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Prenatal exposure to perfluoroalkyl substances is associated with lower hand, foot and mouth disease viruses antibody response in infancy: Findings from the Guangzhou Birth Cohort Study

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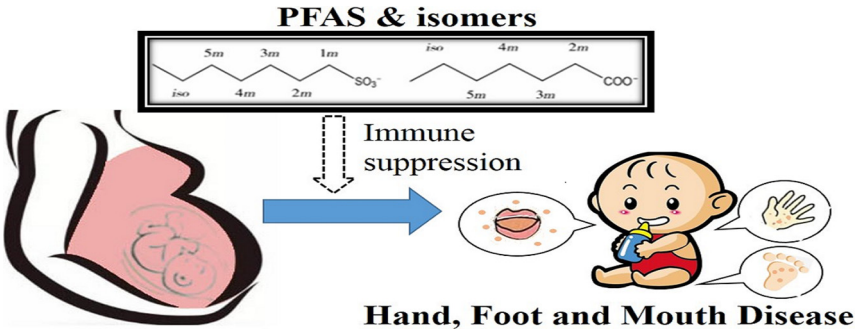
HIGHLIGHTS

- Higher PFAS concentration in cord blood was related to lower hand, foot and mouth disease antibody levels in infants.
- We identified obvious immunosuppressive effects from exposure to branched PFOS isomers.
- This association was apparent in boys at three months of age for CA16.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Perfluoroalkyl substances (PFASs) are synthetic chemicals widely used in industry and for commercial products. Their immunomodulatory effects are a growing health concern in children. Hand, Foot and Mouth Disease (HFMD) is a common childhood viral infection, and increased incidence of which has parallel the rise in PFAS exposure in the Asia-Pacific region.

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Objective: We conducted the first study to assess whether prenatal exposure to PFAS was associated with a reduction in HFMD virus antibodies in infants.
Methods: We enrolled 201 mother-infant pairs from the Guangzhou Birth Cohort Study from July to October 2013. High performance liquid chromatography-mass spectrometry was employed to determine concentrations of specific PFAS isomers in cord blood. Neutralizing antibodies titers were measured against two HFMD viruses, enterovirus 71 (EV71) and coxsackievirus A 16 (CA16), in cord blood serum and blood serum at three months of age.
Results: Higher umbilical cord blood PFAS concentrations were associated with lower EV71 and CA16 antibody concentrations. A doubling in the composite sum of cord blood PFASs in three month old infants was associated with significant increase in the risk of HFMD antibody concentration below clinical protection level (≥1:8 titers) for CA16 (odds ratio, OR: 2.74 [95% confidence interval (CI): 1.33, 5.61] and for EV71 (OR = 4.55, 95% CI: 1.45, 4.28). This association was higher in boys at three months of age for CA16.
Conclusions: Our findings suggest that cord blood PFAS exposure is associated with lower HFMD antibody in infancy. Given the widespread nature of PFAS exposures and the high global incidence of HFMD globally, these findings have substantial public health implications and therefore, these associations need to be replicated in a larger study to more definitively address the risk.

1. Introduction

Perfluoroalkyl substances (PFASs) are one kind of synthetic chemicals which have been widely used as surfactants and repellants in industrial and commercial products since the mid 20th Century (Buck et al., 2011). Considering their ubiquitous and persistent nature and long half-lives in the human body, the potential health effect of PFAS has become a global public health issue (Bartell et al., 2010; Olsen et al., 2007; Zhang et al., 2013). There is evidence that PFASs have immunomodulatory effects on immune function in animals and humans, with effects varied by sex and different isomers of PFAS (Chang et al., 2016; Chen et al., 2015; Grandjean et al., 2012). The immune function of children, in particularly, are more susceptible to PFASs exposure. PFASs can pass through the placenta during pregnancy and lead to suppressed immune responses in early childhood (Chang et al., 2016). A recent Norway birth cohort ($n = 641$) indicated that cord blood PFAS concentration were associated with an increased number of respiratory tract infections in the first 10 years of childhood (Impinen et al., 2018). Goudarzi et al. (2017) also reported that prenatal exposure to PFOS and PFHxS was associated with prevalence of infectious diseases up to 4 years of life in a Japanese birth cohort ($n = 1558$). Additionally, Granum et al. (2013) observed an inverse association between cord blood PFAS concentrations and the level of antirubella antibodies in children at age 3 years in a Norwegian cohort ($n = 99$). Disruption of early life immune function may be manifested as increased incidence of common childhood infectious diseases due to inability to mount a normal antibody response.

Hand, foot and mouth disease (HFMD), is a common and highly infectious disease which causes a substantial disease burden in the Asia-Pacific region, such as China, Australia, Singapore and Vietnam (Koh et al., 2018; Sanders et al., 2006). For example, China reported 13.7 million cases of HFMD between 2008 and 2015, leading to 123,261 severe cases (with neurological complications or pulmonary complications) and 3322 deaths in 31 provinces of mainland China (Xiao et al., 2017). The most vulnerable population is children under 5 years of age (Yang et al., 2017). Specific maternal antibodies could transport across the placenta to against virus infection in infants for the first 12 months after birth (Zinkernagel, 2001). However, several factors can impair immunoglobulin transplacental transfer (Fouda et al., 2018). Enterovirus 71 (EV71) and coxsackievirus A 16 (CA16) are the two main viruses responsible for the majority of HFMD cases. However, no vaccines or effective antivirus therapies are available to curtail these infections, except that a vaccine for EV71 was licensed for use in China in late 2015 (CFDA, 2016). For HFMD, the two viruses EV71 and CA16 are antigens that depend on the T_H1 and T_H2 $CD4^+$ helper T cell subsets for innate and memory immune antibody responses in humans (Wu et al., 2014). The regulation of interleukin-35 involving in the ratio of regulatory T cells (Tregs) and T helper 17 balance may play a critical role in the pathogenesis of EV71-associated HFMD (Huang et al., 2017). Furthermore, experimental study has suggested that the immunotoxic effects of PFAS may depend on the interfering of interleukin expression which is important to the immunoglobulin secretion (Zhang et al., 2014). Although recent studies suggested that HFMD prevalence is associated with climate changes (Zhao et al., 2018), the contributions of environmental pollutant exposure to the immune responses of HFMD remains unknown.

Given the potential impact of prenatal exposure to PFASs on developing immune function, it is critical to identify whether PFAS exposure modifies the antibody immune response of HFMD viruses. We hypothesized that higher cord blood PFAS exposure would suppress the antibody response to HFMD infection. We also aimed to test whether the effects would be different between PFAS isomers and that the impact may vary by sex.

2. Methods

2.1. Study population

The Guangzhou Birth Cohort (GBC) was developed based on one hospital in Guangzhou (Guangdong Province, China) from July to October 2013 (Li et al., 2017). A total of 411 women with singleton pregnancies between ages of 18 to 45 years with no self-reported diagnosed cancer or psychiatric illness were recruited.

Questionnaires were used to collect demographic information including smoking history, family income, education and other variables. Participants provided an umbilical cord blood specimen at delivery and completed one visit three months after the delivery. Both cord and peripheral blood collected at three months of age were sampled for HFMD viruses (EV71 and CA16) antibody measurement.

For the present study, we used data from cord blood samples of 201 children who had both PFAS concentration analysis and Hand, Foot and Mouth Disease (HFMD) viruses antibody measurement. At the three months follow-up, 180 of the 201 children provided blood to measure HFMD viruses antibody concentration in serum (participation rate: 93%). Clinical information was collected from the patient's electronic medical record by trained staff. Children's date of birth, birth weight and height were collected from medical records.

Informed consent was obtained from participants. This study protocol was approved by the Institutional Review Board (Sun Yat-sen University Research Ethics Committee), and all study procedures were in accordance with the principles of Helsinki Declaration.

2.2. Exposure assessment

We determined concentrations of 17 linear PFASs and 10 PFAS isomers in cord blood serum, according to a previously described method (Zhang et al., 2017). In brief, we used high performance liquid

chromatography (HPLC), coupled to an Agilent 6410 Triple Quadrupole (QQQ) mass spectrometer (MS/MS) (Agilent, Palo Alto and Santa Clara, CA). PFAS standards, including 17 linear PFASs, isomers of PFOS/PFOA, and nigh mass-labeled internal standards, were purchased from Wellington Laboratories (Guelph, ON, Canada). Detail regarding the standards and reagents, sample preparation and extraction, instrumental analysis, and quality assurance and quality control are provided in the Supplementary material. We limited our study to seven linear PFASs, including perfluoro-n-decanoic acid (PFDA), perfluoro-n-dodecanoic acid (PFDoDA), sodium perfluoro-1-hexanesulfonate (PFHxS), perfluoro-n-nonanoic acid (PFNA), sodium perfluoro-1-octanesulfonate (PFOS), perfluoro-n-octanoic acid (PFOA), perfluoro-n-undecanoic acid (PFUnDA), and three branched PFOS isomers (i.e., 1 m-PFOS, isoPFOS, and 3 m + 4 m + 5 m-PFOS) with detection rate >45% in specimens (Table S1).

The detail of PFASs and isomer analysis method and isomer nomenclature was specified in Supplementary Material. Briefly, 0.3 ml serum was spiked with 0.5 ng of mass labeled internal standards and vortexed to mix. The Oasis-HLB cartridge (Waters, 200 mg/6 ml) was conditioned with 2 ml of methanol (HPLC grade) followed by 2 ml of 0.1 M formic acid. The prepared serum was then loaded to the column and washed successively with 3 ml of 0.1 M formic acid, 5 ml of 50% 0.1 M formic acid/50% methanol and 1 ml of 1% ammonium hydroxide. PFASs were eluted with 2.0 ml of 1% ammonium hydroxide in acetonitrile. The eluent was evaporated to near-dryness by nitrogen and then resolved with a 100 μl mixture (30%methanol/70%ammonium formate). Samples were centrifuged at 12,000 rpm for 5 min at 4 °C before analysis by HPLC-tandem mass spectrometry (Zhang et al., 2017).

2.3. Hand, foot and mouth disease (HFMD) viruses antibody measurements

As HFMD vaccine was not available in China before 2015, all the children in our study sample did not receive CA16 and EV71 viruses vaccination before blood sample collection. We quantified neutralizing antibody titers against serum CA16 viruses and EV71 viruses, using a modified cytopathogenic effect assay (Fu et al., 2016). Briefly, we inactivated a dilution of 1:8 sera at 56 °C for 30 min, then repeatedly diluted to 1:2048 and mixed with equal volumes of 100 TCID50 EV71 and CA16. The virus-serum mixtures were added to a 96-well microplate and incubated at 37 °C for 2 h. Afterwards, human rhabdomyosarcoma (RD) cells were added to the microplate at a concentration of 10^5 cells/ml, followed by incubation at 35 °C for seven days. Cell control, serum control, virus control, and virus backdrops were included on each plate. The cytopathogenic effect was evaluated by microscopic examination. The test was considered valid if the backdrops showed 32–320 $TCID_{50}$/well. Neutralizing antibody titers were defined as the dilution rate demonstrating 50% inhibition of the cytopathogenic effect. For CA16 and EV71, titers of ≥1:8 in the test were considered as seropositivity for the clinical protection level (Luo et al., 2009).

2.4. Statistical analysis

We first used the Wilcoxon rank-sum test to assess different PFAS concentrations between boys and girls, Spearman correlation coefficients to evaluate their inter-correlations, since the effect of PFAS on human health may differ by sex in previous studies. Neutralization antibody titers were log-transformed to calculate the geometric mean titer and 95% confidence intervals (95% CI). Next, we used multiple linear regression models to assess associations between individual PFAS concentrations as predictors, with continuous antibody concentrations as outcomes, adjusted for sex, age, parental education and occupation, annual family income, parity and birth weight as confounders (Goudarzi et al., 2017). We also created composite sum variables as long as they were detected in samples (including 15 linear PFAS and all isomers) as total PFASs for an individual (set the value of undetected PFAS as 'limit of detection (LOD) / √2') (Zhang et al., 2017). We natural log transformed PFAS and antibody concentrations to achieve a normal distribution of residuals with a homogeneous variance, and the effects were expressed as the relative percent change for a doubling in PFAS concentrations, using ((2^β)-1) * 100 (Stein et al., 2016). Because of the seropositivity of CA16 and EV71 ≥ 1:8 titers in neutralizing antibody titers test, we next used multiple logistic regression to estimate odds ratios (ORs) for the effects of individual PFAS exposures on dichotomized antibody concentration below this limit, adjusted for the aforementioned confounders. To evaluate the robustness of our estimates, additional sensitivity analyses were conducted by excluding preterm neonates (with gestational age < 37 weeks) or low birth weight neonates (with birth weight < 2.5 kg) who may have weaker immune function. Finally, the analyses were sex-stratified to investigate sex-differences in the associations. We defined statistical significance as two-tailed *P*-values <0.05 except for the interaction term where statistical significance was set at *P*-values <0.1 (Qian et al., 2014). All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Inc., Cary, NC, USA).

3. Results

Table 1 shows the characteristics of a total of 201 study participants, stratified by sex. The dominant PFAS in cord blood serum was PFHxS, followed by PFOS and PFOA. There was no difference in PFAS concentration between boys and girls ($P > 0.05$, Table 1). Most cord serum PFASs were positively inter-correlated (Table S2). No difference was observed between boys and girls in serum antibody concentration of cord blood or of three-month infant blood ($P > 0.05$, Fig. 1).

The multiple linear regression analysis showed a consistent pattern of inverse associations between higher cord blood PFAS concentrations and lower CA16 and EV71 antibody concentrations (Table 2). For example, a doubling in total-PFOA exposure was associated with an 18.7% (95% CI: −28.6%, −7.4%) lower EV71 antibody concentration in cord blood and 7.2% (95% CI: −13.2%, −0.8%) lower among infants at three months of age. When the analysis was stratified by sex, the associations appeared to be stronger in boys than in girls for CA16 antibody levels, such as in PFDA ($P_{inter} = 0.084$), PFDoDA ($P_{inter} = 0.010$) and total PFASs exposure ($P_{inter} = 0.023$) in three-month infants (Table 2). In addition, we observed negative association between branched PFOS isomer exposure and serum CA16 and EV71 antibody levels. For EV71, doubling of branched PFOS isomer exposure was associated with reduction of 19.6% (95%CI: −31.8%, −5.2%) in cord blood serum and of 9.4% (95%: −16.7%, −1.6%) in three-month serum.

Doubling of cord blood PFAS concentration was associated with increased odds of antibody seronegativity when adjusted for potential confounders as shown in Table 3. This association is more prominent in three-month infants than in cord blood (Table 3, Fig. 2). For example, we found higher odds for EV71 seronegativity in infants at three months age (OR = 4.55, 95% CI: 2.06, 10.06) than that in cord blood serum (OR = 1.90, 95% CI: 1.14, 3.16) for a doubling in the concentration of PFASs (Table 3). The association was higher in boys (OR = 7.40, 95% CI: 2.18, 25.07) than in girls (OR = 1.44, 95% CI: 0.61, 3.39) for CA16 in total PFASs exposure ($P_{inter} = 0.064$, Table S3). Besides, we also observed consistent positive associations between branched PFAS isomers exposure and risk of antibody seronegativity for HFMD (Table 3, Table S3). The results of the sensitivity analysis that excluded preterm or low birth weight neonates whose immune function may be compromised were coinciding with the main analysis (Table S4). We also analyzed the association between HFMD antibody levels and PFASs with the detection rate <70% (e.g. 1m-PFOS, 3 + 4 + 5 m-PFOS, PFDA, PFDoDA, PFNA, PFUnDA) as categorical: <LOD and > LOD, instead of replacing values blow the LOD with a single value of LOD / √2. Results showed the similar trend in these two different categorical analysis with some variations (Table S5). Furthermore, we categorized the PFASs as perfluoroalkylated carboxylic acid (PFCA) and perfluoroalkylated sulfonate (PFSA) and compared its association with CA16 and EV71 antibody.

Table 1
Characteristics (mean + SD) of Guangzhou Birth Cohort participants and PFAS concentrations [median (Q1, Q3)] in cord blood serum ($n = 201$).

Variables	Total ($n = 201$)	Boys ($n = 106$)	Girls ($n = 95$)
Mother			
Age at parturition (years)	28.1 ± 5.5	28.2 ± 5.8	28.1 ± 5.5
Gestational period (week)	39.2 ± 1.4	39.3 ± 1.3	39.2 ± 1.5
Education (no. %)			
<high school	74 (36.8)	40 (37.4)	34 (35.8)
≥high school	127 (63.2)	66 (62.3)	61 (64.2)
Occupation (no. %)			
White collar	19 (9.5)	8 (7.6)	11 (11.6)
Blue collar	182 (90.6)	98 (92.4)	84 (88.4)
Delivery (no. %)			
Vaginal	137 (68.2)	75 (70.8)	62 (65.3)
Caesarean	64 (31.8)	31 (29.3)	33 (34.7)
Income (RMB/month, no. %)			
<4000	143 (71.1)	79 (74.5)	64 (67.4)
4000–7999	44 (21.9)	20 (18.9)	24 (25.2)
≥8000	14 (7.0)	7 (6.6)	7 (7.4)
Infant			
Birth weight (kg)	3.1 ± 0.5	3.1 ± 0.5	3.2 ± 0.5
Parity (no. %)			
1	106 (52.7)	57 (53.8)	49 (51.6)
2	95 (47.3)	49 (46.2)	46 (48.4)
Preterm (no. %)	11 (5.5)	5 (4.7)	6 (6.3)
Low birth weight (no. %)	14 (7.0)	9 (8.5)	5 (5.3)
Cord blood serum PFASs levels (ng/ml)			
Total–PFOA	1.22 (0.86, 1.74)	1.23 (0.86, 1.94)	1.21 (0.86, 1.66)
n–PFOA	1.03 (0.67, 1.54)	1.03 (0.67, 1.72)	1.02 (0.67, 1.48)
Total–PFOS	3.17 (1.88, 4.94)	3.10 (1.76, 4.88)	3.41 (1.95, 5.23)
n–PFOS	2.20 (1.33, 3.91)	2.10 (1.33, 3.92)	2.23 (1.30, 3.92)
Total–Br PFOS	0.75 (0.39, 1.31)	0.67 (1.76, 4.88)	0.82 (0.41, 1.38)
1 m–PFOS	0.13 (0.06, 0.25)	0.13 (0.06, 0.23)	0.14 (0.06, 0.23)
3m + 4m + 5m–PFOS	0.39 (0.19, 0.72)	0.37 (0.19, 0.71)	0.43 (0.19, 0.73)
iso–PFOS	0.19 (0.10, 0.35)	0.19 (0.06, 0.35)	0.19 (0.10, 0.36)
PFDA	0.12 (0.05, 0.22)	0.12 (0.05, 0.20)	0.13 (0.05, 0.24)
PFDoDA	0.05 (0.05, 0.13)	0.05 (0.05, 0.14)	0.08 (0.05, 0.12)
PFHxS	3.96 (2.32, 5.41)	4.15 (2.22, 5.76)	3.74 (2.35, 5.12)
PFNA	0.16 (0.05, 0.29)	0.17 (0.05, 0.34)	0.15 (0.05, 0.23)
PFUnDA	0.13 (0.05, 0.27)	0.13 (0.05, 0.27)	0.12 (0.05, 0.29)
PFASs	10.56 (8.43, 15.07)	10.52 (8.35, 14.68)	10.87 (8.60, 15.57)

RMB, Chinese Renminbi Yuan; preterm was defined as an infant with gestational age < 37 weeks; low birth weight was defined as an infant with birth weight < 2.5 kg.
PFASs, all perfluoroalkyl substance; Total–PFOA, perfluorooctanoic acid; n–PFOA, perfluoro-n-octanoic acid; Total–PFOS, the sum of n–PFOS and total–Br PFOS; n–PFOS, sodium perfluoro-1-octanesulfonate; 1 m–PFOS, perfluoro-1-methylheptanesulfonate; 3 m + 4 m + 5 m–PFOS, sodium perfluoro-3-methylheptanesulfonate+ sodium perfluoro-4-methylheptanesulfonate+ sodium perfluoro-5-methylheptanesulfonate; iso–PFOS, sodium perfluoro-6-methylheptanesulfonate; Total–BrPFOS, the sum of 1 m–PFOS, 3 m + 4 m + 5 m–PFOS and iso–PFOS; PFDoDA, perfluoro-n-dodecanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluoro-n-nonanoic acid; PFUnDA, perfluoro-n-undecanoic acid.
Percentage may not total 100 because of rounding.

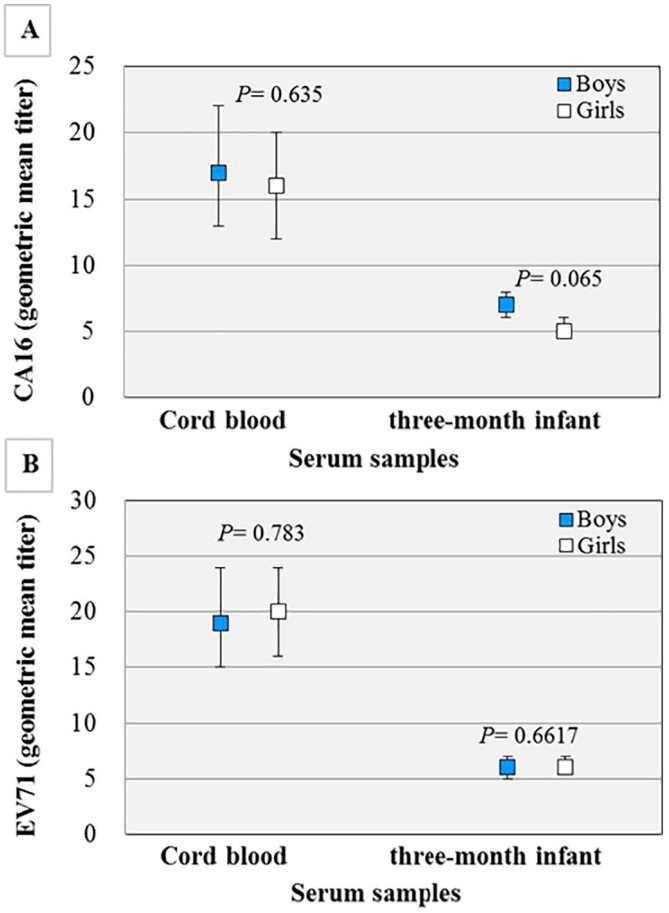

Fig. 1. Antibody concentration of CA16 (A) and EV71 (B) in cord blood and three-month infant serum. Note: CA16 and EV71 antibody level was represented as geometric mean titer (Q1, Q3).

Results did not show consistent difference between PFCA and PFSA (Table S6). However, both PFCA and PFSA was associated with increasing odds of antibody seronegativity (Table S7).

4. Discussion

This prospective study showed that higher levels of cord blood PFAS exposure were associated with diminished HFMD antibody response in early childhood. This association was particularly prominent at three month after birth. Furthermore, we identified immunosuppressive effects from exposure to branched PFOS isomers.

The PFAS concertation measured in cord blood serum in our cohort was similar to those reported in other Asian birth cohort studies, with the exception for PFHxS which was the predominant PFASs (median: 3.96 ng/ml). The concentration of PFHxS in our study was obviously higher than that reported in Korean birth cohort (median: 0.56 ng/ml, $n = 118$, Lee et al., 2016) and other Chinese birth cohorts, such as in Shanghai (median: 0.16 ng/ml, $n = 686$, Wang et al., 2016) and Beijing (mean: 0.23 ng/ml, $n = 170$, Shi et al., 2017). However, consistent with our result, high cord blood PFHxS level was also observed in a Faro Islands study (Needham et al., 2011) and a Taiwan study (Wang et al., 2018) which reported PFHxS concentration of 9.1 ng/ml and 22.8 ng/ml, respectively. The different transplacental transfer efficiency in PFAS which is affected by the functional group and carbon chain length may partly explain the high concentration found in the present study (Beesoon et al., 2011; Chen et al., 2017; Monroy et al., 2008). Furthermore, the fact that PFHxS is produced as one of the major substitutes for PFOS in China may also contribute to the high concentration in human (Gao et al., 2015).

In the past few decades, large outbreaks of HFMD across the Asia-Pacific region and Europe have been responsible for a substantial burden of disease (Jones et al., 2018). Children under five years are the most susceptible to this highly infectious disease. However, it has been reported that HFMD also occurs in immunocompromised adults (Murase and Akiyama, 2018). Some HFMD patients could rapidly develop severe neurological complications and cardiorespiratory failure (Koh et al., 2018). Therefore, unraveling the contributions of environmental factors and host condition to immune responses of HFMD is of great significance from a population health perspective. A literature review suggested that HFMD prevalence was associated with climate changes, temperature, rainfall and lower level of vitamin A in human (Omaña-Cepeda et al., 2016). However, the potential environmental risk factors exposure affecting the specific HFMD viruses antibody responses have not yet been identified.

Table 2
Estimated change (%) in HFMD antibody concentrations in serum of cord blood and three–month blood, per doubling increase in concentration of PFAS in cord blood.

Antibody	Cord blood (%)			P_{inter}[c]	Three-month infant (%)			P_{inter}[c]
	Total[a] ($n = 194$)	Boys[b] ($n = 103$)	Girls[b] ($n = 91$)		Total[a] ($n = 180$)	Boys[b] ($n = 91$)	Girls[b] ($n = 89$)	
CA16								
Total–PFOA	−16.3 (−25.3, −6.1)	−22.0 (−33.1, −8.9)	−8.7 (−22.6, 7.6)	0.191	−6.9 (13.2, 0.0)	−11.1(−20.7, −0.3)	−3.2 (−11.2, 5.5)	0.210
n–PFOA	−13.6 (−21.8, −4.4)	−17.6 (−27.6, −6.4)	−7.2 (−20.4, 8.1)	0.291	−5.8 (−11.5, 0.2)	−9.4(−17.8, −0.2)	−2.4 (−9.7, 5.1)	0.221
Total–PFOS	−20.6 (−30.0, −9.9)	−24.7 (−37.6, −9.1)	−14.0 (−27.5, 1.9)	0.233	−6.9 (−13.9, 0.7)	−12.2 (−23.7, 1.1)	−2.8 (−10.9, 6.2)	0.174
n–PFOS	−16.4 (−24.7, −7.2)	−18.7 (−29.9, −5.7)	−11.6 (−23.5, 2.2)	0.300	−5.1 (−11.0, 1.2)	−8.4 (−18.2, 2.6)	−2.3 (−9.3, 5.1)	0.257
Total–BrPFOS	−15.8 (−27.1, −2.7)	−17.6 (−34.0, 2.9)	−10.2 (−26.1, 9.1)	0.504	−5.2 (−13.2, 3.6)	−10.3 (−23.6, 5.3)	−0.8 (−105, 10.0)	0.292
1m–PFOS	−9.4 (−22.2, 5.5)	−6.3 (−24.3, 15.8)	−7.8 (−26.1, 15.1)	0.851	−2.1 (−10.9, 7.5)	−3.1 (−17.1, 13.2)	0.4 (−10.6, 12.8)	0.770
3m + 4m + 5m–PFOS	−13.9 (−25.3, −0.8)	−17.0 (−33.0, 2.7)	−6.8 (−23.2, 13.2)	0.410	−5.3 (−13.2, 3.3)	−11.2 (−23.8, 3.5)	0.0 (−9.7, 10.8)	0.228
iso–PFOS	−15.4 (−25.5, −3.8)	−16.3 (−31.5, 2.1)	−11.0 (−24.7, 5.2)	0.483	−4.9 (−12.1, 2.8)	−9.3 (−21.6, 4.8)	−1.6 (−9.9, 7.5)	0.284
PFDA	−17.1(−27.4, −5.3)	−26.0(−39.1, −10.2)	−9.9 (−24.9, 8.0)	0.059	−11.2(−18.0, −3.9)	−17.3(−27.6, −5.6)	−5.5 (−14.0, 3.8)	0.084
PFDoDA	−4.2 (−18.00, 11.8)	−16.7 (−33.9, 4.8)	4.0 (−15.5, 28.0)	0.138	−5.7 (−14.3, 3.8)	−18.6 (−30.9, −4.2)	4.5 (−6.4, 16.6)	0.010
PFHxS	−1.9 (−14.2, 12.2)	−8.2 (−22.3, 8.6)	8.9 (−12.2, 35.1)	0.415	−0.2 (−7.6, 7.8)	−5.9 (−15.3, 4.7)	12.3 (0.3, 25.7)	0.037
PFNA	−12.1(−22.2, −0.6)	−13.7 (−25.9, −0.3)	−11.4 (−27.2, 7.8)	0.478	−6.9 (−13.6, 0.4)	−9.0 (−18.3, 1.4)	−13.7 (−25.9, −0.3)	0.426
PFUnDA	−12.4(−21.7, −2.0)	−20.6(−32.1, −7.1)	−4.6 (−18.4, 11.6)	0.065	−7.2 (−13.4, −0.6)	−12.3(−21.8, −1.7)	−2.4 (−10.0, 6.0)	0.132
PFASs	−27.0 (−38.5, −13.4)	−36.5 (−51.0, −17.8)	−17.7 (−34.3, 3.0)	0.131	−11.7 (−20.6, −1.8)	−24.0 (−36.9, −8.4)	−2.9 (−13.9, 9.4)	0.023
EV71								
Total–PFOA	−18.7 (−28.6, −7,4)	−20.6 (−32.5, −6.6)	−14.6 (−30.4, 4.6)	0.479	−7.2 (−13.2, −0.8)	−8.2 (−16.2, 0.5)	−4.9 (−13.7, 4.8)	0.429
n–PFOA	−16.2 (−25.2, −6.0)	−16.6 (−27.2, −4.6)	−13.2 (−28.2, 4.9)	0.614	−5.9 (−11.3, −0.3)	−6.8 (−13.7, 0.7)	−3.8 (−11.9, 5.1)	0.459
Total–PFOS	−23.6 (−33.9, −11.8)	−23.4 (−37.2, −6.6)	−23.5 (−37.9, −5.8)	0.803	−10.6 (−16.9, −3.9)	−12.2 (−21.3, −1.9)	−8.6 (−17.1, 0.9)	0.383
n–PFOS	−18.8 (−28.0, −8.5)	−17.2 (−29.2, −3.1)	−19.7 (−32.8, −4.0)	0.967	−7.9 (−13.3, −2.2)	−9.2 (−16.9, −0.8)	−6.0 (−13.4, 2.0)	0.343
Total–BrPFOS	−19.6 (−31.8, −5.2)	−21.4 (−37.5, −1.0)	−18.2 (−35.7, 4.0)	0.653	−9.4 (−16.7, −1.6)	−11.6 (−22.1, 0.2)	−6.8 (−17.0, 4.7)	0.439
1m–PFOS	−16.2 (−29.5, −0.4)	−15.8 (−32.4, 4.9)	−19.1 (−38.5, 6.3)	0.827	−8.7 (−16.4, −0.2)	−10.1 (−20.4, 1.5)	−6.7 (18.2, 6.6)	0.540
3m + 4m + 5m–PFOS	−16.0 (−28.6, −1.2)	−16.8 (−33.5, 4.0)	−14.5 (−32.8, 8.7)	0.699	−8.1 (−15.4, −0.3)	−9.6 (−19.9, 2.1)	−5.7 (−16.0, 5.9)	0.555
iso–PFOS	−19.8 (−30.6, −7.4)	−25.9 (−39.6, −9.2)	−15.8 (−31.5, 3.6)	0.310	−8.4 (−14.9, −1.4)	−11.2 (−20.8, −0.7)	−6.0 (−14.9, 3.9)	0.305
PFDA	−24.7 (−35.2, −12.5)	−20.1 (−35.1, −1.8)	−26.6 (−41.0, −8.6)	0.563	−9.9 (−16.4, −2.8)	−10.3 (−19.4, −0.1)	−8.5 (−17.8, 1.9)	0.661
PFDoDA	−2.1 (−18.0, 16.8)	−9.8 (−29.2, 15.0)	7.7 (−16.8, 39.4)	0.223	4.6 (−4.5, 14.5)	0.5 (−12.7, 14.8)	8.1 (−4.3, 22.2)	0.305
PFHxS	−4.1 (−17.6, 11.8)	−8.9 (−23.5, 8.5)	5.2 (−19.6, 37.6)	0.208	−2.4 (−9.3, 5.0)	−2.5 (−10.4, 6.1)	−0.6 (−12.9, 13.3)	0.591
PFNA	−17.9(−28.5, −5.7)	−5.1 (−19.2, 11.4)	−34.3 (−47.8, −17.2)	0.017	−3.7 (−10.3, 3.5)	2.0 (−6.5, 11.2)	−11.9 (−21.5, −1.3)	0.065
PFUnDA	−17.9 (−27.7, −6.8)	−12.6 (−26.2, 3.4)	−19.3 (−33.3, −2.3)	0.612	−6.5 (−12.4, −0.2)	−4.6 (−13.0, 4.6)	−6.3 (−14.5, 2.7)	0.950
PFASs	−28.2 (−41.0, −12.7)	−27.1 (−44.8, −3.6)	−27.9 (−45.3, −4.9)	0.845	−12.3 (−20.6, −3.1)	−11.2 (−23.8, 3.5)	−11.8 (−22.8, 0.9)	0.748

[a] Results were adjusted for sex, age, parental education, parental occupation, family income, parity, and birth weight.
[b] Results were adjusted for age, parental education, parental occupation, family income, parity, and birth weight.
[c] P_{inter} indicated the interaction between sex and PFASs on HFMD antibody level.

Table 3
Adjusted odds ratio (95% CIs) for HFMD antibody concentration below clinically protection level in serum of cord blood and three-month blood[a].

Antibody	Cord blood ($n = 194$)	P	Three-month infant ($n = 180$)	P
CA16				
Total–PFOA	1.56 (1.13, 2.14)	0.007	1.73 (1.08, 2.75)	0.022
n–PFOA	1.51 (1.13, 2.01)	0.006	1.53 (1.07, 2.19)	0.022
Total–PFOS	1.75 (1.16, 2.63)	0.007	1.71 (1.12, 2.60)	0.013
n–PFOS	1.53 (1.08, 2.16)	0.016	1.39 (1.02, 1.90)	0.039
Total–BrPFOS	1.64 (1.06, 2.54)	0.028	1.70 (1.03, 2.79)	0.037
1m–PFOS	1.19 (0.79, 1.80)	0.398	1.41 (0.88, 2.25)	0.150
3m + 4 m + 5 m–PFOS	1.49 (0.99, 2.25)	0.054	1.68 (1.04, 2.73)	0.035
iso–PFOS	1.70 (1.14, 2.54)	0.010	1.61 (1.04, 2.49)	0.032
PFDA	1.19 (0.82, 1.71)	0.356	2.22 (1.42, 3.47)	0.003
PFDoDA	1.04 (0.68, 1.58)	0.859	1.64 (0.98, 2.75)	0.060
PFHxS	1.08 (0.74, 1.60)	0.682	1.00 (0.71, 1.43)	0.988
PFNA	1.10 (0.79, 1.54)	0.579	1.50 (1.04, 2.17)	0.032
PFUnDA	1.13 (0.83, 1.53)	0.438	1.45 (1.02, 2.08)	0.040
PFASs	2.24 (1.30, 3.85)	0.004	2.74 (1.33, 5.61)	0.006
EV71				
Total–PFOA	1.49 (1.09, 2.05)	0.014	2.11 (1.27, 3.48)	0.004
n–PFOA	1.44 (1.08, 1.92)	0.013	1.70 (1.18, 2.46)	0.005
Total–PFOS	1.66 (1.12, 2.45)	0.011	2.25 (1.44, 3.51)	0.000
n–PFOS	1.58 (1.12, 2.24)	0.010	1.66 (1.20, 2.31)	0.002
Total–BrPFOS	1.44 (0.97, 2.14)	0.070	2.49 (1.45, 4.28)	0.001
1m–PFOS	1.17 (0.78, 1.76)	0.440	2.12 (1.26, 3.57)	0.005
3m + 4m + 5m–PFOS	1.31 (0.90, 1.91)	0.156	2.22 (1.32, 3.73)	0.003
iso–PFOS	1.53 (1.06, 2.21)	0.024	2.17 (1.36, 3.46)	0.001
PFDA	1.49 (1.03, 2.16)	0.035	2.05 (1.33, 3.18)	0.001
PFDoDA	1.05 (0.69, 1.61)	0.812	0.88 (0.58, 1.35)	0.568
PFHxS	1.38 (0.91, 2.09)	0.128	1.49 (1.06, 2.10)	0.023
PFNA	1.44 (1.02, 2.02)	0.037	1.01 (1.04, 2.16)	0.029
PFUnDA	1.12 (0.83, 1.52)	0.456	1.48 (1.04, 2.10)	0.031
PFASs	1.90 (1.14, 3.16)	0.013	4.55 (2.06, 10.06)	<0.001

[a] Results are adjusted for sex, age, parental education, parental occupation, family income, parity, and birth weight.

To the best of our knowledge, our study is the first to investigate the association between environmental pollutant exposure and protective antibodies against HFMD. Our results support previous studies that reported lower antibodies response for other infectious diseases. A prospective cohort study ($n = 587$), from the Faroe Islands, reported that −49% (95% CI: −67%, −23%) reduction in overall serum antibody concentrations to tetanus and diphtheria toxoids was associated with a two-fold higher concentration of major PFASs in five years old children (Grandjean et al., 2012). At seven years of age, higher PFOS and PFOA exposures were associated with increased odds between 2.38 (95%CI: 0.89, 6.35) and 4.20 (95% CI: 1.54, 11.44) for tetanus and diphtheria seronegativity after receiving a vaccine booster (Grandjean et al., 2012). This evidence of human PFAS immunotoxicity was strengthened by Mogensen et al. (2015) who used structural equation modeling. A similar pattern suggested persistent effects among 13 year olds in a recent Faroe Island Cohort study follow-up (Grandjean et al., 2017a). The BraMat subcohort study from Norway ($n = 99$), reported inverse associations between maternal plasma PFAS conncentrations (PFOS, PFOA, PFNA and PFHxS) and anti-rubella antibody levels in three year olds (n = 99) (Granum et al., 2013). In a Japanese birth cohort study ($n = 1558$), maternal serum PFOS level in the highest quartile was associated with increasing odds of infectious diseases, including otitis media, pneumonia, respiratory syncytial virus infection and varicella in children at the age of 4 years old (Goudarzi et al., 2017). Dalsager et al. (2016) also found association between higher PFOS and PFOA levels in maternal serum and more episodes of fever in children aged 1–4 years in Odense Child Cohort ($n = 359$). Finally, a cross-sectional study ($n = 1191$) using data from the 1999–2000 and 2003–2004 U.S. National Health and Nutrition Examination Survey (NHANES) cycles observed associations between higher PFAS exposures and lower mumps and rubella antibody concentrations in children aged 12–19 years (Stein et al., 2016). Our findings provide new insights of PFAS-associated immunosuppression, and build upon prior evidence.

Electro-chemical fluorination is one of the most commonly used PFAS manufacture processes producing 20%–30% branched PFAS isomers (Benskin et al., 2010). While previous studies reported different pharmacokinetic behaviors (Beesoon et al., 2011; Gao et al., 2015) and transplacental transfer efficiencies (Beesoon et al., 2011; Beesoon and Martin, 2015) for branched PFAS isomers, studies that have investigated the effects of individual PFAS isomers on human immune function are rare. Our data showed obvious effects from cord blood exposure to branched PFOS isomers. In contrast, the Faroe Islands birth cohort study did not find the association between branched PFOS and vaccine antibody response (Grandjean et al., 2012). This discrepancy might relate to life stage differences and critical windows of vulnerability for developmental immunotoxicity in the respective study populations (Cohen Hubal et al., 2014). Our study focused on HFMD viruses antibody levels in neonates and three-month old infants, while the Faroe Islands birth cohort study looked at diphtheria antibody concentrations in five and seven year olds (Grandjean et al., 2012). In fact, a recent Faroe Islands birth cohort publication, indicated that the developing immune system was particularly vulnerable to PFAS immunotoxicity, most specifically during the first six months after birth (Grandjean et al., 2017b). Dysregulation of immune responses at birth predispose to the subsequent risk of childhood infection or allergic disease. Our results was in agreement with this notion, showing that the association between prenatal PFAS exposure and higher odds of HFMD antibody seronegativity was stronger in infants at the age of three months.

The ability of immune-system to respond to pathogens or environmental exposures differs between males and females. In general, females are more resistant to infectious disease, with higher production of antibodies, pro-inflammatory cytokines, and chemokines when compared with males (Edwards et al., 2018). A previous study suggested a higher prevalence of HFMD in males than in females (Wang et al., 2011). Our study found that boys appeared to be more vulnerable to the effects of cord blood PFAS exposure on the reduction in protective antibodies against HFMD. The precise mechanism for sex-dependent effects on the immune response to different stimuli are not yet well understood. A recent review suggested that environmental chemicals may have sex differential effects on the outcome of immune responses through epigenetic alteration, driving hormonal effects directly or indirectly by activation of estrogen receptors (Edwards et al., 2018). Furthermore, a recent study showed that expression of the majority of immune genes induced by viral stimulation in human varied by sex (Piasecka et al., 2018).

Experimental studies have shown that PFAS could induce immunotoxicity via several pathways, including inhibition of T-cell proliferation (Lv et al., 2015), alteration of the ratios of immune cell populations in the spleen and thymus (Rockwell et al., 2017), and cytokine imbalance, such as decreasing in interleukin (IL) -2 (Midgett et al., 2015). Norwegian BraMAT cohort has investigated transcriptomics profiles in cord blood and their association with maternal PFAS exposure, anti-rubella antibody levels at three years of age (Pennings et al., 2016). The authors found that genes associated with PFAS exposure overlapped with those associated with rubella antibody. This finding provides toxicological pathway for the connection of prenatal PFAS exposure and impaired immune function in early childhood (Pennings et al., 2016). The main causative viruses of HFMD are EV71 and CA16. Although HFMD pathogenesis remains largely unclear, studies have shown that inflammatory responses may play an important role in the immune response to EV71, with low T cell proliferation and higher levels of proinflammatory cytokines, T_H2 and T_H17 cytokines (Zhang et al., 2015). However, the specific mechanism involving in the association between PFAS exposure and HFMD antibody suppression remains to be further explored.

Our findings should be interpreted in the context of the limitations of our study. First, we did not incorporate measures for other persistent environmental agents, including polychlorinated biphenyls and toxic trace elements heavy metals, which were also found to be associated

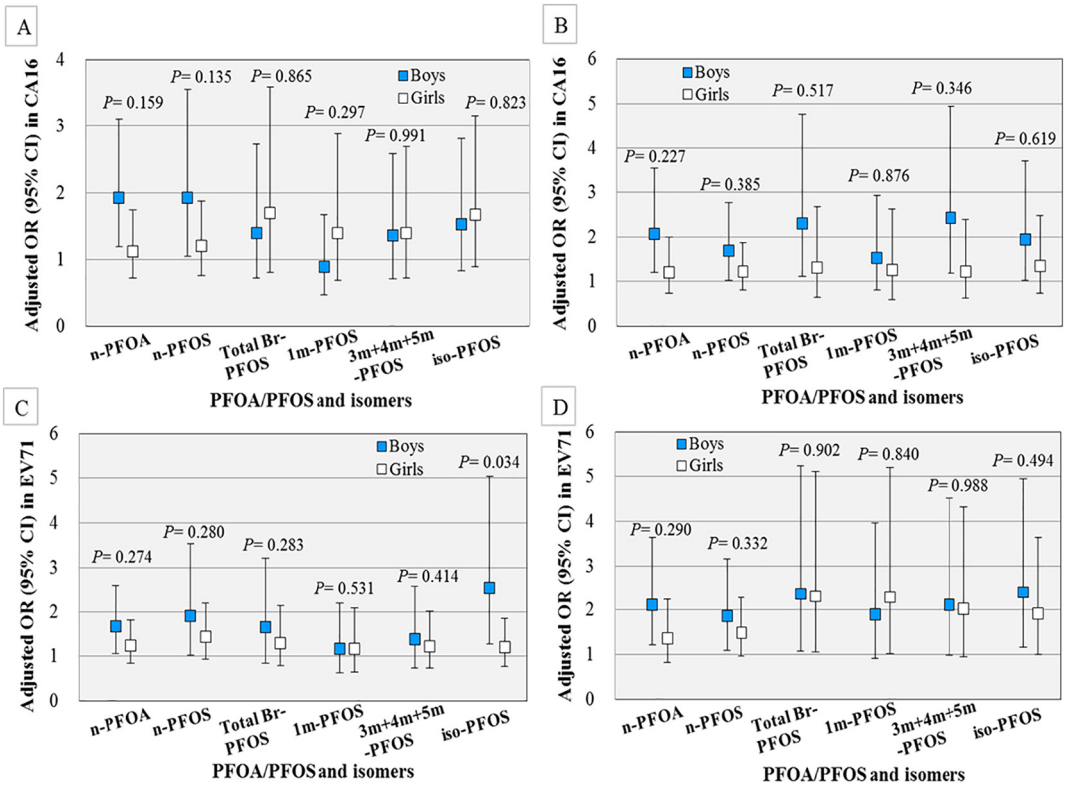

Fig. 2. Adjusted odds ratio (95% CI) for CA16 antibody in cord blood (A) and three-month infants (B), and for EV71 antibody in cord blood (C) and three–month infant (D) below seropositive level with doubling concentration increase of Log PFOA/PFOS and isomers in cord blood, stratified by sex. Note: results are adjusted for age, parental education, parental occupation, family income, parity, and birth weight; Vertical bars indicate the 95% CI. *P* value for the interaction between sex and PFASs on ORs of HFMD.

with immunosuppression in children and might be correlated with prenatal PFAS exposures (Granum et al., 2013; Lin et al., 2017). However, the sources of exposure to these agents tend to be different and so residual confounding effect may be less (Berg et al., 2017). Nevertheless, a more comprehensive exposure assessment is necessary for a more definitive evaluation. Second, we did not examine breast milk PFAS, concentrations. Breast milk serves as an important pathway for PFAS exposure in infants, and breast milk concentrations may increase in association with maternal changes in diet and behavior during lactation (Lee et al., 2018). Thus, we may have misclassified exposure for some participants. However, breast milk PFAS concentrations are likely to be strongly correlated with maternal serum and cord blood serum, given the highly persistent nature of PFASs, and so any misclassification is likely to have been random and led to underestimation of the effects (Liu et al., 2011). However, exposure via breast milk and the period of lactation should be considered in future research. Third, given the small number of study participants, our study may have been underpowered to detect small associations. A larger future investigation will be required to confirm our results. Finally, we conducted numerous statistical tests during this study, without adjustment for inflation of the type-1 error rate, to maximize our ability to detect associations (Goldberg and Silbergeld, 2011). Therefore, a study with comprehensive exposure assessment in a larger sample is required to confirm our results.

5. Conclusions

We found an increased risk of reduction in protective antibodies against HFMD, especially in boys at three months of age. However, a larger and more comprehensive investigation will be necessary to confirm these findings. Given the growing epidemiological evidence and the widespread nature of PFAS exposure, the possibility for PFAS-associated immunosuppression in early life merits attention from public health policy researchers, policy makers, industry, and regulators that oversee manufacturing and usage of PFAS globally, especially in China.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.01.325.

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