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分担研究報告書

Prenatal Exposure to Perfluoroalkyl Acids and Risk of Infectious Diseases in Early Life

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研究要旨

Animal studies have shown that perfluroalkyl acids (PFAAs) have immunotoxic effects. However, epidemiological studies investigating the effects of PFAAs on infectious diseases, are scarce. We examined the relation between prenatal exposure to PFAAs and risk of infectious diseases at 4 years of age. Mother-infant pairs who enrolled in the Hokkaido Study on Environment and Children's Health in 2003–2009 were included in this study. Eleven PFAAs including PFHxA, PFHpA, PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA were measured in maternal plasma taken at third trimester of gestation using ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry (UPLC-MS-MS). Information on characteristics of participants was obtained from medical birth records, and self-administered questionnaires obtained during pregnancy and after delivery. Infectious diseases including otitis media, pneumonia, respiratory syncytial virus (RSV), varicella, and febrile seizure were defined using a mother-reported questionnaire at 4 years of age. For those who have information on allergy at 4 years and PFAA measurements were used for analysis (n=1558). The number of children who developed infectious disorders at 4 years of age were as follows: otitis media, 649 (41.4%); pneumonia, 287 (18.4%); RSV, 197 (12.6%); varicella 589 (37.8%), and febrile seizure, 121 (7.7%), and total infectious disease 1075 (69.0%). PFOS levels in the highest quartile were associated with increased odds ratio of infectious diseases (Q4 vs Q1 OR: 1.56; 95% CI: 1.12, 2.17; p for trend= 0.022) in all children. In addition, PFHxS was associated with higher risk of total infectious diseases only among girls (Q4 vs Q1 OR: 1.56, 95% CI: 0.963, 2.54; p for trend= 0.043). Our findings suggest that prenatal exposure to PFOS and PFHxS may increase risk of infectious diseases at 4 years of age. In addition, we previously reported immunosuppressive effects of PFAAs on allergic symptoms at 2 and 4 years old children. These suggest that prenatal exposure to PFAAs may suppress immune system in next generation.

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A．研究目的

There is a globally contamination of perfluoroalkyl acids (PFAAs) in environment, wild life, and humans. Food is expected to be the main source of human exposure to PFAAs; however people are also exposed to these chemicals through contaminated water, dust and air and various consumer products (ATSDR 2015). Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are the most

commonly used PFAAs. PFAAs are resistant to metabolism; elimination half-life for PFOS and PFOA is 5.4 and 3.8 years, respectively (Olsen et al. 2007). Recently, PFOS and PFOA are being voluntarily phased out by several industries, however they are still present in older products. However, humans are constantly exposed to PFAAs with long-half-lives resulting in bioaccumulation into human tissues overtime which raises human health concerns.

Globally, infectious diseases account for more than one-half of all deaths among children aged less than 5 years, and it also has high burden for health care systems (Elliot and Beason, 2008). Previous laboratory studies showed that exposure to PFAAs have immunotoxic and immunosuppressive effects such as atrophy and reduced cell number of immune organs such as spleen and thymus, lower IgM production, decreases of natural killer-cell activity and change of pro-inflammatory cytokine production (Dewitt 2008, Peden-Adams 2008, Brieger et al. 2011; Qazi et al. 2012).

PFAAs can pass placenta during pregnancy, therefore fetuses are exposed to these chemicals. Pre- and postnatal PFOS/ PFOA concentrations are associated with reduced humoral immune response to diphtheria and tetanus in children aged 5 and 7 years (Grandjean et al. 2012). Also, another report showed inverse association between prenatal exposure to PFOS, PFOA, PFNA and PFHxS and the level of anti-rubella antibodies in the children and the concentrations of the four PFAAs. Furthermore, they found a positive association between the maternal concentrations of PFOA and PFNA and the number of episodes of common cold for the children, and between PFOA and PFHxS and the number of episodes of gastroenteritis (Granum et al. 2013). However, Fei et al. (2010) reported no association between prenatal exposure to PFOS and PFOA with and risk of infectious diseases leading o hospitalization in early childhood.

Previously, in a small cohort, we reported negative association of prenatal exposure to PFOA and cord blood IgE levels among female infant; however we did not observe any association between PFOS and PFOA with risk of allergic diseases at 18 months of age (Okada et al. 2012). We also examined the association of in utero exposure to PFAAs with allergic diseases in early infancy in a large scale cohort and found that PFTrDA levels is inversely associated with risk of eczema among female infants (Okada et al. 2014).

To this date, effects of PFAAs on risk of infectious diseases is not well investigated especially impact of exposure to these chemicals during pregnancy on developing immune system and functions. In this study, we assessed association between prenatal exposure to eleven PFAAs and risk of infectious diseases in early childhood, in a prospective birth cohort.

B．研究方法

The current work is a part Hokkaido Study on Environment and Children’s health, prospective ongoing birth cohort (Kishi et al. 2011 and 2013). This study started in February 2003 and the participants were all native Japanese mother-child pairs. Briefly, pregnant women who had antenatal health care in early pregnancy (>13 weeks of gestational age) at any 37 participating hospitals and clinics in Hokkaido prefecture

in this study were eligible. Health care personnel approached pregnant women and introduced the study. Flowchart of study is shown in Figure. 1.

During the first trimester of pregnancy, participants completed a self-administered baseline questionnaire which included parental information related to age, prepregnancy BMI, previous medical history, educational level, annual household income, parity, alcohol consumption and smoking during pregnancy. Medical birth records from hospitals included the gestational age, infant gender, and birth weight, as well as miscarriage, stillbirth, multiple births, and congenital anomalies. We collected a self-administered questionnaire at 4 months after delivery reported by mothers, including information about maternal smoking status in the third trimester. At 4 years post-delivery, participants completed another self-administered questionnaire including information related to breast feeding, smoking status of parents, parental history of allergic diseases, pets in the home, and environmental tobacco smoke (ETS) exposure and day care attendance. In addition, mothers reported previous or current medical history of infant infectious diseases including pneumonia, otitis media, varicella, respiratory syncytial virus (RSV), and febrile seizure.

Detailed sample preparation and PFAAs measurement methods have been previously described (Okada et al. 2013). Maternal peripheral vein samples were collected and stored at -80ºc until exposure analysis. We used maternal plasma for exposure assessment using ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry instrumentation (UPLC-MS/MS) (Waters, USA). We measured concentrations of 11 PFAAs: PFSAs (perfluoroalkane sulfonates) including PFHxS, PFOS; and PFCAs (perfluorinated carboxylic acids) including perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, perfluorotetradecanoic acid (PFTeDA) in maternal plasma samples obtained at 3rd trimester of pregnancy.

We performed all of the statistical analyses using JMP pro 10 (SAS Institute Inc., NC, USA). The results were considered statistically significant if $p < 0.05$. For participants with PFAA levels less than MDL, a value equal to half of the MDL was substituted. We divided participants to 4 groups according to quartiles (Q) of prenatal PFAA levels. In crude and adjusted logistic regression analyses we examined associations between maternal PFAA concentrations and the risk of infectious diseases. In logistic models, odds ratios (ORs) for the risk of infectious diseases were evaluated with PFAA concentrations in the second through fourth quartiles and compared to those in the lowest quartiles. We selected confounders in analysis according to a review of the literature. Potential confounding variables considered in the analysis were: maternal age (continuous), number of older siblings (0, ≥1), maternal education (≤12, >12 years), parental allergic history (yes/no), infant gender, breast-feeding period (<6, ≥6 months), day care attendance (yes/no), and environmental tobacco smoke (ETS) exposure at 4 years old children (yes/no). The number of older siblings was obtained from parity information. Because of potential sex differences of PFAA health effects, we stratified the results by sex, as

well.

(倫理面への配慮)

This study was conducted with all of the participants' written informed consent during pregnancy up to two years old and also another informed consent was obtained at four years old. The institutional ethical board for epidemiological studies at Hokkaido University Center for Environmental and Health Sciences and Hokkaido University Graduate School of Medicine approved the study protocol.

C. 研究結果

The average of maternal age at birth (SD) was 31.1 (4.4) and 50.9% of infants were male. 54.3 % of mothers were multiparous and 5.9% were smoking during pregnancy (Table 1).

Because of low detection rate, PFHxA, PFHpA and PFTeDA levels were excluded before data analysis. Median of PFAAs were as follows: PFHxS (0.296 ng/mL); PFOS (4.92 ng/mL); PFOA (2.01 ng/mL); PFNA (1.18 ng/mL); PFDA (0.522 ng/mL); PFUnDA (1.43); PFDoDA (0.186 ng/mL); PFTrDA (0.332 ng/mL) (Table 2).

Incidence of infectious diseases symptoms among children at 4 years in our study population is shown in Table 3. The number and percentage of children who developed infectious diseases at 4 years old were: otitis media, 649 (41.6%); pneumonia, 287 (18.4%); RSV, 197 (12.6%); varicella, 589 (37.8%) and febrile seizure, 121 (7.7%). In total, 1075 (69.0%) of children had at least one of infectious diseases. Incidence of infectious diseases was not significantly different among boys than girls.

We assessed the association of PFAAs with total infectious diseases using logistic regression models (Figure 2, Supplementary Table S1). We observed a positive association with total infectious diseases across PFHxS quartiles (Q4 vs Q1 adjusted OR: 1.56, 95% CI: 0.963, 2.54; p for trnd= 0.043) in female but not male children. In addition, adjusted ORs in the highest quartile vs lowest quartile for total infectious diseases were significantly increased for PFOS (Q4 vs Q1 OR: 1.56; 95% CI: 1.12, 2.17; p for trend= 0.022) in all children.

D. 考察

This study is one of few studies which focuses on prenatal exposure to PFAAs and risk of infectious diseases. We measured eleven types of PFAAs including long-chain PFAAs during pregnancy and followed up children until 4 years in a large-scale birth cohort. We observed that prenatal exposure to PFHxS and PFOS were associated with higher risk of infectious diseases in 4 year-old children. However, we did not any significant association of PFCAs including PFOA, PFNA and PFDA with infectious diseases.

Median values of PFAAs with C6-C8 including PFHxS, PFOS and PFOA in this study were low compare to those in the US (Stein et al., 2012), Denmark (Halldorsson et al., 2012), Korea (Lee et al., 2013) and China (Jiang et al., 2014) during pregnancy. However, longer chain PFAA levels (C≥9) were higher than western countries such as Spain, Denmark, Sweden and USA (Harada et al. 2011).

Animal studies showed endocrine disruption, neuro- and immunotoxic properties of PFOS and PFOA (Lau et al. 2003; Seacat 2003; Leubker 2005). Exposure to PFOS and PFOA in animals decreased lymphoid organ weights, reduced number of lymphoid cells and antibody

production (Yang 2001; Peden-Adams 2007). Pre- and post-natal exposure to PFOS and PFOA were associated with reduced antibody levels of tetanus, diphtheria (Grandjean et al. 2012), and rubella (Granum et al. 2013) in children. In adults, elevated PFOA serum concentrations are associated with reduced antibody titer rise, particularly to A/H3N2 influenza virus, and an increased risk of not attaining the antibody threshold considered to offer long-term protection (Looker et al. 2014). These animal and human studies suggest immunosuppressive effects of PFAAs.

There are few conducted studies about the effects of PFAAs, especially in prospective studies, on risk of infectious diseases. A Danish study examined the association of prenatal exposure to PFOS and PFOA with risk of hospitalization for infectious diseases in early childhood, and did not find any association between these PFAAs and risk of infectious diseases leading to hospitalization (Fei et al. 2010). However, Granum et al. (2013) reported a positive association between the prenatal PFOA and PFNA levels and the number of episodes of common cold for the children and between PFOA and PFHxS and the number of episodes of gastroenteritis at 3 years of age. In this study PFAA exposure levels were similar to those we found, and their results are consistent with our result indicating that prenatal exposure to PFAAs are associated with increased risk of infectious diseases in next generation.

Previously we studied association of eleven PFAAs and risk of allergic diseases at 12-24 months of age and found inverse association of prenatal exposure to PFTrDA and risk of eczema among female infants (Okada et al. 2014). Recently, we examined the effects of prenatal PFAAs on risk of allergic diseases at 4 years of age in the same cohort and follow up of the same participants. The result showed that there is an inverse association of prenatal exposure to PFDoDA and PFTrDA with risk of eczema; and inverse association between PFHxS and wheezing (Goudarzi et al. in preparation). Taken together, PFAAs may suppress immune system in humans resulting in higher risk of infectious diseases and reduced allergic reactions.

E．結論

This study suggests inverse association between prenatal exposures to PFOS and PFHxS and risk of infectious diseases in early childhood. It may provide new evidence that PFAAs have immunomodulatory effects on human immune system. However, more studies are necessary to observe long effects of in utero exposure to PFAAs on immune system in later life.

F．研究発表

1.論文発表

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2.学会発表

なし

G．知的財産権の出願・登録状況（予定を含む。）

該当なし

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Figure S1. Flow chart of study participant selection.

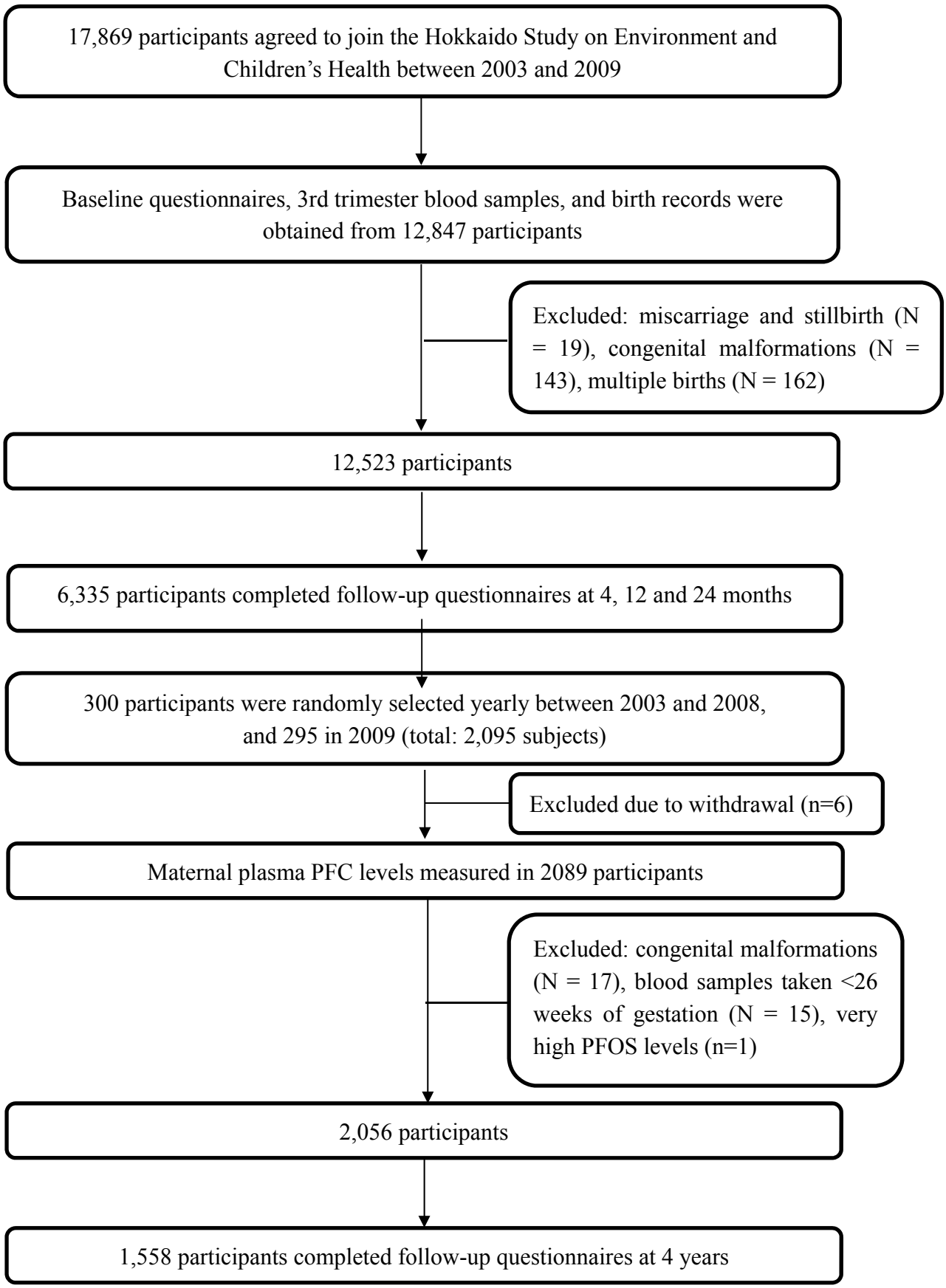


Table 1. Characteristics of study population of the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013.

Characteristics		Participants (n=1558) No. (%)
Parental characteristics		
Maternal age (years) (mean ± SD)		31.12±4.48
Prepregnancy BMI		20.96±2.90
Maternal educational level (years)	≤12	660 (42.36)
	>12	898 (57.64)
Parity (times)	0	702 (45.67)
	≥1	835 (54.32)
	missing	21
Maternal smoking status during pregnancy	Nonsmoker	1465 (94.03)
	Smoker	93 (5.97)
Maternal allergic history	Yes	484 (31.07)
Paternal allergic history	Yes	307 (19.70)
Annual household income (million yen)	<5	880 (56.48)
	≥5	495 (31.77)
	Missing	183 (11.75)
Children characteristics		
Gender	Male	793 (50.9)
	Female	765 (49.1)
Older siblings (numbers)	0	626 (40.18)
	≥1	932 (59.82)
Day care attendance at 4-year-old	Yes	1373 (90.27)
	No	148 (9.73)
	missing	37
ETS[a] exposure at 4-year-old	Yes	724 (48.07)
	No	782 (51.92)
	Missing	52

[a] ETS: environmental tobacco exposure

Table 2. Concentrations of 11 PFAAs in 1558 maternal plasma samples from the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013.

Compound	MDL[a]	%	Mean	Minimum	25th	50th	75th	Maximum
PFHxS	0.2	82.61	0.322	<0.2	0.221	0.296	0.395	3.386
PFHxA	0.1	46.28	0.103	<0.1	<0.1	<0.1	0.145	0.694
PFHpA	0.1	35.24	0.095	<0.1	<0.1	<0.1	0.125	0.757
PFOS	0.3	100	5.456	1.003	3.667	4.925	6.654	30.283
PFOA	0.2	99.94	2.713	<0.2	1.314	2.013	3.346	24.88
PFNA	0.3	99.87	1.402	<0.3	0.908	1.183	1.589	13.189
PFDA	0.1	99.55	0.575	<0.1	0.393	0.522	0.694	2.434
PFUnDA	0.1	99.81	1.534	<0.1	1.037	1.431	1.895	5.89
PFDoDA	0.1	90.69	0.191	<0.1	0.14	0.186	0.233	0.729
PFTrDA	0.1	97.82	0.35	<0.1	0.247	0.332	0.424	1.325
PFTeDA	0.1	15.28	0.061	<0.1	<0.1	<0.1	<0.1	0.303

[a]MDL: method detection limit

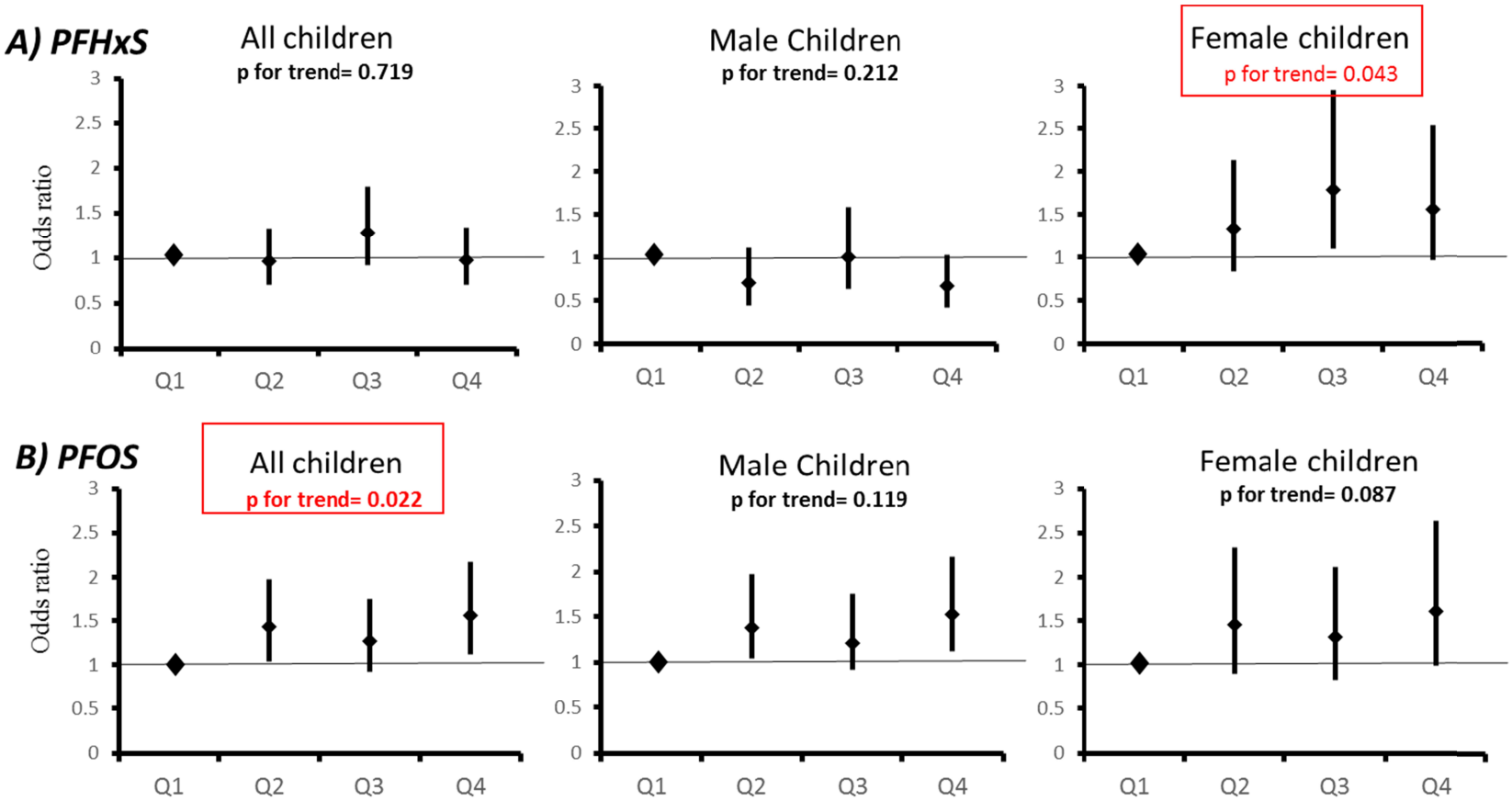
Table 3. Number and proportion of children who developed allergic and infectious diseases during the 4-year-old in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013 (n = 1558).

Symptoms	Total (n=1558)		Male children (n=793)		Female children (n=765)		p[a]
	n	(%)	n	(%)	n	(%)	
Infectious diseases [b]	1075	(69)	534	(67.34)	541	(70.72)	0.149
Otitis media	649	(41.66)	340	(42.88)	309	(40.39)	0.320
Pneumonia	287	(18.42)	151	(19.04)	136	(17.78)	0.520
RS virus	197	(12.64)	92	(11.6)	105	(13.73)	0.207
Febrile seizure	121	(7.77)	59	(7.44)	62	(8.1)	0.624
Varicella	589	(37.8)	284	(35.81)	305	(39.87)	0.099

[a] Chi-square test.

[b] “Infectious diseases” indicates cases with at least one of the listed symptoms.

Figure 2. The association between quartiles of PFHxS (A) and PFOS (B) with risk of total allergic diseases among 4-year old children.



Adjusted ORs in the highest quartile vs lowest quartile for infectious diseases were significantly decreased for PFHxS and PFOS. Q: quartile. Infectious diseases includes otitis media, pneumonia, respiratory syncytial virus (RSV), varicella, febrile seizure and were collected using a mother-reported questionnaire at 4 years of age. Logistic models were adjusted for maternal age, maternal educational level, parental allergic history, parity, children gender, day care attendance and ETS exposure in at 4-year-old, and breast feeding.

Supplementary Table S1. Odds ratio (95% CI) between PFAA concentrations in maternal plasma and total infectious diseases during the 4 year-old in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013 (n= 1558).

Compound	Total (n = 1558)					Male children (n = 793)					Female children (n = 765)				
	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															
Quartile 1	267	1		1		143	1		1		124	1		1	
Quartile 2	267	0.898	(0.663, 1.21)	0.963	(0.697, 1.33)	127	0.710	(0.465, 1.08)	0.705	(0.446, 1.11)	140	1.14	(0.743, 1.77)	1.33	(0.835, 2.13)
Quartile 3	280	1.10	(0.811, 1.51)	1.28	(0.919, 1.79)	140	0.945	(0.612, 1.46)	1.00	(0.630, 1.59)	140	1.30	(0.836, 2.04)	1.79	(1.10, 2.95)
Quartile 4	261	0.858	(0.634, 1.16)	0.974	(0.703, 1.34)	124	0.647	(0.425, 0.987)	0.663	(0.421, 1.03)	137	1.16	(0.750, 1.80)	1.56	(0.963, 2.54)
p for trend		0.596		0.719			0.131		0.212			0.416		**0.043**	
PFOS															
Quartile 1	251	1		1		130	1		1		121	1		1	
Quartile 2	276	1.31	(0.969, 1.77)	1.43	(1.04, 1.98)	134	1.23	(0.813, 1.86)	1.38	(0.883, 2.17)	142	1.39	(0.900, 2.16)	1.45	(0.903, 2.33)
Quartile 3	264	1.15	(0.856, 1.55)	1.27	(0.921, 1.75)	127	1.11	(0.736, 1.68)	1.21	(0.775, 1.90)	137	1.18	(0.773, 1.82)	1.32	(0.834, 2.11)
Quartile 4	284	1.46	(1.07, 1.98)	1.56	(1.12, 2.17)	143	1.40	(0.924, 2.13)	1.52	(0.968, 2.41)	141	1.52	(0.978, 2.39)	1.61	(0.995, 2.63)
p for trend		0.039		**0.022**			0.171		0.119			0.123		0.087	
PFOA															
Quartile 1	266	1		1		129	1		1		137	1		1	
Quartile 2	272	1.04	(0.774, 1.42)	1.13	(0.814, 1.57)	137	0.927	(0.611, 1.40)	0.934	(0.593, 1.46)	135	1.232	(0.787, 1.92)	1.42	(0.875, 2.32)

Quartile 3	277	1.13	(0.835, 1.54)	1.18	(0.850, 1.66)	144	1.23	(0.802, 1.90)	1.22	(0.765, 1.95)	133	1.04	(0.673, 1.60)	1.16	(0.717, 1.89)
Quartile 4	260	0.917	(0.679, 1.23)	1.16	(0.826, 1.65)	124	0.851	(0.559, 1.29)	0.986	(0.606, 1.60)	136	0.993	(0.646, 1.52)	1.38	(0.838, 2.31)
p for trend		0.699		0.363			0.766		0.743			0.802		0.346	
PFNA															
Quartile 1	273	1		1		140	1		1		133	1		1	
Quartile 2	271	0.984	(0.723, 1.33)	1.17	(0.849, 1.62)	134	0.926	(0.605, 1.41)	1.14	(0.725, 1.80)	137	1.04	(0.674, 1.63)	1.23	(0.775, 1.97)
Quartile 3	276	1.01	(0.743, 1.37)	1.20	(0.869, 1.67)	125	0.812	(0.531, 1.24)	0.971	(0.616, 1.52)	151	1.32	(0.844, 2.07)	1.53	(0.952, 2.50)
Quartile 4	255	0.808	(0.597, 1.09)	0.987	(0.711, 1.37)	135	0.815	(0.537, 1.23)	0.973	(0.617, 1.53)	120	0.777	(0.503, 1.20)	1.02	(0.634, 1.66)
p for trend		0.204		0.983			0.272		0.745			0.441		0.704	
PFDA															
Quartile 1	277	1		1		142	1		1		135	1		1	
Quartile 2	275	0.941	(0.69, 1.28)	0.989	(0.712, 1.37)	133	0.799	(0.524, 1.21)	0.799	(0.508, 1.25)	142	1.14	(0.720, 1.81)	1.28	(0.787, 2.08)
Quartile 3	266	0.851	(0.625, 1.15)	0.893	(0.645, 1.23)	130	0.817	(0.533, 1.25)	0.791	(0.501, 1.24)	136	0.886	(0.568, 1.38)	1.00	(0.625, 1.60)
Quartile 4	257	0.744	(0.549, 1.00)	0.851	(0.614, 1.17)	129	0.775	(0.507, 1.18)	0.865	(0.545, 1.36)	128	0.711	(0.460, 1.10)	0.865	(0.541, 1.38)
p for trend		0.042		0.266			0.280		0.547			0.066		0.365	
PFUnDA															
Quartile 1	262	1		1		131	1		1		131	1		1	
Quartile 2	270	1.08	(0.799, 1.46)	1.11	(0.804, 1.53)	146	1.03	(0.683, 1.56)	1.04	(0.672, 1.63)	124	1.15	(0.738, 1.80)	1.16	(0.722, 1.89)
Quartile 3	271	1.06	(0.790, 1.44)	1.08	(0.788, 1.49)	122	0.94	(0.618, 1.44)	0.984	(0.621, 1.56)	149	1.19	(0.779, 1.83)	1.19	(0.756, 1.88)
Quartile 4	272	1.10	(0.812, 1.49)	1.07	(0.779, 1.48)	135	1.04	(0.686, 1.59)	1.07	(0.682, 1.68)	137	1.16	(0.750, 1.79)	1.04	(0.660, 1.66)
p for trend		0.577		0.703			0.942		0.836			0.485		0.801	
PFDoDA															
Quartile 1	264	1		1		127	1		1		137	1		1	

Quartile 2	262	0.908	(0.671, 1.22)	0.957	(0.694, 1.32)	145	1.089	(0.714, 1.66)	1.16	(0.747, 1.83)	117	0.747	(0.484, 1.15)	0.718	(0.448, 1.14)
Quartile 3	275	1.06	(0.780, 1.44)	1.01	(0.736, 1.40)	130	0.976	(0.638, 1.49)	1.03	(0.657, 1.61)	145	1.16	(0.744, 1.81)	0.956	(0.592, 1.54)
Quartile 4	274	1.02	(0.752, 1.38)	1.10	(0.801, 1.53)	132	0.962	(0.630, 1.46)	1.13	(0.724, 1.78)	142	1.09	(0.703, 1.70)	1.05	(0.653, 1.69)
p for trend		0.655		0.473			0.730		0.719			0.320		0.548	
PFTrDA															
Quartile 1	261	1		1		121	1		1		140	1		1	
Quartile 2	270	1.10	(0.816, 1.49)	1.10	(0.801, 1.52)	150	1.45	(0.951, 2.22)	1.64	(1.04, 2.59)	120	0.829	(0.536, 1.28)	0.710	(0.444, 1.13)
Quartile 3	272	1.14	(0.842, 1.54)	1.16	(0.841, 1.61)	137	1.18	(0.779, 1.80)	1.30	(0.834, 2.04)	135	1.11	(0.716, 1.74)	0.992	(0.614, 1.60)
Quartile 4	272	1.05	(0.784, 1.43)	1.08	(0.789, 1.49)	126	1.04	(0.685, 1.58)	1.23	(0.789, 1.93)	146	1.08	(0.700, 1.66)	0.922	(0.577, 1.47)
p for trend		0.676		0.563			0.889		0.567			0.464		0.907	

[a] Adjusted for maternal age, maternal educational level, parental allergic history, parity, children gender, breast-feeding period, day care attendance at 4-year-old, and ETS exposure in children at 4-year-old.

[b] Adjusted for all the covariates except children gender.

[c] OR: odds ratio. [d] CI: confidence interval.

*Indicates number of cases with infectious diseases.