of the Hokkaido Study analyses to assess the effects of antenatal exposure to polychlorinated biphenyls and dioxins on infant development [21]. The BSID-II consists of a mental development index (MDI) for assessing cognitive, language and personal/social development and a psychomotor development index (PDI) for assessing fine and gross motor development. MDI and PDI scores range from 50 to 150. In the USA, a mean value of 100 has been established as the cut-off point for each index. However, because the cut-off for Japanese infants requires further investigation [23], we used the total PDI and MDI scores in our study.

For the assessment, infants were brought to the community centre in Sapporo where they were tested in a quiet, private room in the presence of one or both parents. Each evaluation was performed by one of three occupational therapists with clinical experience in the field of developmental disabilities. The examiners were unaware of the antenatal EPDS scores of the mothers. In all cases, the therapist who performed the examination calculated the infant's score which was then double-checked by the other two examiners based on a video recording of the examination. The final score was decided through discussion and agreement by all three examiners.

Confounder measures

Characteristics of participants

Participants completed a self-administered questionnaire at the time of recruitment (between 23 and 35 weeks of gestation) which included questions on maternal smoking, caffeine intake, alcohol intake, drug use, working status during pregnancy, educational level of both parents and household income. Information on the anamnesis of thyroid disease and mental illness was also obtained through the questionnaire. Maternal smoking was categorised as either "no" (non-smokers who did not smoke throughout pregnancy or who quit smoking during the first trimester) or "yes" (smokers who continued to smoke during pregnancy, including women who quit after the first trimester). Modified self-administered questionnaires described by Nagata et al. [16, 17] were used to estimate caffeine and alcohol intake. Information on drug use and anamnesis of parents included medication taken at the time of study and a complete disease history. Perinatal information was obtained from obstetrical records and included age of parents at childbirth, pregnancy complications, gestational age, and infant sex, parity, disease, birth weight and birth size (length, head circumference, chest circumference). Information on maternal working status at 6 months after delivery was obtained using the self-reported questionnaire at the 6-month infant assessment.

Maternal psychological status before and during pregnancy

At the time of recruitment, pregnant women were also asked to complete self-rating questionnaires which were originally developed in this study to determine psychological status before and during pregnancy. Women answered of "yes" or "no" to questions on (1) stressful life events during the year before pregnancy ("Have you experienced stressful life events during the past year?"); (2) maternal neuroses, including past depressive symptoms ("Have you felt continuous depression or unhappiness every day for more than 2 weeks before pregnancy?"), worrying ("Do you think of yourself as a worrier?") and obsessiveness ("Do you think of yourself as obsessive?"); (3) readiness for pregnancy, including planned pregnancy ("Did you plan to be pregnant?") as well as wanted pregnancy ("Did you want to be pregnant?").

Maternal postnatal depression

The EPDS was used to evaluate postpartum depression and was mailed to mothers at 1 month after delivery and returned within 4 months

Child care environment

The self-rating questionnaire of the Evaluation of Environmental Stimulation (EES) was used to evaluate the child care environment. Mothers were asked to answer the questionnaire in the 6-month assessment period for infant development. The EES was devised based on the Home Observation for Measurement of the Environment (HOME) [24] and the Home Screening Questionnaire (HSQ) [25] as adapted for the Japanese cultural and social contexts of the child care environment [26]. The EES is composed of 30 items comprising six subscales, including "human involvement" (varied involvement in daily life, scored 0–9), "responsiveness" (maternal response to the child, scored 0–2), "avoidance of restriction and punishment" (avoidance of neglect of infant, scored 0–1), "physical involvement" (appropriate maternal physical stimulus of the infant, scored 0–4), "social involvement" (opportunities for social interaction outside the home, scored 0–6), "organisation of the environment" (organisation of the physical environment, scored 0–3) and "social support" (social support in child rearing, scored 0–5). Higher scores indicate better child care environments.

Statistical analysis

A series of univariable and multivariable analyses was conducted using the following procedure. (1) In order to detect confounding variables that were possibly correlated to maternal depression during pregnancy, univariable analyses exploring correlation between the antenatal EPDS score and potential confounders (factors adjusted in previous studies, including characteristics of mothers, fathers, infants and child care environments) were carried out using a Spearman's correlation test, a Mann–Whitney *U* test and a Kraskal–Wallis test; (2) the same nonparametric tests were conducted between BSID-II (MDI, PDI) scores and potential confounders to detect confounders that were possibly related to infant development; (3) univariable

analyses using a Spearman's correlation test and a Mann–Whitney U test were carried out to identify any correlation between maternal antenatal depression and infant development; (4) as a final justification, multivariable analyses entering the antenatal EPDS score as an independent variable and the MDI and PDI scores as outcome variables were conducted with and without adjusting for confounders, the results of which indicated a significant association of $p < 0.01$ in steps (1) and (2) of the univariable analyses. In this final process, case–control comparison between depressed and non-depressed women during pregnancy was not possible because there were only nine (5.8 %) depressed women in this study. Therefore, we applied linear regression analyses using the total score of antenatal EPDS as a continuous variable, which minimised the influence of the low number of depressed women during pregnancy. The MDI and PDI scores were transformed into log 10 scales because the distributions were skewed, while the independent variable of the antenatal EPDS score was hypothesised to follow a normal distribution according to the central limit theorem based on the sample size of over 100 [27].

Based on the results of analyses (1)–(4), gestational age and intrauterine growth restriction (IUGR) were thought to be significant confounders between depression during pregnancy and infant development. The correlation of the antenatal EPDS score with gestational age and IUGR was therefore analysed. Further linear regression analyses as well as logistic regression analyses were carried out, entering gestational age and IUGR as outcome variables and the antenatal EPDS score as an independent variable. We found no multicollinearity in a series of regression analyses. The goodness-of-fit for all regression models was evaluated by using adjusted R^2 and F test.

Informed consent and ethical review

This study was conducted after obtaining written informed consent from all participants and was approved by the Institutional Ethics Board for epidemiologic studies at the Hokkaido University Graduate School of Medicine.

Results

Table 1 presents the characteristics of the mothers, fathers, infants and the child care environment. The mean (± standard deviation, SD) maternal age at delivery was 31.4 ± 4.9 years. Twenty-six (16.9 %) mothers had a low annual household income (<3,000,000 yen), 22 (14.3 %) mothers smoked during pregnancy and 60 (39.0 %) mothers reported stressful life events during the year before pregnancy. There were 78 male (50.6 %) and 76 female (49.4 %) infants and 71 first-born infants (46.1 %). The mean (+SD) gestational age was 275.7 ± 8.5 days, and the mean infant birth weight was 3090.5 ± 361.1 g. In total, five (3.2 %) and three (1.9 %), infants were preterm and small for gestational age (SGA), respectively, and three (1.9 %) and 12 (7.8 %) infants had a low birth weight or IUGR, respectively. None of the women assessed had diabetes during pregnancy; however, the cohort included 17 women with pregnancy-induced hypertension, seven with thyroid disease, and two with mental diseases, one of whom was prescribed a minor tranquilliser.

Table 2 presents data on antenatal and postnatal depression of mothers and data for infant development. The EPDS identified nine mothers with depression during pregnancy (5.8 %) and 21 mothers with depression after delivery (13.6 %). The median MDI and PDI scores were 90 [interquartile range (IQR, 25th–75th percentile) 88–94] and 88 (IQR 82–97), respectively.

Table 3 presents the results of the univariable analyses between the antenatal EPDS score, BSID-II (MDI, PDI) scores, and potential confounders. Potential confounding variables during pregnancy that showed significant association ($p < 0.10$) with the antenatal EPDS score were maternal education level ($p = 0.055$), household income ($p = 0.076$), past depressive symptoms ($p < 0.000$), worrying ($p < 0.000$), obsessiveness ($p < 0.000$), father's age ($r = -0.14$, $p = 0.088$) and father's education level ($p = 0.096$). Postnatal EPDS was also found to be statistically significantly related to antenatal EPDS ($r = -0.48$, $p < 0.000$) (Table 4). Potential confounding factors that were significantly associated with MDI included infant sex ($p = 0.067$), IUGR ($p = 0.059$), gestational age ($r = 0.19$, $p = 0.019$), birth weight ($r = 0.15$, $p = 0.068$), infant length ($r = 0.15$, $p = 0.067$) and head circumference ($r = 0.13$, $p = 0.097$). Potential confounding variables significantly related to PDI included caffeine intake during pregnancy ($r = -0.16$, $p = 0.043$), gestational age ($r = 0.24$, $p = 0.002$), birth weight ($r = 0.14$, $p = 0.079$), infant length ($r = 0.14$, $p = 0.079$), age at 6-month assessment ($r = 0.16$, $p = 0.046$) and "avoidance of restriction and punishment" ($r = 0.18$, $p = 0.025$). Maternal smoking during pregnancy and maternal age, which were adjusted in previous studies, did not show statistical significance in correlation with antenatal EPDS, MDI or PDI.

Results of the univariable analyses for the MDI and PDI scores in relation to the antenatal and postnatal EPDS scores are shown in Table 4. Maternal antenatal EPDS tended to be significantly correlated to MDI ($r = -0.15$, $p = 0.057$), while there was no significant association between maternal postnatal depression and infant development.

We conducted linear regression analyses between the antenatal EPDS score and the MDI and PDI scores and

Table 1 Characteristics of mothers, fathers, infants, and childcare environments

Characteristic	Mean ± SD, *n* (%)
Maternal characteristics	
Age (years)	31.4 ± 4.9
Education level (years)	
≤9	5 (3.2)
10–12	54 (35.1)
13–16	92 (59.7)
≥17	3 (1.9)
Household income (yen/year)	
<3,000,000	26 (16.9)
3,000,000–5,000,000	68 (44.2)
5,000,000–7,000,000	40 (26.0)
>7,000,000	20 (13.0)
Worked during pregnancy	23 (14.9)
Smoked during pregnancy	22 (14.3)
Caffeine intake during pregnancy (mg/day)	123.4 (80.2–183.1)[a]
Alcohol intake during pregnancy (g/day)	0.0 (0.0–0.9)[a]
Stressful life events before pregnancy	60 (39.0)
Self-reported psychological status	
Past depressive symptoms	18 (11.7)
Worrying	70 (45.5)
Obsessiveness	45 (29.2)
Readiness for pregnancy	
Planned pregnancy	77 (50.0)
Wanted pregnancy	131 (85.1)
Worked at 6 months postpartum	17 (11.0)
Paternal characteristics	
Age (years)	33.2 ± 5.8
Education level (years)	
≤9	4 (2.6)
10–12	53 (34.4)
13–16	80 (51.9)
≥17	17 (11.0)
Infant characteristics	
Male	78 (50.6)
First born (parity = 0)	71 (46.1)
Preterm birth	5 (3.2)
SGA	3 (1.9)
LBW	3 (1.9)
IUGR	12 (7.8)
Gestational age (days)	275.7 ± 8.5
Birth weight (g)	3,090.5 ± 361.1
Length (cm)	48.3 ± 1.7
Head circumference (cm)	33.3 ± 1.3
Chest circumference (cm)	31.5 ± 1.4
Age at 6-month assessment (days)	190.3 ± 8.7
Child care environment	
EES subscores at 6 months	
Humanistic involvement	7 (7–8)[a]
Responsiveness	2 (2–2)[a]
Avoidance of restriction and punishment	1 (1–1)[a]
Physical involvement	3 (2–3)[a]
Social involvement	4 (3–5)[a]
Organisation of environment	2 (2–3)[a]
Social support	5 (4–5)[a]

EES Evaluation of environmental stimulation, *IUGR* intrauterine growth restriction, *LBW* low birth weight, *SD* standard deviation, *SGA* small for gestational age

[a] These data are presented as the median with the interquartile range (IQR, 25th–75th percentile) given in parenthesis

Table 2 Antenatal and postnatal maternal depression and infant development

Maternal depression/infant development	Median (IQR), *n* (%)
Maternal depression	
Antenatal EPDS[a]	
Total score	1 (0–3)
≤8	145 (94.2)
≥9	9 (5.8)
Postnatal EPDS[b]	
Total score	3 (1–6)
≤8	133 (86.4)
≥9	21 (13.6)
Infant development[c]	
BSID-II mental development index (MDI)	90 (88–94)
BSID-II psychomotor development index (PDI)	88 (82–97)

BSID-II Bayley Scales of Infant Development II, *EPDS* Edinburgh Postnatal Depression Scale

[a] Maternal depression between the second and the third trimesters (23–35 gestational weeks)

[b] Maternal depression after delivery (1–4 months)

[c] Infant development at 6 months (from 5 months and 16 days to 6 months and 15 days after birth)

adjusted for any factors with an association of $p < 0.10$ in univariable analyses (Tables 5, 6). Model 1 was adjusted for infant factors, namely, infant sex, IUGR, gestational age, birth weight, length, head circumference and age at 6-month assessment. Model 2 was adjusted using these same parameters as well as maternal caffeine intake during pregnancy and the child care factor "avoidance of restriction and punishment". Model 3 was a full model that adjusted for all covariants with a significant association of $p < 0.10$ in the univariable analyses, namely, father's age

Table 3 Maternal antenatal depression (EPDS) and infant development (BSID-II, MDI and PDI) in relation to potential confounding variables

	n	Antenatal EPDS[a]		MDI[b]		PDI[b]	
		Mean ± SD	*p*	Mean ± SD	*p*	Mean ± SD	*p*
Maternal characteristics							
Age (years)[c]		$r = -0.11$	0.184	$r = 0.01$	0.867	$r = 0.01$	0.865
Education level (years)[d]							
≤12	55	3.07 ± 3.70	0.055	91.39 ± 4.96	0.147	90.80 ± 10.95	0.492
≥13	87	2.02 ± 2.67		90.95 ± 5.98		89.73 ± 10.41	
Household income (yen/year)[e]							
<3,000,000	26	3.85 ± 3.87	0.076	89.38 ± 5.25	0.415	89.15 ± 11.18	0.807
3,000,000–5,000,000	68	2.18 ± 2.68		91.69 ± 5.25		90.76 ± 10.27	
5,000,000–7,000,000	40	1.78 ± 2.57		90.10 ± 5.12		90.05 ± 11.19	
>7,000,000	20	2.42 ± 3.14		91.45 ± 7.16		89.45 ± 10.42	
Worked during pregnancy[d]							
No	131	2.58 ± 3.32	0.342	90.63 ± 5.37	0.301	90.16 ± 10.41	0.754
Yes	23	1.52 ± 1.59		92.13 ± 6.81		90.00 ± 11.89	
Smoked during pregnancy[d]							
No	132	2.34 ± 2.97	0.913	90.83 ± 5.64	0.856	90.33 ± 10.55	0.619
Yes	22	2.91 ± 4.05		90.05 ± 5.59		89.00 ± 11.09	
Caffeine intake during pregnancy (mg/day)[c]		$r = 0.18$	0.827	$r = -0.04$	0.662	$r = -0.16$	0.043
Alcohol intake during pregnancy (g/day)[c]		$r = 0.11$	0.160	$r = -0.07$	0.383	$r = -0.04$	0.671
Stressful life events before pregnancy[d]							
No	94	2.27 ± 3.14	0.220	91.14 ± 5.22	0.450	89.34 ± 11.47	0.162
Yes	60	2.67 ± 3.16		90.42 ± 6.19		90.38 ± 9.03	
Self-reported psychological status							
Past depressive symptoms[d]							
No	136	1.96 ± 2.58	<0.001	90.75 ± 5.73	0.337	90.17 ± 10.68	0.861
Yes	18	5.94 ± 4.56		91.67 ± 4.67		89.89 ± 10.31	
Worrying[d]							
No	84	1.29 ± 1.74	<0.001	91.21 ± 5.67	0.383	89.37 ± 10.13	0.387
Yes	70	3.79 ± 3.84		90.43 ± 5.56		91.06 ± 11.15	
Obsessiveness[d]							
No	109	1.76 ± 2.29	0.001	90.66 ± 5.85	0.686	89.84 ± 10.49	0.556
Yes	45	4.02 ± 4.21		91.33 ± 5.03		90.84 ± 10.94	
Readiness for pregnancy							
Planned pregnancy[d]							
No	77	2.66 ± 3.44	0.677	90.92 ± 6.28	0.880	90.12 ± 11.52	0.912
Yes	77	2.18 ± 2.81		90.79 ± 4.90		90.16 ± 9.68	
Wanted pregnancy[d]							
No	23	3.39 ± 4.20	0.238	91.65 ± 6.78	0.945	91.22 ± 10.04	0.548
Yes	131	2.25 ± 2.90		90.72 ± 5.40		89.95 ± 10.72	
Worked at 6 months[d]							
No	137	2.48 ± 3.27	0.988	90.92 ± 5.62	0.772	89.92 ± 10.52	0.504
Yes	17	1.94 ± 1.89		90.35 ± 5.71		91.88 ± 11.43	
Paternal characteristics							
Age (years)[c]		$r = -0.14$	0.088	$r = -0.02$	0.845	$r = -0.09$	0.273
Education level (years)[d]							
≤12	57	2.79 ± 2.21	0.096	90.65 ± 5.74	0.891	91.19 ± 9.62	0.167
≥13	97	2.21 ± 3.15		90.98 ± 5.56		89.52 ± 11.14	
Infant characteristics							

Table 3 continued

	n	Antenatal EPDS[a]		MDI[b]		PDI[b]	
		Mean ± SD	*p*	Mean ± SD	*p*	Mean ± SD	*p*
Sex[d]							
Male	78	2.31 ± 2.82	0.617	91.72 ± 5.71	0.067	90.81 ± 10.03	0.337
Female	76	2.54 ± 3.45		89.97 ± 5.61		89.45 ± 11.12	
Parity[d]							
0	71	2.61 ± 3.49	0.963	91.17 ± 5.62	0.725	89.82 ± 10.99	0.553
≥1	83	2.27 ± 2.82		90.59 ± 5.63		90.41 ± 10.31	
Preterm birth							
No	149	2.37 ± 3.06	0.725	90.87 ± 5.67	0.992	90.09 ± 10.49	0.890
Yes	5	4.00 ± 5.15		90.40 ± 3.85		91.40 ± 15.13	
SGA[d]							
No	151	2.43 ± 3.16	0.957	90.88 ± 5.66	0.659	90.18 ± 10.70	0.803
Yes	3	2.00 ± 2.65		89.67 ± 2.08		88.00 ± 3.00	
LBW[d]							
No	151	2.38 ± 3.13	0.165	90.83 ± 5.63	0.757	90.24 ± 10.61	0.351
Yes	3	4.33 ± 3.51		92.00 ± 5.29		85.00 ± 10.39	
IUGR[d]							
No	142	2.45 ± 1.42	0.859	90.62 ± 5.55	0.059	90.32 ± 10.84	0.477
Yes	12	2.08 ± 2.28		93.67 ± 5.77		88.00 ± 7.12	
Gestational age (days)[c]		$r = 0.22$	0.006	$r = 0.19$	0.019	$r = 0.24$	0.002
Birth weight (g)[c]		$r = 0.06$	0.479	$r = 0.15$	0.068	$r = 0.14$	0.079
Length (cm)[c]		$r = 0.15$	0.065	$r = 0.15$	0.067	$r = 0.14$	0.079
Head circumference (cm)[c]		$r = 0.14$	0.094	$r = 0.13$	0.097	$r = 0.07$	0.365
Chest circumference (cm)[c]		$r = 0.07$	0.391	$r = 0.13$	0.113	$r = 0.09$	0.294
Age at 6-month assessment (days)[c]		$r = 0.07$	0.385	$r = 0.09$	0.275	$r = 0.16$	0.046
Childcare environment							
EES subscores at 6 months							
Human involvement[c]		$r = 0.20$	0.018	$r = 0.04$	0.610	$r = -0.04$	0.958
Responsiveness[c]		$r = 0.05$	0.530	$r = 0.10$	0.200	$r = 0.03$	0.705
Avoidance of restriction and punishment[c]		$r = -0.56$	0.491	$r = 0.93$	0.252	$r = 0.18$	0.025
Physical involvement[c]		$r = -0.11$	0.165	$r = -0.04$	0.667	$r = -0.09$	0.251
Social involvement[c]		$r = 0.20$	0.018	$r = -0.03$	0.740	$r = -0.13$	0.120
Organisation of environment[c]		$r = 0.10$	0.242	$r = -0.07$	0.378	$r = -0.02$	0.821
Social support[c]		$r = -0.21$	0.011	$r = -0.06$	0.474	$r = 0.03$	0.694

Potential confounding variables including characteristics of mothers, fathers, infants, and childcare environments

EES evaluation of environmental stimulation, *EPDS* Edinburgh Postnatal Depression Scale, *IUGR* intrauterine growth restriction, *LBW* low birth weight, *MDI* mental development index, *PDI* psychomotor development index, *SGR* small for gestational age

[a] Maternal antenatal depression between the second and the third trimesters (23–35 gestational weeks)

[b] Infant mental and psychomotor development at 6 months (from 5 months and 16 days to 6 months and 15 days after birth); Statistical analyses

[c] Spearman correlation

[d] Mann–Whitney *U* test

[e] Kruskal–Wallis test

and father's educational level in addition to factors adjusted for in Model 2. In the linear regression analyses, $p < 0.05$ was considered to indicate a significant association.

Table 5 presents the MDI score in relation to the antenatal EPDS score and confounding variables based on the crude model (goodness of fit: adjusted $R^2 = 0.007$, $F = 2.07$, $p = 0.153$), model 1 (adjusted $R^2 = 0.087$,

Table 4 Infant development (BSID-II, MDI and PDI) in relation to antenatal and postnatal maternal depression (EPDS)

Maternal characteristics	*n*	Antenatal EPDS[a]		MDI[b]		PDI[b]	
		Mean ± SD	*p*	Mean ± SD	*p*	Mean ± SD	*p*
Antenatal EPDS							
Total score[c]				$r = -0.15$	0.057	$r = -0.1$	0.881
≤8[d]	145			90.88 ± 5.71	0.756	90.06 ± 10.51	0.841
≥9	9			90.44 ± 3.92		91.33 ± 12.57	
Postnatal EPDS at 1 month							
Total score[c]		$r = 0.48$	<0.000	$r = -0.16$	0.679	$r = -0.03$	0.216
≤8[d]	133	1.88 ± 2.54	<0.000	90.96 ± 5.80	0.302	89.71 ± 10.68	0.798
≥9	21	5.86 ± 4.30		90.19 ± 4.33		92.81 ± 9.94	

EPDS Edinburgh Postnatal Depression Scale, *MDI* Mental Development Index, *PDI* Psychomotor Development Index

[a] Maternal antenatal depression between the second and the third trimesters (23–35 gestational weeks)

[b] Infant mental and psychomotor development at 6 months (from 5 months and 16 days to 6 months and 15 days after birth); Statistical analyses

[c] Spearman correlation

[d] Mann–Whitney *U* test

$F = 2.81$, $p = 0.006$), model 2 (adjusted $R^2 = 0.080$, $F = 2.34$, $p = 0.014$) and model 3 (adjusted $R^2 = 0.069$, $F = 1.94$, $p = 0.034$). Even though the validity of all statistical models except the crude model was assured at the level of $p < 0.05$, the adjusted R^2 was highest in model 1. A significant association between antenatal EPDS and MDI was not found in the crude model or in any of the adjusted models (crude: $\beta = -0.00$, 95 % CI [−0.00, 0.00], $p = 0.153$; model 1: $\beta = -0.05$, 95 % CI [−0.00, 0.00], $p = 0.500$; model 2: $\beta = -0.05$, 95 % CI [−0.00, 0.00], $p = 0.552$; model 3: $\beta = -0.05$, 95 % CI [−0.00, 0.00], $p = 0.585$). To the contrary, gestational age showed a significant relation to MDI with consistently larger regression coefficients than those of the other factors even though the statistical model had been changed (model 1: $\beta = 0.23$, 95 % CI [0.00, 0.00], $p = 0.013$; model 2: $\beta = 0.22$, 95 % CI [0.00, 0.00], $p = 0.019$; model 3: $\beta = 0.23$, 95 % CI [0.00, 0.00], $p = 0.018$). Similarly, IUGR showed a significant relation to MDI in all models (model 1: $\beta = 0.19$, 95 % CI [0.00, 0.04], $p = 0.020$; model 2: $\beta = 0.21$, 95 % CI [−0.00, 0.04], $p = 0.015$; model 3: $\beta = 0.21$, 95 % CI [0.00, 0.04], $p = 0.017$).

Table 6 presents the PDI score in relation to the antenatal EPDS score and confounding variables based on the crude model (goodness of fit: adjusted $R^2 = -0.007$, $F = 0.01$, $p = 0.927$), model 1 (adjusted $R^2 = 0.092$, $F = 2.93$, $p = 0.005$), model 2 (adjusted $R^2 = 0.133$, $F = 3.34$, $p = 0.001$) and model 3 (adjusted $R^2 = 0.141$, $F = 3.09$, $p = 0.001$). Each adjusted model was validated at the level of $p < 0.01$; however, model 3 showed the highest value of adjusted R^2. While there was no significant correlation between antenatal EPDS and PDI in all models, PDI did show an association with gestational age (model 1: $\beta = 0.28$, 95 % CI [0.00, 0.00], $p = 0.003$; model 2: $\beta = 0.25$, 95 % CI [0.00, 0.03], $p = 0.006$; model 3: $\beta = 0.23$, 95 % CI [0.00, 0.00], $p = 0.012$), infant age at the 6-month assessment (model 1: $\beta = 0.25$, 95 % CI [0.00, 0.00], $p = 0.002$; model 2: $\beta = 0.24$, 95 % CI [0.00, 0.00], $p = 0.003$; model 3: $\beta = 0.24$, 95 % CI [0.00, 0.00], $p = 0.002$), and "avoidance of restriction and punishment" (model 2: $\beta = 0.20$, 95 % CI [0.01, 0.07], $p = 0.010$; model 3: $\beta = 0.23$, 95 % CI [0.02, 0.08], $p = 0.004$).

Although we found no significant relation between antenatal EPDS and MDI or PDI in our linear regression analyses, the Spearman's correlation test detected a trend towards a correlation between antenatal EPDS and MDI ($r = -0.15$, $p = 0.057$) (Table 3). To the contrary, antenatal EPDS was significantly associated with gestational age in Spearman's correlation ($r = 0.22$, $p = 0.006$) (Table 3) and, moreover, gestational age and IUGR were significantly related to MDI or PDI in the linear regression analyses. In order to explore the association between all of these variables in detail, we conducted further multiple linear regression analyses on gestational age and logistic regression analyses on IUGR in relation to antenatal EPDS (Table 7), adjusting for all potential confounders before delivery. The factors adjusted were: maternal factors (age, education level, household income, worked during pregnancy, smoked during pregnancy, caffeine intake during pregnancy, alcohol intake during pregnancy, stressful life events before pregnancy, past depressive symptoms, worrying, obsessiveness, planned pregnancy, wanted pregnancy), paternal factors (age and education level) and infant factors (sex and parity).

As a consequence, antenatal EPDS was significantly correlated to gestational age in the crude model

Table 5 Linear regression analyses for mental development (BSID-II, MDI) in relation to maternal antenatal depression (EPDS) and confounding variables

Antenatal EPDS	Crude[a] adjusted $R^2 = 0.007$ $F = 2.07$, $p = 0.153$			Model 1[b] adjusted $R^2 = 0.087$, $F = 2.81$, $p = 0.006$			Model 2[c] adjusted $R^2 = 0.080$, $F = 2.34$, $p = 0.014$			Model 3[d], adjusted $R^2 = 0.069$, $F = 1.94$, $p = 0.034$		
	β	95 % CI	p	β	95 % CI	p	β	95 % CI	p	β	95 % CI	p
Antenatal EPDS	−0.00	[−0.00, 0.00]	0.153	−0.05	[−0.00, 0.00]	0.500	−0.05	[−0.00, 0.00]	0.552	−0.05	[−0.00, 0.00]	0.585
Infant factors												
Sex				−0.14	[−0.02, 0.00]	0.107	−0.14	[−0.02, 0.00]	0.105	−0.14	[−0.02, 0.00]	0.105
IUGR				0.19	[0.00, 0.04]	0.020	0.21	[−0.00, 0.04]	0.015	0.21	[0.00, 0.04]	0.017
Gestational age				0.23	[0.00, 0.00]	0.013	0.22	[0.00, 0.00]	0.019	0.23	[0.00, 0.00]	0.018
Birth weight				0.04	[0.00, 0.00]	0.790	0.05	[0.00, 0.00]	0.725	0.05	[0.00, 0.00]	0.730
Length				0.01	[0.00, 0.00]	0.928	0.02	[−0.00, 0.00]	0.864	0.02	[−0.00, 0.00]	0.878
Head circumference				0.08	[0.00, 0.01]	0.408	0.07	[−0.00, 0.01]	0.505	0.07	[−0.00, 0.01]	0.522
Age at 6-month assessment				0.09	[0.00, 0.00]	0.233	0.09	[0.00, 0.00]	0.252	1.00	[0.00, 0.00]	0.230
Childcare factor												
Avoidance of restriction and punishment							0.08	[−0.01, 0.03]	0.349	0.07	[−0.01, 0.03]	0.384
Maternal factor												
Caffeine intake during pregnancy							−0.20	[0.00, 0.00]	0.841	−0.02	[0.00, 0.00]	0.849
Paternal factors												
Age										0.00	[−0.00, 0.00]	0.980
Education level										0.04	[−0.01, 0.01]	0.627

CI confidence interval, *EPDS* Edinburgh Postnatal Depression Scale, *IUGR* intrauterine growth restriction, *PDI* Psychomotor Development Index

[a] $n = 154$ in linear regression analyses

[b] Model 1: Adjusted for infant factors (sex, IUGR, gestational age, birth weight, length, head circumference and age at 6-month assessment)

[c] Model 2: Adjusted as in Model 1 as well as for childcare factor (avoidance of restriction and punishment) and maternal factor (caffeine intake during pregnancy)

[d] Model 3: Adjusted as in Model 2 as well as for paternal factors (age and education level)