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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.

Key words: Perfluoroalkyl acids, perfluorotridecanoic acid, prenatal exposure, allergic diseases, eczema

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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.

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

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 IJ et al., 2011). 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 IJ et al., 2011). In the C8 Health Project, which was a cross-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 M et al., 2011). 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 M et al., 2011). 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 6,335 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 6,335 participants, we randomly extracted 300 participants per year from 2003 to 2008 and 295 participants in 2009 to give a total of 2,095 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 concentrations due to increased maternal blood volume during gestation. Thus, a final total of 2,063 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). 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 2,095 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 with a maternal allergic history was 31.6%. Our population consisted of 1,044 (50.6%) male infants and 1,018 (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 2,062 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 of 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 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 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 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 IJ et al., 2011). 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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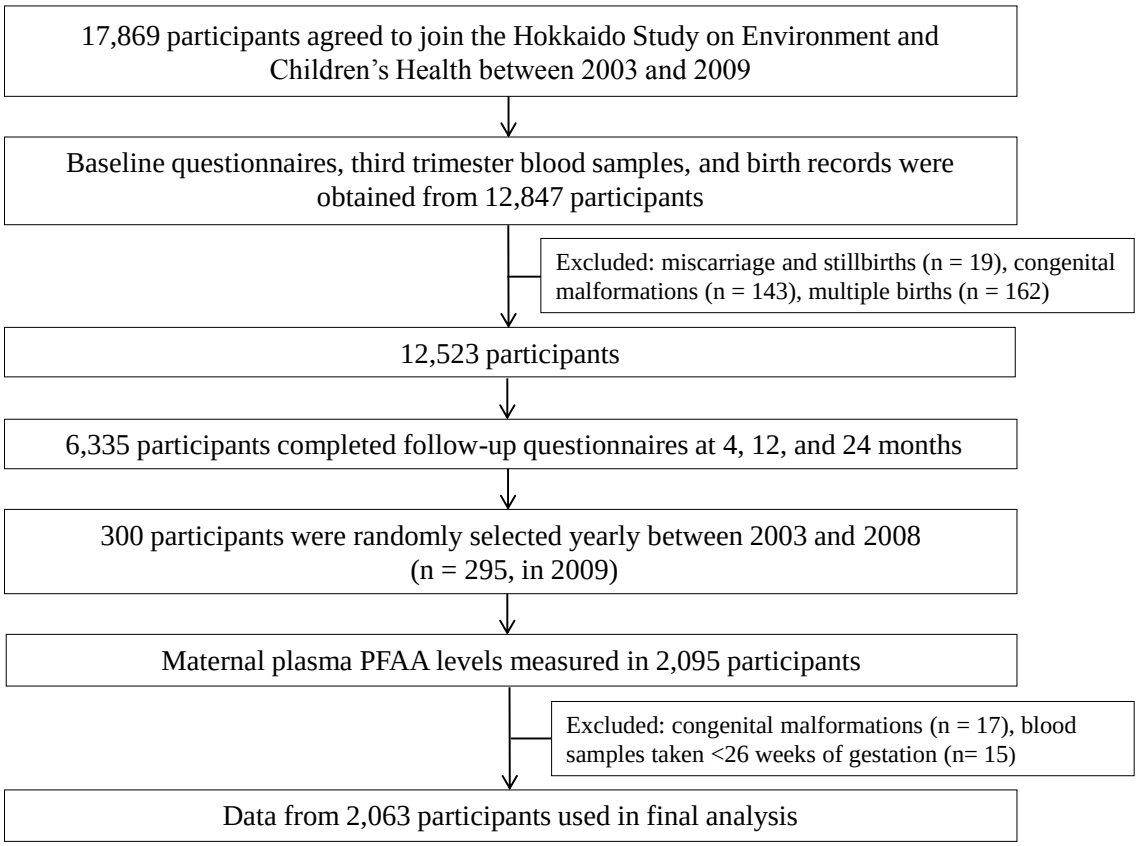
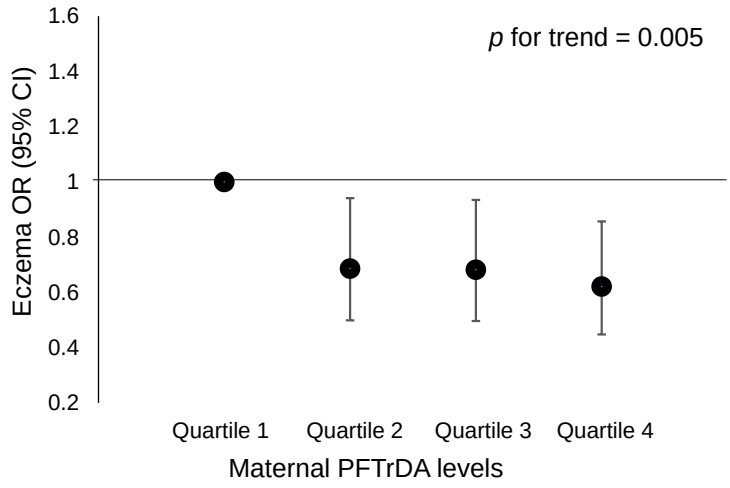
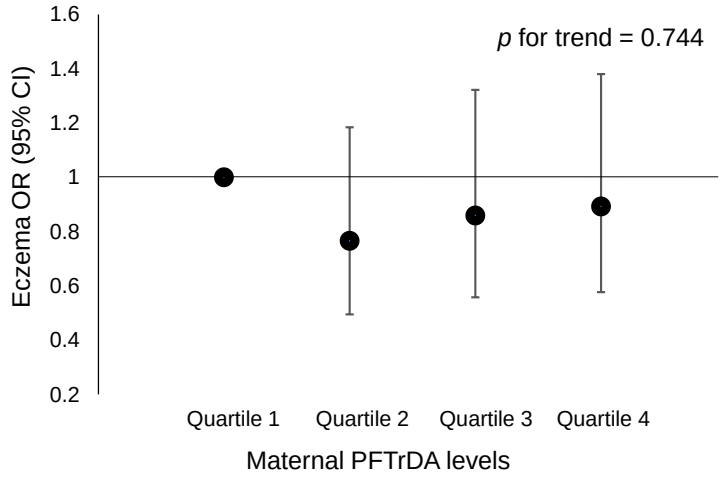


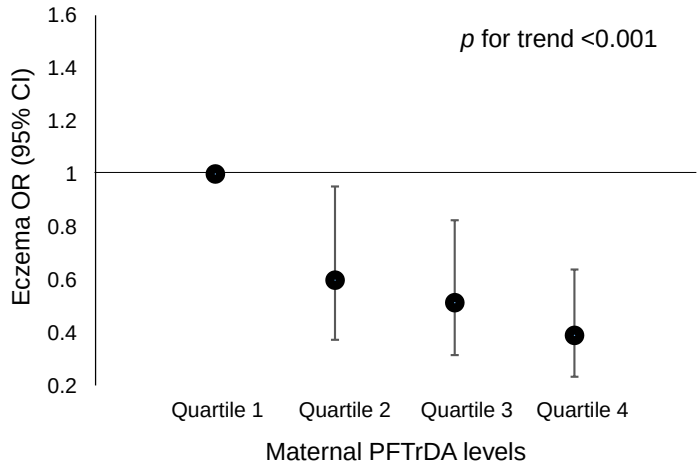
Fig. 1. Flow chart of study participant selection.



A; All infants



B; Male infants



C; Female infants

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*) Among female infants.

Table 1

Characteristics of 2,062 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	1,155	(61.8)
	≥5	640	(34.5)
Maternal educational level (years)	<13	911	(44.2)
	≥13	1,151	(55.8)
Parity (times)	0	944	(45.8)
	≥1	1,118	(54.2)
Maternal smoking status during pregnancy	Nonsmoker	1,912	(92.7)
	Smoker	150	(7.3)
Maternal allergic history	Yes	652	(31.6)
Paternal allergic history	Yes	385	(18.7)
Infant characteristics			
Gender	Male	1,044	(50.6)
	Female	1,018	(49.4)
Older siblings (number)	0	944	(45.8)
	≥1	1,118	(54.2)
Breast-feeding period (months)[a]	<6	420	(20.4)
	≥6	1,640	(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 2,062 maternal plasma samples from the Hokkaido Study on Environment and Children's Health, Japan, 2003–2009.

Compound (carbon chain length)	MDL[a]	Detection		Concentration (ng/mL)						
		No.	(%)	Geometric mean	Mean	Minimum	25th	50th	75th	Maximum
PFHxS (C6)	0.2	1,688	(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	2,062	(100)	5.01	5.56	1.00	3.71	5.02	6.83	30.3
PFOA (C8)	0.2	2,061	(100.0)	2.08	2.67	<0.2	1.31	2.01	3.26	24.9
PFNA (C9)	0.3	2,059	(99.9)	1.19	1.36	<0.3	0.873	1.15	1.57	13.2
PFDA (C10)	0.1	2,049	(99.4)	0.501	0.563	<0.1	0.382	0.510	0.684	2.43
PFUnDA (C11)	0.1	2,055	(99.7)	1.34	1.50	<0.1	1.02	1.40	1.87	5.89
PFDoDA (C12)	0.1	1,857	(90.1)	0.168	0.188	<0.1	0.138	0.182	0.230	0.729
PFTrDA (C13)	0.1	2,012	(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.

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 = 2,062).

Symptoms	Total (n = 2,062)		Male infants (n = 1,044)		Female infants (n = 1,018)		p[a]
	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 = 2,062).

Compound (carbon chain length)	Total (n = 2,062)					Male infants (n = 1,044)					Female infants (n = 1,018)				
	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.

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 = 2,062).

Compound (carbon chain length)	Total (n = 2,062)					Male infants (n = 1,044)					Female infants (n = 1,018)				
	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	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.