# Effects of prenatal exposure to perfluoroalkyl acids on risk of allergic diseases at 4 years old children

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**研究要旨**

Perfluoroalkyl acids (PFAAs) as emerged chemicals are extremely resistant chemicals widespread in environment and frequently detected in human blood samples. Animal studies showed exposure to PFAAs cause immunotoxicity. However, the association between PFAAs including long chain PFAAs and allergy in humans are not well understood. We examined whether prenatal exposure to PFAAs is associated with allergic symptoms in 4-year old children in a large-scale prospective birth cohort, Hokkaido, Japan. 1558 mother-child pairs were analyzed in this study and prenatal levels of 11 PFAAs were measured in maternal plasma samples obtained between 28 and 32 weeks of pregnancy by ultra-performance liquid chromatography-tandem mass spectrometry. Information of participants' characteristics were obtained from self-administered pre-, postnatal questionnaires and medical birth records. Infant allergies including eczema, wheezing, and allergic rhinoconjunctivitis were assessed by Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three questionnaire obtained at 4 years post-delivery. Associations of PFAA quartiles with allergic outcomes were examined using logistic models. Adjusted odds ratios (ORs) in the 4th quartile vs 1st quartile (Q4 vs Q1) for total allergic diseases (including at least one of allergic outcomes) were significantly decreased for PFDoDA (Q4 vs Q1 OR: 0.621; 95% CI: 0.454, 0.847) and PFTrDA (Q4 vs Q1 OR: 0.712; 95% CI: 0.524, 0.966) in all children. We found the same results between PFAAs and eczema. The adjusted OR (Q4 vs Q1) for wheezing in association with higher maternal PFHxS levels was 0.728 (95% CI: 0.497, 1.06) in all children.

Although adjusted OR for allergic outcomes in 2nd to 4th of examined PFAA quartiles reduced compare to first quartile in both sexes, the associations were statistically significant only in boys after sex stratification (p for trend<0.05). In conclusion, prenatal exposure to PFAAs, especially long chain ones, may have immunosuppressive effects on allergic diseases in 4 year old children especially among boys.

**研究協力者**

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## A．研究目的

Perfluoroalkyl acids (PFAAs) are ubiquitous chemicals with widespread contamination in environment, animals and humans. Main route of exposure to PFAAs are contaminated food and water, and house dust (Kato et al., 2009). The most used PFAAs are perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). While PFOS and PFOA are being voluntarily phased out by several industries,

they are still present in older products. PFAAs are resistant to metabolism with long elimination half-lives for example 3.8, 5.4 and 8.5 years for PFOA, PFOS perfluorohexane sulfonate (PFHxS) in humans, respectively (Olsen et al., 2007). The stability and long half-lives of PFAAs result in continued presence in environment and human exposure.

Exposure to PFOS and PFOA in animals decreased lymphoid organ weights, reduced number of lymphoid cells and antibody production (Yang et al. 2001; Peden-Adams et al., 2007). In animals, PFOS and PFOA inhibit T-cell-dependent Immunoglobulin M (IgM) antibody response (TDAR) which is one of essential and predictor of immune system function.

PFAAs can pass placenta during pregnancy (Inoue et al., 2004); therefore, fetuses and children are continuously exposed to PFAAs. Exposure to PFAAs during these critical window of susceptibility may affect several aspects of health in later life including immune function. Previous epidemiological studies propose immunomodulatory effects of PFAAs indicating that prenatal exposure to PFOS and PFOA were associated with IgE levels of cord blood in different directions (Okada et al., 2012; Wang et al., 2011). Also pre- and post-natal exposure to PFOS and PFOA are associated with reduced antibody levels of tetanus, and diphtheria (Grandjean et al., 2012), and rubella (Granum et al., 2013) in children.

We previously reported declining trend for PFOS and PFOA, however we observed increasing trend for perfluorononanoic acid (PFNA, C9) and perfluorodecanoic acid (PFDA, C10) levels among pregnant women between 2003 and 2011, Hokkaido, Japan (Okada et al. 2013); worthy to note that PFAAs with longer carbon chains including perfluoroundecanoic acid (PFUnDA, C11), perfluorododecanoic acid (PFDoDa, C12), and perfluorotridecanoic acid (PFTrDA, C13) were detectable in more than 90% of maternal plasma samples obtained at 3rd trimester of pregnancy in our cohort. Our group assessed association of prenatal exposure to 11 types of PFAAs and allergic symptoms at 12 to 24 months of age, reported negative association between prenatal exposure to PFTrDA and eczema among female infants (Okada et al., 2014). Although some animal experiments suggest prenatal PFC exposures modified postnatal immune response throughout the period of early childhood (Keil et al. 2008); to this date, effects of PFAAs including long-chain PFAAs on allergic diseases in childhood long observations especially in prospective birth cohorts are not well understood. Therefore, in this study, we followed mother-child pairs in the same cohort of report of Okada et al. (2014) and assessed the association of prenatal PFAAs with allergic disease at 4 year-old children.

## B．研究方法

The current work is a part Hokkaido Study on Environment and Children’s health, prospective ongoing birth cohort. The details of this study have previously described (Kishi et al. 2011 and 2013). 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. 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

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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.

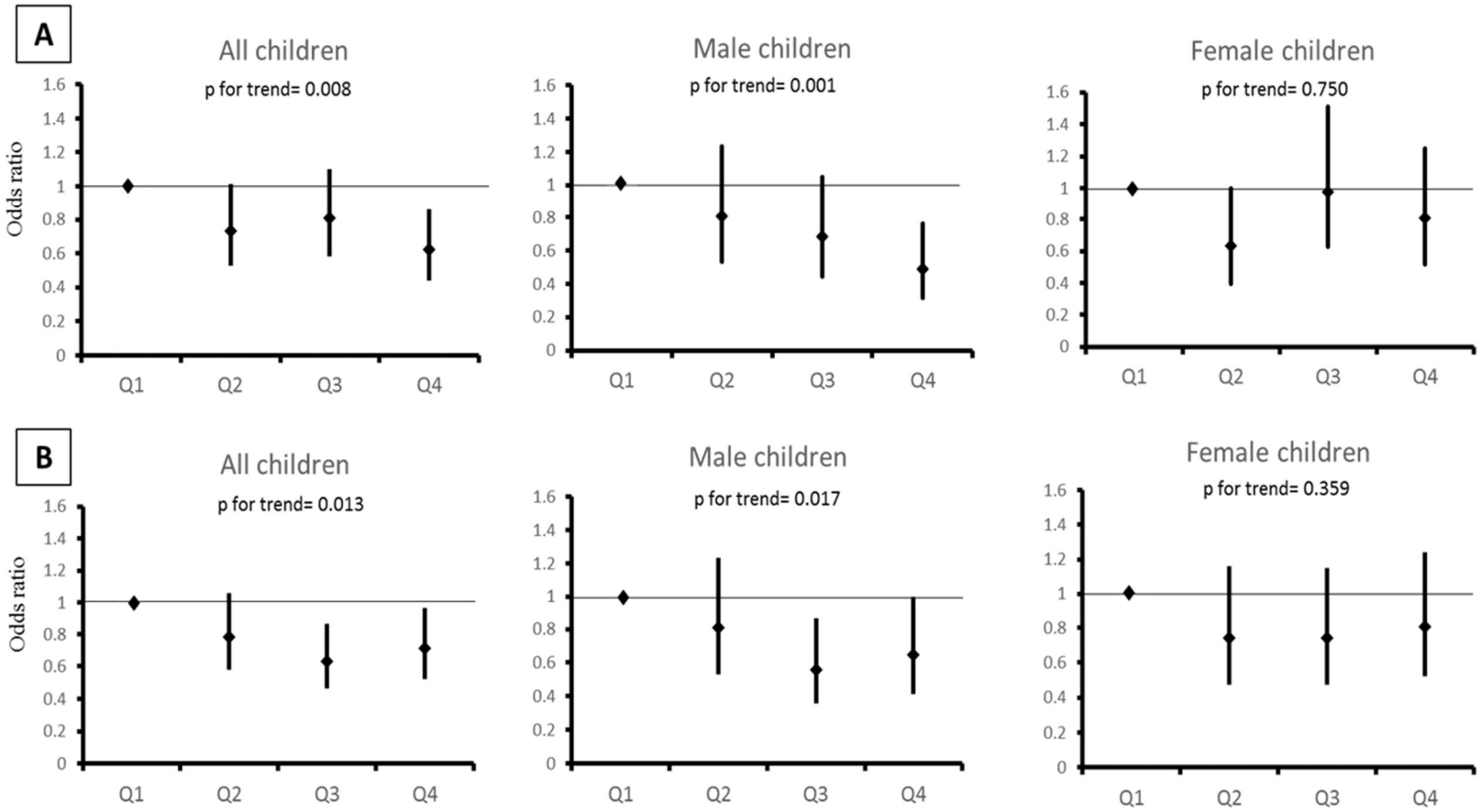

Figure 2. The association between quartiles of PFDoDA (A) and PFTrDA (B) with risk of eczema among 4-year old children. Adjusted for maternal age, maternal educational level, parental allergic history, number of older siblings, children gender, breast feeding, day care attendance and ETS exposure at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