in this study were eligible. Health care personnel approached pregnant women and introduced the study. Between 2003 and 2009, 17,869 agreed to participate in large-scale Hokkaido cohort. Of these, 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. After exclusion of cases with miscarriage and stillbirth (n = 19), congenital malformation (n = 143), and multiple births (n = 162), we extracted 6335 participants who had completed all three postnatal questionnaires at 4, 12, and 24 months after birth for long-term follow-up. From these, we randomly extracted 300 participants per year from 2003 to 2008 and 295 participants in 2009 (n=2095) for the PFAA measurement in maternal plasma samples (Okada et al., 2014). Finally, a total of 1,558 mother-child pairs sent us 4-year old questionnaires and were included in the current study.

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, and medication. 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 birth size, maternal complications during pregnancy, and maternal smoking status in the third trimester. At 4 years post-delivery, participants completed another self-administered questionnaire including information related to breast feeding, infant size, smoking status of parents, parental history of allergic diseases, environmental tobacco smoke (ETS) exposure and day care attendance. In addition, mothers reported previous or current medical history of infant allergic diseases including eczema, wheezing, and allergic rhinoconjunctivitis symptoms.

Detailed sample preparation and PFAAs measurement methods have been previously described (Okada et al., 2013). We used maternal plasma for exposure assessment using ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry instrumentation (UPLC-MS/MS). 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 third trimester of pregnancy.

Infant allergies were assessed based on the mothers' self-administered questionnaires obtained 4 years 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 (Asher et al., 2006). Eczema was defined based on positive answers to all three following questions: “Have your child had this itchy rash at any time in the past 12 months?”, “Have 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 defined based on a positive answer to the question: "Have your child had wheezing or whistling in the chest in the past 12 months?". Current allergic rhinoconjunctivitis symptoms were assessed based on all positive answers to both of following questions: "In the past 12 months, 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 the answer is positive, "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 symptoms of eczema, wheezing, and allergic rhinoconjunctivitis.

Correlations between PFAA concentrations were analyzed using the Spearman's rank correlation coefficient (ρ). 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 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 lowest quartiles. We examined the effects on total allergic diseases and also each allergic symptoms, separately. 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.

（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

We assessed the association of prenatal exposure to PFAAs and allergic diseases in 4-year old children in totally 1,558 mother-child pairs. The average of maternal age (SD) was 31.1 (4.4), and prepregnancy BMI (SD) was 20.9 (2.9). 45.7 % of mothers were nulliparous and 5.7% were smoking during pregnancy. 50.9% of infants were male (Table 1).

Because of low detection rate, we excluded PFHxA, PFHpA and PFTeDA before data analysis. Among left 8 PFAAs, PFHxS and PFDoDA had detection rate of 82.6 and 90.6%, respectively (Table 2). Other PFAAs had detection rate were more than 97%. PFOS had the highest median exposure levels (4.92 ng/mL) followed by PFOA (2.01 ng/mL), PFUnDA (1.43 ng/mL), and PFNA (1.18 ng/mL).

Table 3 shows the incidence of allergic symptoms among children at 4 years in our study population. The number and percentage of children who developed

allergic diseases in the preceding 12 months were: wheezing, 291 (18.7%); eczema, 296 (19.0%), and rhinoconjunctivitis 84 (5.4%). Totally, 536 (34.4%) had at least one of allergic symptoms. Incidence of allergic symptoms were a little bit higher among boys than girls but it was not statistically significant.

We analyzed the association of PFAAs with total allergic diseases (Figure 1), eczema (Figure 2), wheezing (Table 4) using logistic regression models. Adjusted ORs in the highest quartile vs lowest quartile for total allergic diseases were significantly decreased for PFDoDA (Q4 vs Q1 OR: 0.621; 95% CI: 0.454, 0.847; p for trend= 0.008) and PFTrDA (Q4 vs Q1 OR: 0.712; 95% CI: 0.524, 0.966; p for trend= 0.013). After sex stratification, we observed significant association of PFDoDA and PFTrDA with total allergic diseases among boys not girls (Figure 1).

Figure 2 shows the association of PFAAs with risk of eczema. Adjusted ORs for eczema and PFOA decreased significantly for the three highest quartiles compared with lowest quartile as reference only among boys (Q4 vs Q1 OR: 0.592; 95% CI: 0.319, 1.08, p for trend= 0.022). Adjusted ORs for the highest vs lowest quartile were 0.566 (95% CI: 0.383, 0.831) for PFDoDA, and 0.672 (95% CI: 0.465, 0.968) for PFTrDA in all children. Effects of these long PFAAs were prominent among boys, for examples adjusted ORs of eczema in boys across second to forth quartile compared with lowest quartile of PFDoDA were 0.877 (95% CI: 0.536, 1.43), 0.828 (95% CI: 0.500, 1.36), and 0.451 (95% CI: 0.253, 0.785) with a dose-response relationship (p for trend= 0.008).

Among PFAAs, PFHxS were significantly associated with risk of wheezing (Table 4); the adjusted OR of PFHxS in the fourth quartile vs first quartile was 0.728 (95% CI: 0.497, 1.06, p for trend= 0.038) in all children. After sex stratification, this association was prominent among boys (Q4 vs Q1 OR: 0.650; 95% CI: 0.391, 1.07; p for trend= 0.063).

We also assess the association between PFAAs and rhinoconjunctivitis (data not shown). PFNA showed significant association with monotonic reduced risk of (Q4 vs Q1 OR: 0.409; 95% CI: 0.192, 0.825; p for trend= 0.019), after sex stratification we observed reduced OR of quartile forth vs first quartile of PFNA in box sexes but p for trend did not meet significance statistically. In addition, adjusted ORs for rhinoconjunctivitis were decreased for the three highest quartiles of PFUnDA (Q4 vs Q1 OR: 0.285; 95% CI: 0.099, 0.714; p for trend= 0.030) and PFDoDA (Q4 vs Q1 OR: 0.430; 95% CI: 0.176, 0.985; p for trend= 0.045) compared with the lowest quartile only among boys.

D．考察

In the current study, we focused on the effects of prenatal exposure to 11 PFAAs, including long-chain ones, on allergic diseases of next generation at 4 years in a prospective birth cohort. We found that prenatal exposure to long chain PFAAs including PFDoDA and PFTrDA were associated with reduced risk of total allergic diseases in 4 year-old children. We observed that PFDoDA and PFTrDA were associated with a decline in the risk of eczema, also PFHxS showed association with reduced risk of wheezing. Although, almost all adjusted OR of allergic diseases across 2nd to 4th quartiles of PFAAs were less than one

compare with first quartile as reference among girls, we observed the associations of PFAAs with allergic diseases at 4 year-old children were statistically significant only in boys.

Several previous animal studies suggest that PFAAs have immunotoxic effects including suppression of cytokine production such as TNF-α, IL4 and IFN-γ (Qazi et al., 2010), and reduced IgM production and humoral immunity (Dewitt et al., 2009; Peden-Adams et al., 2007). Epidemiological studies also have reported suppression of antibody production in individuals exposed to higher PFAA levels. Prenatal exposure to PFAAs were negatively associated with anti-rubella antibody among 3-year old children (Granum et al., 2013). In Granum's study, only 4 types of PFAAs were examined and strength of inverse association between PFAAs with reduced antibody were as follows: PFNA>PFOA>PFHxS>PFOS, indicating that PFCAs have stronger impact on antibody production compare with PFSAs. We also observed stronger association of PFCAs with longer carbon chain on allergic outcomes compare with PFSAs. In another study, higher pre- and postnatal exposure to PFOS and PFOA were inversely associated with tetanus and diphtheria antibody concentrations at 5 and 7 years old children (Grandjean et al., 2012); Results of these birth cohort are consistent with our results suggesting association of PFAAs with reduced immune response.

Our group previously analyzed the association of prenatal exposure to PFOS and PFOA with cord blood IgE and allergic diseases at 18 months of age in another birth cohort of Hokkaido Study with small sample size (Okada et al., 2012). We did not find any association of PFOS and PFOA with allergic diseases; however, PFOA levels were negatively associated with cord blood IgE among female infants suggesting immunomodulatory effects of this chemical. In addition, we reported the association of PFAAs with infant allergic diseases at 12 and 24 months of age using ISAAC questionnaires (Okada et al., 2014), showing association of prenatal exposure to PFTrDA and reduced risk of eczema (n=2,062). After sex stratification, higher PFTrDA and PFUnDA were negatively associated with risk of eczema only among female infants. In the current study, we followed those infants to 4 years including 1558 mother-child pairs and found that not only PFTrDA but also other long chain PFAAs including PFNA, PFUnDA, PFDoDA are negatively associated with reduced risk of allergic outcomes. However, impact of PFAAs on male infants were prominent in this current study. We conducted further examination and comparison to clarify difference of populations in Okada et al. (2014) (n=2,062) and current analyses (n=1558), we looked at characteristics and exposure levels between two studies (data not shown). PFAA exposure levels of participants in these two studies were similar in range. Demographic characteristics including maternal age, parity, child gender and parental allergy history were similar. However, maternal smoking rate during pregnancy was lower in the current analysis compare to report of Okada et. al. (5.7% vs 7.3%). Also, we looked at the characteristics of population with loss of between 2 vs 4 years (n=498); 10.6% of mothers were smoker during pregnancy and mothers had lower educational levels in loss of follow up group. Day care attendance in current analysis was significantly higher compare with that of Okada et al. (90.3% vs

28.3%), suggesting that high percentage of children at 4 year old have day care attendance. Taken together, although we observed some few differences in characteristic between these Okada et al. study and current study, these two reports suggest consistent immunosuppressive effects of prenatal exposure to PFAAs in infancy and early childhood.

E．結論

This study suggest inverse association between prenatal exposures to long chain PFCAs with risk of allergic diseases in early childhood with sex differences. It may provide new evidence in humans that PFAAs have immunosuppressive effects consistent with animal studies. However, more studies with longer observations need to be conducted in prospective studies.

F．研究発表

1.論文発表

Houman Goudarzi, Chihiro Miyashita, Emiko Okada, Ikuko Kashino, Sumitaka Kobayashi, Chi-Jen Chen, Sachiko Ito , Atsuko Araki , Hideyuki Matsuura, Yoichi M. Ito, Reiko Kishi. Effects of prenatal exposure to perfluoroalkyl acids on risk of allergic diseases at 4 years old children. In preparation.

2.学会発表

Houman Goudarzi, Sumitaka Kobayashi, Chi-Jen Chen, Atsuko Araki, Chihiro Miyashita, Sachiko Ito, Reiko Kishi. Effects of prenatal exposure to perfluoroalkyl acids on risk of allergic diseases at 4 years old children: The Hokkaido Study. 第 67 回北海道公衆衛生学会,旭川。平成 27 年 11 月 21 日。

G．知的財産権の出願・登録状況（予定を含む。）

該当なし

参考文献

1) Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK. 2006. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 368:733–43.

2) DeWitt JC, Shnyra A, Badr MZ, Loveless SE, Hoban D, Frame SR, et al. 2009. Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. Crit Rev Toxicol 39:76–94.

3) Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, et al. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA 307:391–7.

4) Granum B, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, et al. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol 10(4):373-9.

5) Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S et al. 2004. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during

pregnancy. Environ Health Perspect 112:1204-1207.

6) Kato K, Calafat AM, Needham LL. 2009. Polyfluoroalkyl chemicals in house dust. Environ Res. 109:518-523.

7) Keil D. E., Mehlmann T., Butterworth L., and Peden-Adams M. M. 2008. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. Toxicol Sci 103, 77–85.

8) Kishi R, Kobayashi S, Ikeno T, Araki A, Miyashita C, Itoh S, et al. 2013. Ten years of progress in the Hokkaido birth cohort study on environment and children's health: cohort profile--updated 2013. Environ Health Prev Med. 18:429–50.

9) Kishi R, Nakajima T, Goudarzi H, Kobayashi S, Sasaki S, Okada E, et al. 2015. The Association of Prenatal Exposure to Perfluorinated Chemicals with Maternal Essential and Long-Chain Polyunsaturated Fatty Acids during Pregnancy and the Birth Weight of Their Offspring: The Hokkaido Study. Environ Health Perspect. 123(10):1038-45.

10) Okada E, Sasaki S, Saijo Y,Washino N,Miyashita C, Kobayashi S, et al. 2012. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environ Res. 112:118–25.

11) Okada E, Kashino I, Matsuura H, Sasaki S, Miyashita C, Yamamoto J, et al. 2013. Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003-2011. Environ Int 60:89-96.

12) Okada E, Sasaki S, Kashino I, Matsuura H, Miyashita C, Kobayashi S, Itoh K, Ikeno T, Tamakoshi A, Kishi R. 2014. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environ Int. 65:127-34.

13) Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect 115:1298-1305.

14) Peden-Adams M. M., EuDaly J. G., Dabra S., EuDaly A., Heesemann L., Smythe, J., et al. 2007. Suppression of humoral immunity following exposure to the perfluorinated insecticide sulfluramid. J Toxicol Environ Health A 70: 1130–141.

15) Qazi MR, Abedi MR, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2010. Dietary exposure to perfluorooctanoate or perfluorooctane sulfonate induces hypertrophy in centrilobular hepatocytes and alters the hepatic immune status in mice. Int Immunopharmacol. 10(11):1420-7.

16) Wang IJ, Hsieh WS, Chen CY, Fletcher T, Lien GW, Chiang HL, et al. 2011. The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. Environ Res. 111: 785–91.

17) Yang Q, Xie, Y, Eriksson A. M., Nelson B. D., and DePierre J. W. 2001. Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice. Biochem Pharmacol 62: 1133–40.

Table 1. Characteristics of study population of the Hokkaido Study on Environment and Children's Health, Japan (n=1558).

Characteristics		4-year postpartum assessment (n=1558), mean±SD or No. (%)
Parental characteristics		
Maternal age (years) (mean ± SD)		31.1±4.4
Prepregnancy BMI		20.9±2.9
Maternal educational level (years)	≤12	660 (42.4)
	>12	898 (57.6)
Parity (times)[a]	0	702 (45.7)
	≥1	835 (54.3)
Maternal smoking status during pregnancy	Nonsmoker	1468 (94.3)
	Smoker	90 (5.7)
Maternal allergic history	Yes	484 (31.0)
Paternal allergic history	Yes	307 (19.7)
Annual household income (million yen)[a]	<5	880 (64.0)
	≥5	495 (36.0)
Children characteristics		
Gender	Male	793 (50.9)
	Female	765 (49.1)
Breast feeding (months)	<6	289 (18.6)
	≥6	1269 (81.4)
Older siblings (numbers)	0	702 (45.7)
	≥1	835 (54.3)
Day care attendance at 4-year-old[a]	Yes	1373 (90.3)
	No	148 (9.7)
ETS exposure at 4-year-old[a, b]	Yes	724 (48.0)
	No	782 (52.0)

[a]Missing data: parity (n=21), annual household income (n=183), day care attendance (N=37), and ETS exposure (n=52).

[b]ETS: environmental tobacco smoke.

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	Detection			Concentration (ng/mL)						
	MDL[a]	No.	%	Geometric mean	Mean	Minimum	25th	50th	75th	Maximum
PFHxS (C6)	0.2	1287	82.6	0.275	0.322	<0.2	0.221	0.296	0.395	3.386
PFHxA (C6)	0.1	721	46.2	0.085	0.103	<0.1	<0.1	<0.1	0.145	0.694
PFHpA (C7)	0.1	549	35.2	0.076	0.095	<0.1	<0.1	<0.1	0.125	0.757
PFOS (C8)	0.3	1558	100	4.932	5.456	1.003	3.667	4.925	6.654	30.283
PFOA (C8)	0.2	1557	99.9	2.105	2.713	<0.2	1.314	2.013	3.346	24.88
PFNA (C9)	0.3	1556	99.8	1.23	1.402	<0.3	0.908	1.183	1.589	13.189
PFDA (C10)	0.1	1551	99.5	0.514	0.575	<0.1	0.393	0.522	0.694	2.434
PFUnDA (C11)	0.1	1555	99.8	1.368	1.534	<0.1	1.037	1.431	1.895	5.89
PFDoDA (C12)	0.1	1413	90.6	0.172	0.191	<0.1	0.14	0.186	0.233	0.729
PFTrDA (C13)	0.1	1524	97.8	0.316	0.35	<0.1	0.247	0.332	0.424	1.325
PFTeDA (C14)	0.1	238	15.2	0.057	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 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 (%)	
Total allergic diseases [b]	536 (34.4)	285 (35.9)	251 (32.8)	0.194
Wheezing	291 (18.7)	162 (20.4)	129 (16.8)	0.071
Eczema	296 (19.0)	153 (19.2)	143 (18.6)	0.762
Allergic rhinoconjunctivitis symptoms	84 (5.4)	46 (5.8)	38 (4.9)	0.467

[a] Chi-square test.

[b] "Total allergic diseases" indicates cases with at least one of the listed symptoms.

Table 4. Prenatal PFAA concentrations and risk of wheezing at 4 years 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	80	1		1		49	1		1		31	1		1	
Quartile 2	83	1.00	(0.711, 1.41)	0.895	(0.624, 1.28)	42	0.829	(0.519, 1.32)	0.705	(0.430, 1.15)	41	1.28	(0.764, 2.14)	1.21	(0.706, 2.10)
Quartile 3	61	0.702	(0.486, 1.01)	0.652	(0.443, 0.954)	33	0.612	(0.374, 1.00)	0.582	(0.346, 0.966)	28	0.842	(0.483, 1.47)	0.811	(0.448, 1.46)
Quartile 4	67	0.778	(0.543, 1.11)	0.728	(0.497, 1.06)	38	0.722	(0.448, 1.16)	0.650	(0.391, 1.07)	29	0.867	(0.499, 1.50)	0.889	(0.494, 1.59)
p for trend		0.056		**0.038**			0.097		0.063			0.320		0.398	
PFOS															
Quartile 1	78	1		1		43	1		1		35	1		1	
Quartile 2	67	0.822	(0.572, 1.18)	0.753	(0.514, 1.09)	33	0.758	(0.458, 1.25)	0.751	(0.439, 1.27)	34	0.899	(0.533, 1.51)	0.753	(0.433, 1.30)
Quartile 3	79	1.01	(0.714, 1.43)	0.980	(0.680, 1.41)	47	1.21	(0.758, 1.94)	1.18	(0.718, 1.94)	32	0.826	(0.487, 1.40)	0.809	(0.467, 1.39)
Quartile 4	67	0.824	(0.574, 1.18)	0.770	(0.526, 1.12)	39	0.901	(0.555, 1.46)	0.889	(0.530, 1.48)	28	0.740	(0.429, 1.27)	0.676	(0.379, 1.19)
p for trend		0.527		0.398			0.855		0.921			0.259		0.238	
PFOA															
Quartile 1	66	1		1		32	1		1		34	1		1	
Quartile 2	74	1.13	(0.79, 1.64)	1.09	(0.743, 1.60)	44	1.33	(0.805, 2.20)	1.22	(0.722, 2.09)	30	0.94	(0.549, 1.61)	0.982	(0.557, 1.72)
Quartile 3	76	1.18	(0.823, 1.70)	1.10	(0.749, 1.62)	44	1.40	(0.845, 2.32)	1.29	(0.762, 2.22)	32	0.977	(0.575, 1.66)	0.969	(0.544, 1.72)
Quartile 4	75	1.16	(0.806, 1.67)	1.09	(0.729, 1.65)	42	1.37	(0.824, 2.28)	1.25	(0.711, 2.22)	33	0.971	(0.574, 1.64)	1.00	(0.555, 1.82)
p for trend		0.411		0.699			0.235		0.427			0.948		0.992	

PFNA														
Quartile 1	70	1		1		36	1		1		34	1		1	
Quartile 2	78	1.14	(0.801, 1.64)	1.16	(0.803, 1.67)	47	1.43	(0.883, 2.34)	1.49	(0.908, 2.49)	31	0.878	(0.514, 1.49)	0.860	(0.493, 1.49)
Quartile 3	67	0.945	(0.654, 1.36)	0.910	(0.617, 1.33)	34	0.987	(0.588, 1.65)	0.911	(0.526, 1.57)	33	0.906	(0.535, 1.53)	0.918	(0.528, 1.59)
Quartile 4	76	1.10	(0.767, 1.57)	1.11	(0.760, 1.63)	45	1.27	(0.781, 2.07)	1.23	(0.732, 2.09)	31	0.918	(0.537, 1.56)	1.04	(0.587, 1.85)
p for trend		0.872		0.875			0.658		0.852			0.788		0.820	
PFDA															
Quartile 1	76	1		1		40	1		1		36	1		1	
Quartile 2	65	0.816	(0.566, 1.17)	0.785	(0.537, 1.14)	36	0.873	(0.529, 1.43)	0.794	(0.468, 1.34)	29	0.755	(0.441, 1.29)	0.785	(0.451, 1.35)
Quartile 3	82	1.09	(0.768, 1.54)	1.08	(0.756, 1.56)	52	1.45	(0.909, 2.32)	1.53	(0.943, 2.51)	30	0.762	(0.447, 1.29)	0.728	(0.415, 1.26)
Quartile 4	68	0.853	(0.594, 1.22)	0.879	(0.602, 1.28)	34	0.834	(0.503, 1.38)	0.859	(0.503, 1.45)	34	0.874	(0.521, 1.46)	0.918	(0.532, 1.58)
p for trend		0.755		0.917			0.966		0.743			0.637		0.702	
PFUnDA															
Quartile 1	72	1		1		37	1		1		35	1		1	
Quartile 2	70	0.96	(0.667, 1.38)	0.994	(0.682, 1.44)	45	1.13	(0.695, 1.83)	1.20	(0.725, 2.01)	25	0.753	(0.43, 1.31)	0.793	(0.444, 1.40)
Quartile 3	77	1.06	(0.748, 1.52)	1.10	(0.762, 1.60)	41	1.216	(0.739, 2.00)	1.32	(0.783, 2.25)	36	0.944	(0.565, 1.57)	0.918	(0.541, 1.56)
Quartile 4	72	0.991	(0.69, 1.42)	1.04	(0.714, 1.51)	39	1.047	(0.635, 1.72)	1.19	(0.709, 2.03)	33	0.931	(0.551, 1.57)	0.906	(0.522, 1.56)
p for trend		0.889		0.706			0.803		0.462			0.980		0.843	
PFDoDA															
Quartile 1	71	1		1		34	1		1		37	1		1	
Quartile 2	71	0.972	(0.675, 1.39)	0.962	(0.659, 1.40)	50	1.42	(0.874, 2.32)	1.41	(0.851, 2.36)	21	0.553	(0.31, 0.987)	0.556	(0.303, 1.00)
Quartile 3	79	1.109	(0.776, 1.58)	1.12	(0.778, 1.63)	41	1.21	(0.731, 2.01)	1.22	(0.728, 2.08)	38	1.01	(0.613, 1.67)	1.02	(0.604, 1.73)
Quartile 4	70	0.946	(0.657, 1.36)	0.999	(0.684, 1.45)	37	1.04	(0.622, 1.74)	1.14	(0.668, 1.95)	33	0.859	(0.512, 1.44)	0.864	(0.502, 1.48)
p for trend		0.960		0.794			0.903		0.781			0.950		0.533	

PFTrDA														
Quartile 1	78	1		1		44	1		1		34	1		1	
Quartile 2	73	0.918	(0.643, 1.31)	0.966	(0.669, 1.39)	38	0.737	(0.452, 1.199)	0.810	(0.487, 1.34)	35	1.16	(0.691, 1.96)	1.19	(0.696, 2.04)
Quartile 3	65	0.800	(0.556, 1.15)	0.805	(0.550, 1.17)	39	0.788	(0.485, 1.281)	0.813	(0.486, 1.35)	26	0.789	(0.453, 1.37)	0.801	(0.449, 1.41)
Quartile 4	75	0.926	(0.650, 1.31)	0.944	(0.653, 1.36)	41	0.883	(0.545, 1.43)	0.978	(0.590, 1.61)	34	0.976	(0.579, 1.64)	0.919	(0.531, 1.58)
p for trend		0.526		0.565			0.694		0.931			0.614		0.474	

[a] Adjusted for maternal age, maternal educational level, parental allergic history, number of older siblings, children gender, breast feeding, day care attendance and ETS exposure at 4-years old.

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

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

*Indicates number of cases with wheezing.

Figure 1. The association between quartiles of PFDoDA (A), PFTrDA (B) with risk of total allergic diseases among 4-year old children. Total allergic diseases were defined as cases with at least one of the following symptoms: eczema, wheezing, allergic rhinoconjunctivitis symptoms. Adjusted for maternal age, maternal educational level, parental allergic history, number of older siblings, children gender, breast feeding, day care attendance and ETS exposure at 4-years old. Q: quartile.

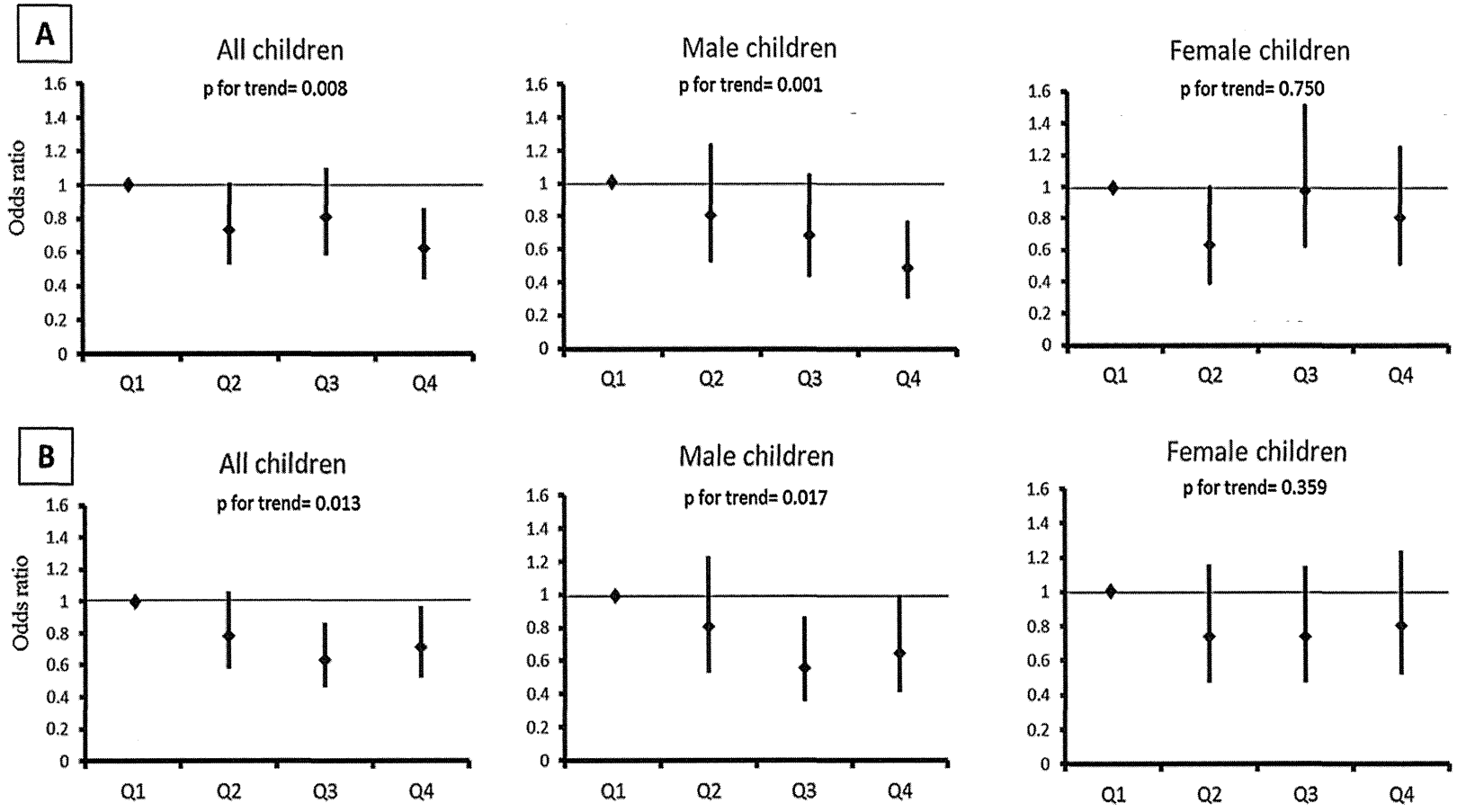
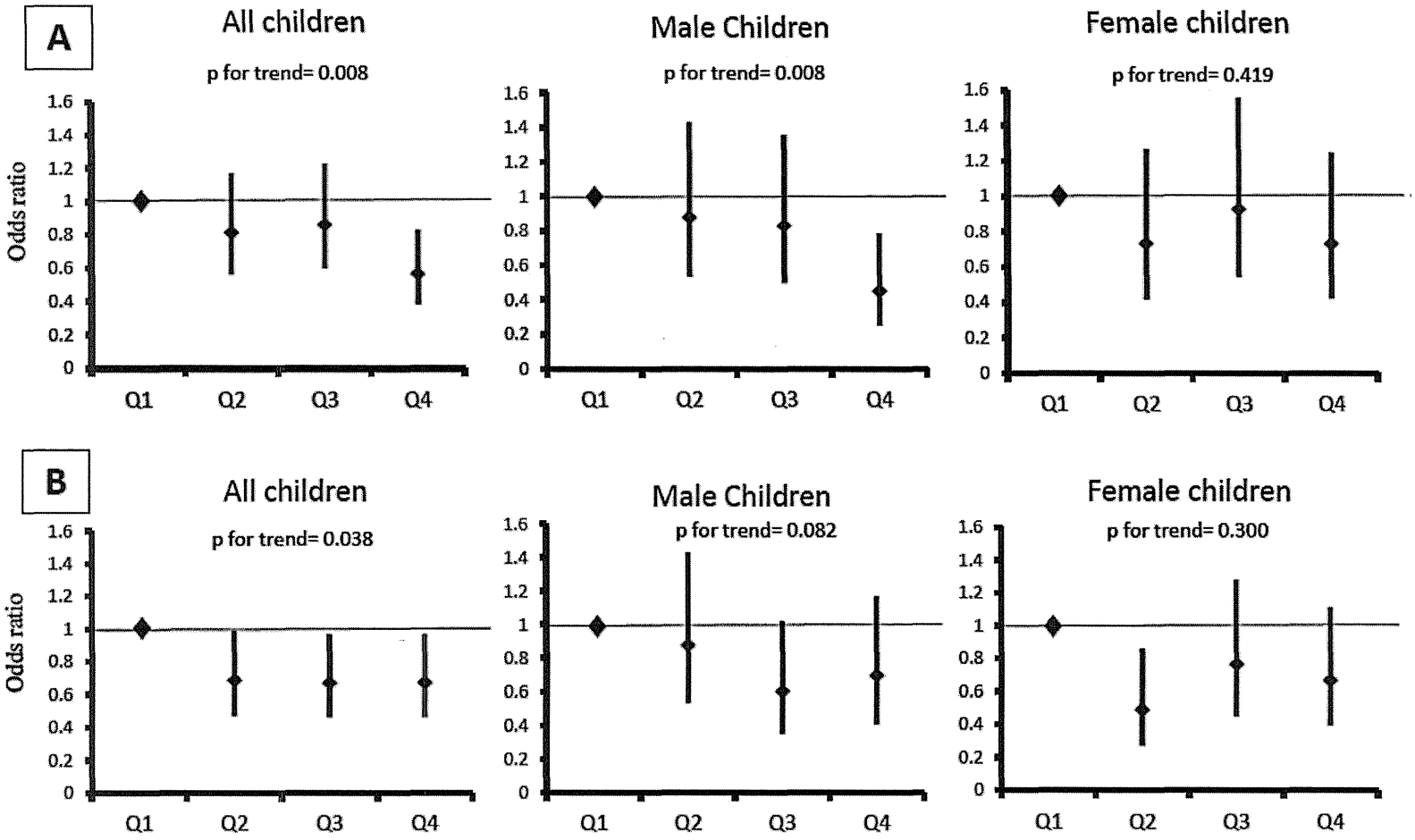


Figure 2. The association between quartiles of PFDoDA (A) and PFTrDA (B) with risk of eczema among 4-year old children. Adjusted for maternal age, maternal educational level, parental allergic history, number of older siblings, children gender, breast feeding, day care attendance and ETS exposure at 4-years old. Q: quartile.



厚生労働科学研究費補助金（化学物質リスク研究事業）
分担研究報告書

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

研究代表者 岸　玲子　北海道大学環境健康科学研究教育センター　特別招へい教授
研究分担者 荒木　敦子　北海道大学環境健康科学研究教育センター　准教授
研究分担者 宮下ちひろ　北海道大学環境健康科学研究教育センター　特任講師

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.

研究協力者
Houman Goudarzi（外国人特別研究員）

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 -80°c 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

Houman Goudarzi, Chihiro Miyashita, Emiko Okada, Ikuko Kashino, Chi-Jen Chen, Sachiko Ito, Atsuko Araki, Hideyuki Matsuura, Reiko Kishi. Prenatal Exposure to Perfluoroalkyl Acids and Risk of Infectious diseases in early life.

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

参考文献

1) ATSDR, 2015. Agency for Toxic Substances and Disease Registry. Draft Toxicological Profile for Perfluoroalkyls. US Department of Health and Human Services, Public Health Service, Atlanta, GA.
2) Brieger A, Bienefeld N, Hasan R, Goerlich R, Haase H. 2011. Impact of perfluoro- octanesulfonate and perfluorooctanoic acid on human

peripheral leukocytes. Toxicol In Vitro. 25(4):960-8.

3) DeWitt, J.C., Copeland, C.B., Strynar, M.J., Luebke, R.W., 2008. Perfluorooctanoic acid induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. Environ. Health Perspect. 116, 644–650.
4) Elliott S.R., Beeson, J.G. 2008. Estimating the burden of global mortality in children aged 5 years by pathogen-specific causes. Clin. Infect. Dis. 46(11),1794–1795.
5) Fei C, McLaughlin JK, Lipworth L, Olsen J. 2010. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ Res. 110(8):773-7.
6) Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, et al. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA 307:391–7.
7) Granum B, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, et al. 2013. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol 10(4):373-9.
8) Houman Goudarzi, Chihiro Miyashita, Emiko Okada, Ikuko Kashino, Sumitaka Kobayashi, Chi-Jen Chen, et al. 2015. Effects of prenatal exposure to perfluoroalkyl acids on risk of allergic diseases at 4 years old children. Submitted to Environment International
9) Halldorsson TI, Rytter D, Haug LS, Bech BH, Danielsen I, Becher G et al. 2012. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ Health Perspect 120:668-673.
10) Harada KH, Hitomi T, Niisoe T, Takanaka K, Kamiyama S, Watanabe T, et al. 2011. Odd-numbered perfluorocarboxylates predominate over perfluorooctanoic acid in serum samples from Japan, Korea and Vietnam. Environ Int. 37:1183–9.
11) Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S et al. 2004. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect 112:1204-1207.
12) Jiang W, Zhang Y, Zhu L, Deng J. 2014. Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. Environ Int 64:40-47.
13) Kannan K, Perrotta E, Thomas NJ. 2006. Association between perfluorinated compounds and pathological conditions in southern sea otters. Environ Sci Technol. 15;40(16):4943-8.
14) Kishi R, Kobayashi S, Ikeno T, Araki A, Miyashita C, Itoh S, et al. 2013. Ten years of progress in the Hokkaido birth cohort study on environment and children's health: cohort profile--updated 2013. Environ Health Prev Med. 18:429–50.
15) Kishi R, Nakajima T, Goudarzi H, Kobayashi S, Sasaki S, Okada E, et al. 2015. The Association of Prenatal Exposure to Perfluorinated Chemicals with Maternal Essential and Long-Chain Polyunsaturated Fatty Acids during