Prenatal Exposure to Perfluoroalkyl Acids and Prevalence of Infectious Diseases up to 4 Years of Age

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Abstract

Perfluoroalkyl acids (PFAAs) are synthetic chemicals with ability to repel oils and water, and have been widely used in many industrial and household applications such as adhesives and water- and stain-repellent surfaces to nonstick coatings. Animal studies have shown that PFAAs have immunotoxic effects. However, few epidemiological studies have investigated the effects of PFAAs on infectious diseases occurrence. We examined the relationship between prenatal exposure to PFAAs and prevalence of infectious diseases up to 4 years of life. A total of 1558 mother-child pairs, who were enrolled in the Hokkaido Study on Environment and Children's Health, were included in this data analysis. 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. Participant characteristics were obtained from medical birth records and self-administered questionnaires during pregnancy and after delivery. Physicians' diagnosis of common infectious diseases including otitis media, pneumonia, respiratory syncytial virus infection, and varicella up to 4 years were extracted from the mother-reported questionnaires. The number of children who developed infectious diseases up to 4 years of age was as follows: otitis media, 649 (41.4%); pneumonia, 287 (18.4%); respiratory syncytial virus infection, 197 (12.6%); varicella 589 (37.8%). A total of 1046 (67.1%) children had at least one of the diseases defined as total infectious diseases. After adjusting for appropriate confounders, PFOS levels in the highest

quartile were associated with increased odds ratios (ORs) of total infectious diseases (Q4 vs. Q1 OR: 1.61; 95% CI: 1.18, 2.21; p for trend = 0.008) in all children. In addition, perfluorohexane sulfonate (PFHxS) was associated with a higher risk of total infectious diseases only among girls (Q4 vs. Q1 OR: 1.55, 95% CI: 0.976, 2.45; p for trend = 0.045). We found no association between infectious diseases and other examined PFAAs. Our findings suggest that prenatal exposure to PFOS and PFHxS may associated with infectious diseases occurrence in early life. Therefore, prenatal exposure to PFAAs may be immunotoxic for the immune system in offspring.

1. Introduction

Perfluoroalkyl acids (PFAAs) are synthetic chemicals with very high thermal and physical stability. Worldwide, PFAA pollutants are present in the environment, wildlife, and humans. Food is thought to be the primary source of human exposure to PFAAs; however, humans are also exposed to these chemicals through contaminated water, dust, and air as well as various consumer products (ATSDR 2015). Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are the most commonly used PFAAs worldwide. PFAAs are resistant to metabolic processes; the elimination half-lives of PFOS and PFOA are 5.4 and 3.8 years, respectively. Additionally, perfluorohexane sulfonate (PFHxS) has a half-life of approximately 8.5 years (Olsen et al. 2007). PFOS was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009. Recently, PFOS and PFOA have been voluntarily phased out by several industries, but they remain present in older products. Therefore, humans are constantly exposed to PFOS, PFOA, and the recently emerged PFAAs, resulting in their bioaccumulation into human tissues over time, increasing human health concerns.

Globally, infectious diseases account for more than half of all deaths among children aged less than 5 years and are a large burden on health care systems (WHO 2002; Elliot and Beason, 2008). Previous laboratory studies showed that PFAAs have immunotoxic and immunosuppressive effects, such as atrophy and reduced cell numbers in immune organs such as the spleen and thymus, lower IgM production, decreased natural killer-cell activity,

and changes in pro-inflammatory cytokine production (Dewitt 2008, Peden-Adams 2008, Brieger et al. 2011; Qazi et al. 2012). Additionally, Kannan et al. (2006) reported significant associations between infectious diseases and high concentrations of PFOS/PFOA in the livers of sea otters. In animals, even low-dose exposure to PFOS/PFOA results in immune response impairment and suppression at doses potentially relevant to highly exposed humans to PFAAs (Guruge et al. 2009; DeWitt et al. 2012).

PFAAs can pass through the placenta during pregnancy, exposing the fetus to these chemicals (Inoue et al. 2004). Pre- and postnatal PFOS/PFOA concentrations are associated with a reduced humoral immune response to diphtheria and tetanus in children (Grandjean et al. 2012). A recent Danish study reported association of prenatal exposure to PFOS and PFOA with increased proportion of days with fever in early life (Dalsager et al. 2016). Additionally, another report found an inverse association between prenatal exposure to PFOS, PFOA, PFNA, and PFHxS and the level of anti-rubella antibodies in the children's sera at age 3 years. Furthermore, they found a positive association between the maternal concentrations of PFOA and PFNA and the number of episodes of common cold in the children, and between PFOA and PFHxS and the number of episodes of gastroenteritis (Granum et al., 2013; Chang et al. 2016). In contrast, Fei et al. (2010) reported no association between prenatal exposure to PFOS and PFOA and the risk of infectious diseases leading to hospitalization during childhood.

We previously reported the negative association between prenatal exposure to PFOA and cord blood IgE levels among female infants in another cohort of the Hokkaido Study; however, we found no association between PFOS and PFOA and the risk of allergic and infectious diseases at 18 months of age (Okada et al. 2012). We also examined the association of *in utero* PFAAs with allergic diseases in the first 24 months of life in a large-scale cohort and found that perfluorotridecanoic acid (PFTrDA) levels were inversely associated with the risk of eczema (Okada et al., 2014). In the same cohort, we found negative associations between PFHxS, PFTrDA, and perfluorododecanoic acid (PFDoDA) and allergic symptoms (Goudarzi et al. 2016). These finding suggest that prenatal exposure to PFAAs may suppress immune system allergic reactions during early life.

However, the effects of PFAAs on the immune system and risk of infectious diseases has not been thoroughly investigated in humans, particularly the impact of exposure to these chemicals during pregnancy on the developing immune system and functions in later life. In this study, we evaluated the association between prenatal exposure to eleven PFAAs and the risk of infectious diseases during early childhood in a prospective large birth cohort study.

2. Methods

2.1 Study population

The current work was a part of the Hokkaido Study on Environment and Children's health, a

prospective ongoing birth cohort study. The details of this study have been previously described (Kishi et al. 2011 and 2013). This study began 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 of the 37 hospitals and clinics in the Hokkaido prefecture participating in this study were eligible. Healthcare personnel approached pregnant women and introduced the study. Among the 33,500 women eligible to participate in the study from 2003 to 2009, 17,869 agreed to participate. All participants completed a baseline questionnaire and mailed follow-up questionnaires. Among 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. Cases with miscarriage and stillbirth (n = 19), congenital malformation (n = 143), and multiple births (n = 162) were excluded. From the selected 12,523 participants, 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 PFAA measurement in maternal plasma samples (Okada et al., 2014). Among these 2095 participants, we excluded cases of congenital malformations that became apparent from the follow-up questionnaire at 12 months (n = 17), maternal blood samples taken before 26 weeks of gestation (n = 15), an extremely high PFOS level (n = 1), and withdrawal (n = 6).

Among the remaining 2056 mother-child pairs, a total of 1558 mother-child pairs sent us 4-year-old questionnaires and were included in the current study (Supplementary Fig. 1).

This study was conducted with the written informed consent of all of participants for a period comprising pregnancy and 2 years after childbirth, and additional informed consent was obtained at 4 years after childbirth. The institutional ethical board for epidemiological studies at the Hokkaido University Center for Environmental and Health Sciences and Hokkaido University Graduate School of Medicine approved the study protocol.

2.2 Data collection

During the first trimester of pregnancy, participants completed a self-administered baseline questionnaire that included parental information related to age, pre-pregnancy body mass index, 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 sex, 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 the mothers, including information such as 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, having pets, cooling/heating system in homes, environmental tobacco smoke (ETS) exposure, and day care attendance. In the 4-year follow up questionnaire, 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 4 years of age.

2.3 Exposure assessment

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 (Waters, Milford, MA, USA). We measured the concentrations of two groups of PFAAs: perfluoroalkane sulfonates, including PFHxS, PFOS; and perfluorinated carboxylic acids, including perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and perfluorotetradecanoic acid (PFTeDA) for a total of eleven PFAAs in maternal plasma samples obtained between 28 and 32 weeks of pregnancy.

2.4 Outcome assessment

Child infectious diseases that developed up to 4 years of life were assessed based on mothers' self-administered questionnaire. Medical doctor's diagnosis of common infectious diseases including otitis media, pneumonia, varicella, and respiratory syncytial virus infection were

asked in 4-year old postnatal questionnaires reported by the mothers. We defined total infectious diseases as cases with at least one of these four common infectious diseases up to 4 years of age.

2.5. Data analysis

We performed all 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 method detection limit, a value equal to half of this limit was substituted. We divided participants into 4 groups according to the quartiles (Q) of prenatal PFAA levels. In crude and adjusted logistic regression analyses, we examined the 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 based on the PFAA concentrations in the second through fourth quartiles and compared to those in the lowest quartiles. To calculate the trend in the p-value, we used linear contrast coefficients -3, -1, +1, and +3 assigned to quartiles 1, 2, 3, and 4, respectively (Kishi et al., 2015; Goudarzi et al., 2015). We examined the effects on total infectious diseases and on each infectious disease. We selected confounders in the analysis according to a review of the literature and on the change in estimate criteria, which were set to more than 10%. Potential confounding variables considered in the analysis were maternal age (continuous), number of older siblings (0, ≥1), maternal smoking during pregnancy (yes/no), maternal education (≤12, >12 years),

infant sex, and breast-feeding period (<6, ≥6 months). For sensitivity analysis, we also added day care attendance (yes/no) and environmental tobacco smoke (ETS) exposure at 4 years old children (yes/no) into the adjusted model. The number of older siblings was obtained from parity information. Because of the potential sex differences in PFAA health effects (Okada et al. 2014; Goudarzi et al. 2015), we also stratified the results by sex.

Results

A total of 1558 mother-child pairs were included in this study. The average of maternal age at birth (SD) was 31.1 (4.4) with average body mass index of 20.9 (2.9). Maternal smoking rate during pregnancy was 6.2%, and 54.3% of mothers were multiparous. A total of 50.9% of infants were male (Table 1).

Because of the low detection rate, perfluorohexanoic acid, perfluoroheptanoic acid, and perfluorotetradecanoic acid levels were excluded before data analysis. The detection rates of the other 8 PFAAs were higher than 97%, except for PFDoDa (90.6%) and PFHxS (82.6%). PFOS showed 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).

The number and percentage of children who developed infectious diseases during the first 4 years of life were otitis media, 649 (41.6%); pneumonia, 287 (18.4%); respiratory syncytial virus infection, 197 (12.6%); varicella, 589 (37.8%). A total of 1046 (67.1%) of children had

at least one infectious disease. Varicella was reported at a greater rate among girls than in boys; however, the prevalence of infectious diseases was not significantly different in boys and girls.

We assessed the association between PFAAs and total infectious diseases using logistic regression models. We observed a positive association with total infectious diseases across the PFHxS quartiles (Q4 vs. Q1 adjusted OR: 1.55, 95% CI: 0.976, 2.45; p for trend = 0.045) in female but not in male children. In addition, adjusted ORs in the highest quartile vs. the lowest quartile for total infectious diseases were significantly increased for PFOS (Q4 vs. Q1 OR: 1.61; 95% CI: 1.18, 2.21; p for trend = 0.008) in all children. After sex stratification, we observed the same trend for boys and girls, but the p-value for trend was significant only among girls. However, p-values for PFAA*sex interaction were not statistically significant. We also assessed the association between PFAAs and individual infectious outcomes and found the same trend; however, the p-value for the trend was not significant, likely because of the small sample size (data not shown). The results remained consistent with those of the primary models when we conducted additional sensitivity analyses including postnatal day care attendance and ETS at 4 years of age (data not shown).

Discussion

Few studies have focused on prenatal exposure to PFAAs and prevalence of infectious diseases. We measured 11 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 a higher risk of infectious diseases in early life. However, we found no significant association between other PFAAs, including PFOA, PFNA, and PFDA, and infectious diseases. This is a unique study because of the number of subjects examined for prenatal exposure to PFAAs, its prospective design, and large sample size.

In current study, we found that perfluoroalkane sulfonic acids (PFSAs) including PFHxS, and PFOS, but not perfluorinated carboxylic acids (PFCAs; such as PFOA, PFNA) showed significant association with increased risk of infectious diseases. PFSAs and PFCAs have different carbon length, half-lives and functional groups. Also, we previously reported significant negative association of prenatal exposure to PFOS, but not PFOA, with cord blood cortisol and cortisone in another birth cohort (Goudarzi et al. 2016). We also observed a positive association between PFOS and dehydroepiandrosterone (DHEA) levels, whereas PFOA was inversely associated with DHEA levels (Goudarzi et al. 2016). Additionally, prenatal PFOS, but not PFOA, showed positive association with cord blood estradiol and estradiol/testosterone ratio among male infants. Also, we observed negative association of PFOS and progesterone and prolactin in cord blood samples among female infants; however

PFOA did not show any association with these hormones (Itoh et al. 2016). Steroid hormones have very important immunoregulatory role in humans, and PFAAs my disrupt immune system function in humans by changing balance of steroid hormones. Furthermore, these results may suggest differential effects of PFSAs vs. PFCAs on immune system, and more studies need to explore this issue in the future.

Varicella vaccine first was developed in Japan; however, it was not in mandatory vaccination program in Japan until 2014. In current study, only 457 children had varicella vaccination among 1,558 children. It may partly explain relatively high prevalence of varicella in this study. We stratified the results of association between PFAAs and infectious diseases by varicella vaccination status but the results remained persistent. Although we did not have information on air pollution in this study, we had information about home environment and indoor air quality including owning pets and having carpets, heating/cooling systems (electrical systems vs. fuel systems), and the presence of mold and dew condensation in homes in 4 years questionnaires, however including these covariates into adjusted model did not change the results.

During pregnancy, the median values of PFAAs with C6–C8 including PFHxS, PFOS, and PFOA in this study were low compared to those in the USA (Stein et al., 2012), Denmark (Halldorsson et al., 2012), Korea (Lee et al., 2013), and China (Jiang et al., 2014). However,

PFAA levels with longer carbon chains (C ≥ 9) were higher than in western countries such as Spain, Denmark, Sweden, and the USA compared with the current study (Harada et al. 2011).

Animal studies have demonstrated the endocrine disruption 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 and reduced the number of lymphoid cells and antibody production (Yang 2001; Peden-Adams 2007). Few epidemiological studies have examined the effects of PFAAs on the risk of infectious diseases, particularly prospective studies. Granum et al. (2013) reported a positive association between prenatal PFOA and PFNA levels and the number of episodes of common cold in children, and between PFOA and PFHxS and the number of episodes of gastroenteritis at 3 years of age. PFAA exposure levels were similar to those found in this study, and their results are consistent with our results, indicating that prenatal exposure to PFAAs is associated with an increased risk of infectious diseases in the next generation. Additionally, pre- and post-natal exposure to PFOS and PFOA were associated with reduced antibody levels of tetanus and diphtheria (Grandjean et al., 2012) in 5- and 7-year-old children. In adults, elevated PFOA serum concentrations were found to be associated with reduced influenza antibody response and an increased risk of not attaining the antibody threshold considered to offer long-term protection (Looker et al., 2014). These results suggest the suppressive effects of PFAAs on immune function and reactions. A recent Danish study showed that higher maternal

concentrations of PFOS and PFOA during pregnancy is associated with increased number of episodes of co-occurrence of fever and coughing and fever and nasal discharge among children aged 1-4 years (Dalsager et al. 2016).

The immunotoxic mode of action of PFAAs is not clearly understood. Steroid hormones are essential for immune system functions. Additionally, lymphocytes and primary lymphoid organs express androgen and estrogen receptors (Tanriverdi et al. 2003). Endocrine-disrupting chemicals, including PFAAs, can change the levels of estrogens and androgens and interact with their receptors (Kjeldsen and Bonefeld-Jorgensen 2013). Therefore, the endocrine-disrupting properties of PFAAs may explain the mechanism of action of PFAAs on immune outcomes. In addition, PFAAs are known agonists for peroxisome proliferator-activated receptor α (PPARα), which is expressed in gestational tissues and lymphocytes important in placental maturation, as well as the regulation of the immune system and hormones (Berry et al., 2003; Tarrade et al., 2001; Jones et al., 2002). A recent study found the same results, showing that PFAAs suppressed the secretion of these cytokines in human immune cells through the nuclear factor-kappa B gene, but not PPARα (Crosini et al. 2012). Interestingly, Crosini et al. reported higher sensitivity of leukocytes obtained from female donors compared with those from male donors to the immunotoxicity of PFAAs, which agrees with our results.

We previously examined the association between 11 PFAAs and the risk of allergic diseases at 12–24 months of age and found an inverse association between prenatal exposure to PFTrDA and the risk of eczema among female infants (Okada et al. 2014). Recently, we examined the effects of prenatal PFAAs on the risk of allergic diseases at 4 years of age in the same cohort and follow-up of the same participants. The results revealed an inverse association of prenatal exposure to PFDoDA and PFTrDA with eczema and an inverse association between PFHxS and wheezing (Goudarzi et al. 2016). Taken together, PFAAs may suppress the immune system in humans, resulting in a higher risk of infectious diseases and reduced allergic reactions.

We examined 11 PFAAs during pregnancy using a sensitive and standard method with large sample size and prospective design. We used information on several covariates related to exposure and/or outcome to control for these factors in the adjusted models. However, there were some limitations to this study. We assessed infectious diseases based on maternal reports that were not proved by medical records, also we have not conducted any studies on the validity of self-reported doctor diagnosed infections. It may have resulted in some level of outcome misclassification which generally bias toward the null, or if an association is demonstrated, the true effect might be slightly greater. Also, we have not performed co-exposure assessment for other environmental chemicals influencing immune system such as dioxins in this population. Compared to the original cohort (n > 20,000), participants in the

current study had a higher maternal education levels, and lower maternal smoking rates during pregnancy and postnatal exposure to tobacco smoke (Goudarzi et al. 2016). This may suggest the possibility of selection bias in the current study. Furthermore, we did not examine the levels of postnatal PFAAs and biomarkers of immune system functions.

We found an association between prenatal exposures to PFOS and PFHxS and a higher risk of infectious diseases during early childhood. Our results suggest that PFAAs have immunomodulatory effects on the human immune system. However, additional studies are necessary to determine the long-term effects of *in utero* exposure to PFAAs on human immune outcomes in later life.

References

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.

Chang ET, Adami HO, Boffetta P, Wedner HJ, Mandel JS. 2016. A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans. Crit Rev Toxicol. 46(4):279-331. doi: 10.3109/10408444.2015.1122573.

Corsini E, Sangiovanni E, Avogadro A, Galbiati V, Viviani B, Marinovich M, et al. 2012. In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). Toxicol Appl Pharmacol. 15;258(2):248-55.

Dalsager L, Christensen N, Husby S, Kyhl H, Nielsen F, Høst A, Grandjean P, Jensen TK. 2016. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort. Environ Int. 96:58-64. doi: 10.1016/j.envint.2016.08.026.

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.

DeWitt JC, Peden-Adams MM, Keller JM, Germolec DR. 2012. Immunotoxicity of perfluorinated compounds: recent developments. Toxicol Pathol 40:300–11.

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.

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.

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.

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.

Goudarzi H, Nakajima S, Ikeno T, Sasaki S, Kobayashi S, Miyashita C, et al. 2015. Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study. Sci Total Environ. 541:1002-1010.

Goudarzi H, Miyashita C, Okada E, Kashino I, Kobayashi Su, Chen CJ, et al. 2016. Effects of prenatal exposure to perfluoroalkyl acids on risk of allergic diseases at 4 years old children. Environ Int. 94:124-32. doi: 10.1016/j.envint.2016.05.020.

Goudarzi H, Araki A, Itoh S, Sasaki S, Miyashita C, Mitsui T, et al. 2017. The Association of Prenatal Exposure to Perfluorinated Chemicals with Glucocorticoid and Androgenic Hormones in Cord Blood Samples: The Hokkaido Study. Environ Health Perspect. 125(1):111-118. doi: 10.1289/EHP142.

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.

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.

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.

Itoh S, Araki A, Mitsui T, Miyashita C, Goudarzi H, Sasaki S,et al. 2016. Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. Environ Int. 94:51-9. doi: 10.1016/j.envint.2016.05.011.

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.

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.

Kishi R, Sasaki S, Yoshioka E, Yuasa M, Sata F, Saijo Y, et al. 2011. Cohort profile: The Hokkaido study on environment and children's health in Japan. Int J Epidemiol 40:611-618.

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.

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.

Kjeldsen LS, Bonefeld-Jorgensen EC. 2013. Perfluorinated compounds affect the function of sex hormone receptors. Environmental science and pollution research international 20:8031-8044.).

Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, et al. 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: Postnatal evaluation. Toxicological sciences: an official journal of the Society of Toxicology 74:382-392.

Lee YJ, Kim MK, Bae J, Yang JH. 2013. Concentrations of perfluoroalkyl compounds in maternal and umbilical cord sera and birth outcomes in Korea. Chemosphere 90:1603-1609.

Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, et al. 2014 Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci. 138(1):76-88.

Luebker DJ, York RG, Hansen KJ, Moore JA, Butenhoff JL. 2005. Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters. Toxicology 215:149-169.

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.

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.

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.

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.

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.

Peden-Adams M.M., Keller J.M., Eudaly J.G., Berger J., Gilkeson G.S., Keil D.E. 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. Toxicol. Sci. 104, 144–154.

Qazi MR, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2012. High-dose dietary exposure of mice to perfluorooctanoate or perfluorooctane sulfonate exerts toxic effects on myeloid and B-lymphoid cells in the bone marrow and these effects are partially dependent on reduced food consumption. Food Chem Toxicol. 50(9):2955-63.

Seacat AM, Thomford PJ, Hansen KJ, Clemen LA, Eldridge SR, Elcombe CR, et al. 2003. Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. Toxicology 183:117-131.

Stein CR, Wolff MS, Calafat AM, Kato K, Engel SM. 2012. Comparison of polyfluoroalkyl compound concentrations in maternal serum and amniotic fluid: a pilot study. Reprod Toxicol 34:312-316.

Tanriverdi, F., Silveira, L. F., MacColl, G. S., and Bouloux, P. M. (2003). The Hypothalamic-pituitary-gonadal axis: Immune function and autoimmunity. J. Endocrinol. 176, 293–304.

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.

Abbreviations:

PFAAs, perfluoroalkyl 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; ETS, environmental tobacco smoke.

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Table 1. Characteristics of study population (n = 1558) of the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013.

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Characteristics		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)	0	702 (45.7)
	≥1	835 (54.3)
Maternal smoking status during pregnancy	Non smoker	1461 (93.8)
	Smoker	97 (6.2)
Maternal allergic history	Yes	484 (31.0)
Paternal allergic history	Yes	307 (19.7)
Annual household income (million yen)	<5	880 (64.0)
	≥5	495 (36.0)
Child characteristics		
Sex	Male	793 (50.9)
	Female	765 (49.1)
Older siblings (numbers)	0	702 (45.7)
	≥1	835 (54.3)
Day care attendance at 4-year-old	Yes	1373 (90.3)
	No	148 (9.7)
ETS[a] exposure at 4-year-old	Yes	724 (48.1)
	No	782 (51.9)

Missing: parity (n = 21), annual income (n = 183), day care attendance at 4 y (n = 37), and ETS at 4 y (n = 52).

Table 2. Distribution of maternal plasma PFAA concentrations at third trimester of pregnancy (n = 1558).

	MDL[a]	No.	Detection rate (%)	Mean	Minimum	25th	50th	75th	Maximum
PFHxS (C6)	0.2	1287	82.61	0.322	<0.2	0.221	0.296	0.395	3.386
PFHxA (C6)	0.1	721	46.28	0.103	<0.1	<0.1	<0.1	0.145	0.694
PFHpA (C7)	0.1	549	35.24	0.095	<0.1	<0.1	<0.1	0.125	0.757
PFOS (C8)	0.3	1558	100	5.456	1.003	3.667	4.925	6.654	30.283
PFOA (C8)	0.2	1557	99.94	2.713	<0.2	1.314	2.013	3.346	24.88
PFNA (C9)	0.3	1556	99.87	1.402	<0.3	0.908	1.183	1.589	13.189
PFDA (C10)	0.1	1551	99.55	0.575	<0.1	0.393	0.522	0.694	2.434
PFUnDA (C11)	0.1	1555	99.81	1.534	<0.1	1.037	1.431	1.895	5.89
PFDoDA (C12)	0.1	1413	90.69	0.191	<0.1	0.14	0.186	0.233	0.729
PFTrDA (C13)	0.1	1524	97.82	0.35	<0.1	0.247	0.332	0.424	1.325
PFTeDA (C14)	0.1	238	15.28	0.061	<0.1	<0.1	<0.1	<0.1	0.303

[a]MDL: method detection limit

Table 3. Prevalence of infectious diseases during the first 4 years of life in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013.

Symptoms	Total (n=1558)		Male children (n=793)		Female children (n=765)		p[a]
	n	(%)	n	(%)	n	(%)	
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
Varicella	589	(37.8)	284	(35.81)	305	(39.87)	0.099
Total Infectious diseases [b]	1046	(67.1)	522	(65.82)	524	(68.49)	0.261

[a] Chi-square test.

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

Table 4. Risk of total infectious diseases in early life according to quartiles of prenatal PFAA levels 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[c]	Adjusted[a]			Adjusted[b]			Adjusted[b]	
		OR	(95% CI)		OR	(95% CI)		OR	(95% CI)
PFHxS									
Quartile 1	267	1			1			1	
Quartile 2	267	1.03	(0.764, 1.41)		0.780	(0.508, 1.19)		1.46	(0.938, 2.29)
Quartile 3	280	1.23	(0.905, 1.69)		0.947	(0.614, 1.45)		1.81	(1.14, 2.88)
Quartile 4	261	0.957	(0.703, 1.30)		0.708	(0.461, 1.08)		1.55	(0.976, 2.45)
p for trend		0.928			0.223			**0.045**	
P for sex interaction		0.134							
PFOS									
Quartile 1	251	1			1			1	
Quartile 2	276	1.44	(1.06, 1.96)		1.45	(0.954, 2.22)		1.42	(0.916, 2.23)
Quartile 3	264	1.28	(0.949, 1.73)		1.25	(0.825, 1.91)		1.32	(0.855, 2.06)
Quartile 4	284	1.61	(1.18, 2.21)		1.59	(1.03, 2.46)		1.71	(1.08, 2.72)
p for trend		**0.008**			0.071			**0.036**	
P for sex interaction		0.561							
PFOA									

Quartile 1	266	1		1		1	
Quartile 2	272	1.17	(0.865, 1.60)	1.02	(0.666, 1.56)	1.45	(0.918, 2.30)
Quartile 3	277	1.32	(0.966, 1.82)	1.34	(0.865, 2.11)	1.37	(0.869, 2.19)
Quartile 4	260	1.11	(0.806, 1.54)	0.952	(0.606, 1.49)	1.37	(0.855, 2.21)
p for trend		0.393		0.854		0.242	
P for sex interaction		0.826					
PFNA							
Quartile 1	273	1		1		1	
Quartile 2	271	1.04	(0.770, 1.42)	1.03	(0.673, 1.58)	1.09	(0.704, 1.70)
Quartile 3	276	1.14	(0.842, 1.56)	0.899	(0.585, 1.38)	1.55	(0.984, 2.46)
Quartile 4	255	0.918	(0.672, 1.25)	0.902	(0.587, 1.38)	0.975	(0.617, 1.54)
p for trend		0.748		0.520		0.711	
P for sex interaction		0.597					
PFDA							
Quartile 1	277	1		1		1	
Quartile 2	275	0.996	(0.731, 1.35)	0.891	(0.583, 1.36)	1.16	(0.734, 1.83)
Quartile 3	266	0.891	(0.655, 1.21)	0.869	(0.567, 1.33)	0.952	(0.609, 1.48)
Quartile 4	257	0.799	(0.588, 1.08)	0.832	(0.542, 1.27)	0.810	(0.518, 1.26)
p for trend		0.114		0.402		0.249	
P for sex interaction		0.590					

PFUnDA							
Quartile 1	262	1		1		1	
Quartile 2	270	1.03	(0.764, 1.40)	0.996	(0.656, 1.50)	1.06	(0.676, 1.66)
Quartile 3	271	1.06	(0.783, 1.43)	0.960	(0.625, 1.47)	1.18	(0.771, 1.83)
Quartile 4	272	1.03	(0.764, 1.40)	1.04	(0.680, 1.59)	1.03	(0.667, 1.61)
p for trend		0.786		0.899		0.756	
P for sex interaction		0.859					
PFDoDA							
Quartile 1	264	1		1		1	
Quartile 2	262	0.921	(0.680, 1.24)	1.05	(0.689, 1.60)	0.767	(0.493, 1.19)
Quartile 3	275	1.03	(0.763, 1.40)	1.02	(0.669, 1.57)	1.02	(0.658, 1.59)
Quartile 4	274	1.07	(0.790, 1.46)	0.999	(0.652, 1.52)	1.17	(0.750, 1.83)
p for trend		0.502		0.968		0.285	
P for sex interation		0.308					
PFTrDA							
Quartile 1	261	1		1		1	
Quartile 2	270	1.04	(0.770, 1.41)	1.48	(0.972, 2.28)	0.711	(0.457, 1.10)
Quartile 3	272	1.10	(0.813, 1.49)	1.21	(0.795, 1.84)	1.01	(0.645, 1.59)
Quartile 4	272	1.01	(0.753, 1.38)	1.07	(0.701, 1.63)	0.965	(0.621, 1.49)
p for trend		0.816		0.999		0.728	
P for sex		0.108					

interaction									

[a] Adjusted for maternal age, maternal educational level, number of elder siblings, child sex, breast-feeding period, and smoking during pregnancy.

[b] Adjusted for all covariates except child sex.

[c] Indicates number of cases with infectious diseases.

*$p < 0.05$ compared with quartile 1.

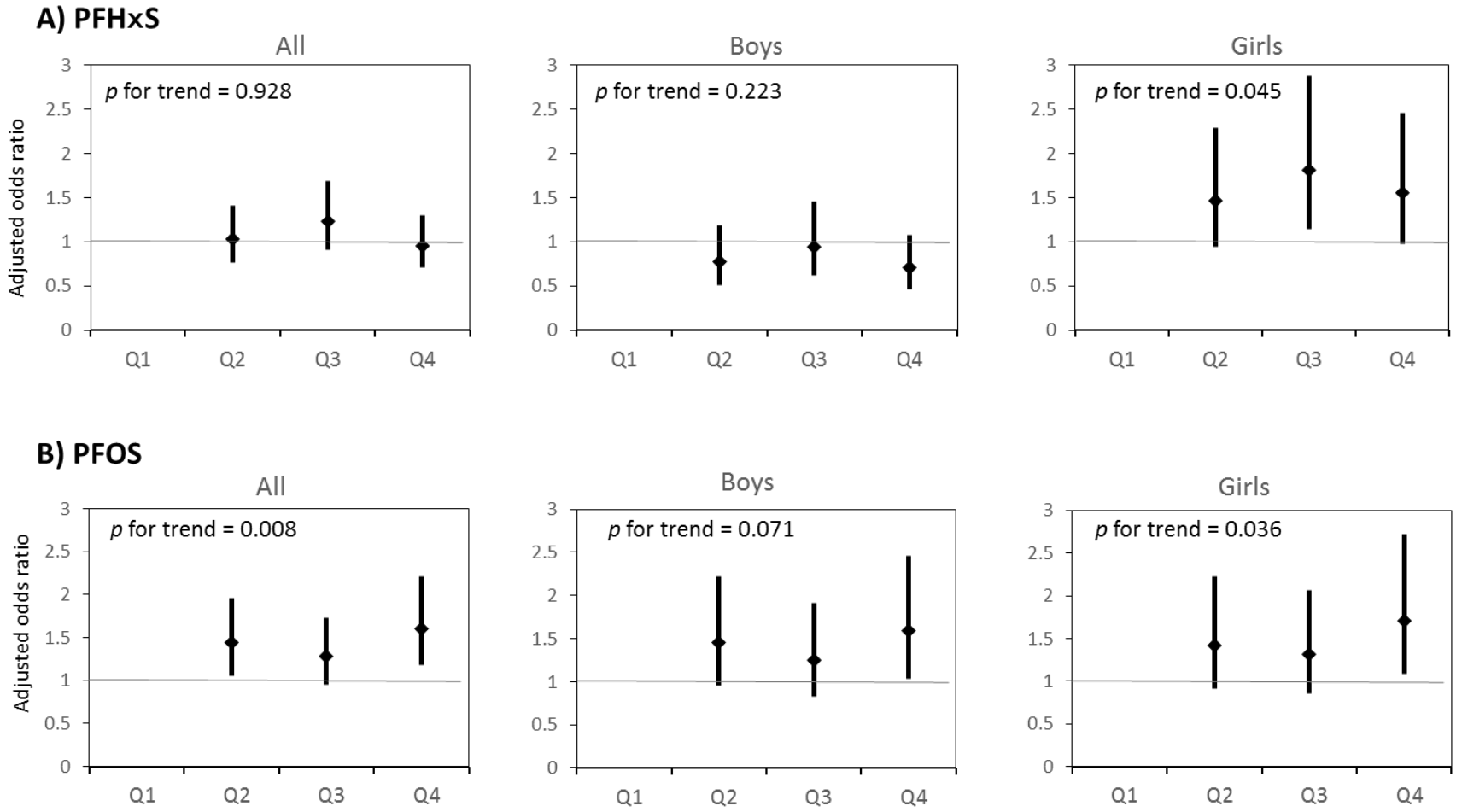

Figure 2. Association between maternal prenatal PFHxS (A) and PFOS (B) concentrations with risk of infectious diseases during early life. The data are expressed as the odds ratio and 95% CI adjusted for maternal age, maternal educational level, number of elder siblings, breast-feeding period, and smoking during pregnancy. In addition, we included the child sex into the adjusted model for all children before sex stratification. P-Values for the trend were calculated using the linear contrast coefficient test. Q: quartile.