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Modeled prenatal exposure to per- and polyfluoroalkyl substances in association with child autism spectrum disorder: a case-control study

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Abstract

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Author contributions

HS, DB, and IH conceived the study and oversaw its coordination. HS conducted data analyses and drafted the initial manuscript. DB and IH helped oversee the study. AC analyzed PFAS in maternal serum samples. DT advised on data analysis, interpretation and reporting. SO selected protocols and oversaw assessment of children for ASD. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The CHARGE study protocol and this study were approved by the institutional review boards for the State of California, the University of California-Davis (UC-Davis), and the University of Texas-Arlington (UT-Arlington). Participants provided written informed consent before collection of any data. The analysis of coded specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined by CDC not to constitute engagement in human subject research.

Availability of data and material

The part of datasets generated and analyzed during this study are publicly available in the National Institute of Mental Health (NIMH) Data Archive. The whole non-identifiable data are however available from the authors upon reasonable request and with permission from the IRBs at UT-Arlington and UC-Davis.

Competing interests

All authors declare they have no actual or potential competing financial interests.

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Competing interests

All authors declare they have no actual or potential competing financial interests.

Background/Objective: Per- and polyfluoroalkyl substances (PFAS) display neurobehavioral toxicity in laboratory animal studies. We examined associations of modeled prenatal maternal exposure to PFAS with child diagnosis of autism spectrum disorder (ASD).

Methods: Participants were 453 mother-child pairs from CHARGE (***CH***ildhood ***A***utism ***R***isk from ***G***enetics and ***E***nvironment), a population-based case-control study. Children underwent psychometric testing and were clinically confirmed for ASD (n = 239) or typical development (TD, n = 214). At the end of the clinic visit, maternal blood specimens were collected. We quantified nine PFAS in maternal serum samples collected when their child was 2 to 5 years old. As surrogate in utero exposure, we used a model built from external prospective data in pregnancy and 24 months post-partum and then reconstructed maternal PFAS serum concentrations during pregnancy in this case-control sample. We used logistic regression to evaluate associations of modeled prenatal maternal PFAS concentrations with child ASD.

Results: Modeled prenatal maternal perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) were borderline associated with increased odds of child diagnosis of ASD (per nanogram per milliliter increase: odds ratio [OR] = 1.46; 95% confidence interval [CI]: 0.98, 2.18 for PFHxS, OR = 1.03; 95% CI: 0.99, 1.08 for PFOS). When compared to the lowest quartile (reference category), the highest quartile of modeled prenatal maternal PFHxS was associated with increased odds of child diagnosis of ASD (OR = 1.95; 95% CI: 1.02, 3.72).

Conclusions: In analyses where modeled prenatal maternal PFAS serum concentrations served as in utero exposure, we observed that prenatal PFHxS and PFOS exposure, but not other PFAS, were associated with increased odds of child diagnosis of ASD. Further studies in which PFAS concentrations are prospectively measured in mothers and children at a range of developmental stages are needed to confirm these findings.

Keywords

autism spectrum disorder; case-control; exposure reconstruction; maternal serum; per- and polyfluoroalkyl substances

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic fluorine-containing compounds widely used in industrial and consumer products, including stain resistant coatings used on fabrics, carpets, paper products, and non-stick coatings on cookware [1]. Because of widespread use of PFAS-containing products, a number of PFAS have been detected in serum of most of the U.S. general population [2]. Although serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), the two most prevalent PFAS detected in the U.S. general population, showed a downward trend from 1999 to 2016 in the USA, concentrations of other PFAS might be increasing [3, 4]. Even after the reduction in U.S. production of PFOA and PFOS in early 2000s, they were detected in raw and finished public drinking water systems [5–7], in private wells near a chemical plant [8, 9], in effluents of wastewater treatment facilities [10], and in grazing fields [11]. In addition, U.S. drinking water near fire- or crash-training sites has been contaminated from the use of PFAS-containing firefighting foam [12, 13], suggesting that local residents are potentially exposed. Thus, exposure via drinking water and the food web is likely to continue.

Of particular concern, there is evidence that PFAS can cross the placenta as they are detected in cord blood [14–22] and amniotic fluid [23, 24]. Evidence also supports transfer to infants via breastfeeding [20, 25–28], a primary excretion route for lactating mothers and an exposure route for nursing infants [29, 30]. Maternal PFAS serum concentrations during pregnancy were weakly to moderately correlated with 3-year-old children's serum concentrations [31], whereas concurrent PFAS serum concentrations between child and mother from the same family were moderately correlated [32, 33]. PFAS have been shown to have potential neurobehavioral toxicity in studies with laboratory animals [34–41]. In humans, the associations between exposures to PFAS and neurodevelopmental or behavioral problems are still inconclusive [42–46]. In a cross-sectional study, an association was found for attention deficit hyperactivity disorder (ADHD) with children's serum concentrations of PFOS and PFOA [43], and perfluorohexane sulfonate (PFHxS) [44], suggesting the possibility of PFAS's neurobehavioral impact. In a prospective cohort study that used the Social Responsiveness Scale as a measure of autistic behaviors (higher scores indicate more autistic behaviors), maternal prenatal serum PFOS concentrations were associated with higher scores in boys but not in girls [42], suggesting that PFOS deserves additional scrutiny for associations with childhood autistic behaviors, and for sexually dimorphic effects more generally. Modeled in utero serum concentrations of PFOS and perfluorononanoate (PFNA) were associated with impairment of thyroid function among children (1–17 years of age) [47]. As thyroid hormone is critical in promoting fetal brain development [48], maternal thyroid dysfunction during pregnancy has been associated with language delay [49], delayed cognitive function [49, 50], and ADHD [51, 52].

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction and communication and by the presence of restricted, stereotyped interests, and repetitive behaviors [53]. Three studies examined prenatal exposure to PFAS in association with ASD [54, 55]. Liew et al. found no consistent evidence to suggest that prenatal maternal plasma PFAS concentrations were associated with increased risk of ASD in a Danish population [54]. Lyall et al. found that higher prenatal maternal serum PFOS and PFOA concentrations were associated with decreased risk of ASD in a Southern California population [55]. Long et al. found that higher maternal PFOS concentrations in amniotic fluid were associated with decreased risk of ASD in a Danish population [24]. Supplemental folic acid and vitamin intake near conception are known to protect against ASD development of certain environmental chemicals [56–60]. Maternal pre-pregnancy body mass index (BMI) and gestational weight gain are modifiable risk factors for ASD [61] or autistic-like traits [62]. However, none of the three studies adjusted for these factors in their models. Hence, additional investigations which both include unmeasured confounders in previous studies and consider an antagonistic interaction of PFAS with periconceptional supplemental folic acid and vitamin intake are needed.

Because ASD is rare (about 1 in 59 U.S. children) [63], the most efficient design to examine potential causal factors is through a large case-control study. However, blood samples that were collected at the time of enrollment (typically when the child is 2 to 5 years old) in a case-control study such as this present study may not capture true in utero exposure to PFAS. Changes in PFAS serum concentrations over time are relatively slow, as the half-life in the body for commonly detected PFAS (e.g., PFOA, PFOS, PFHxS, PFNA) is 2–7 years

[64, 65]. With advances in pharmacokinetic modeling of PFAS [66, 67] and known predictors of maternal serum PFAS concentrations during pregnancy and early childhood [31], we now have the opportunity to enhance retrospective exposure assessment during pregnancy in large case-control studies. In the present study, we reconstructed maternal PFAS serum concentrations at the time of pregnancy using a simple pharmacokinetic model and maternal blood samples collected when the child was 2 to 5 years old in a population-based case-control study. The goal of this study was to determine whether modeled prenatal PFAS serum concentrations were higher among mothers whose children were diagnosed with ASD as compared to mothers whose children were not diagnosed with ASD (controls).

2. Methods

2.1. Study population

CHARGE (***CH***ildhood ***A***utism ***R***isk from ***G***enetics and ***E***nvironment) is a population-based case-control study that has enrolled over 1800 index children and their families, with a goal to identify causes and contributing factors for autism [68]. Children with autism are primarily recruited from lists of children receiving services for autism through the California Department of Developmental Services, as well as from other studies, by self- or provider referrals and from various clinics. Children are eligible if they are between 2 and 5 years old, born in California, live with a biological parent who speaks either English or Spanish, and reside in the catchment areas of a specified list of California Regional Centers that coordinate services for persons with developmental disabilities [57]. The general population controls are identified from state birth files and are frequency matched to the expected age, sex, and Regional Center catchment area distribution of the autism cases. Details of study design, recruitment, eligibility, sample size, exposure data, and developmental diagnosis are available elsewhere [68].

Enrollment of CHARGE began in 2002, but CHARGE started collecting serum in 2009. For the present study, among all CHARGE participants who enrolled since 2009, we selected 450 mothers who (1) provided blood samples with sufficient volume of available serum for quantification of PFAS, and (2) had a child who completed the study with a final diagnosis of ASD or typical development (TD) by February 2017. One mother participated with twins, and another mother participated with triplets. Thus, a total of 453 children were included in the present study (see Figure S1 for a flow chart of participant and sample selection). Basic demographic information of the participants who were eligible for inclusion in the current study and ineligible is described in the supplemental material (see Table S1).

2.2. Child neurodevelopmental assessment

All children included in this study were administered the Mullen Scales of Early Learning (MSEL) to assess cognitive development [69, 70], and the Vineland Adaptive Behavior Scales (VABS) to assess adaptive function [71]. ASD diagnoses were confirmed by licensed clinical psychologists using two gold standard instruments: the Autism Diagnostic Interview-Revised (ADI-R) [72–74], and the Autism Diagnostic Observation Schedules-Generic (ADOS-G) [75]. Children in the general population group were screened for ASD symptoms using the Social Communication Questionnaire (SCQ) [76]. For those who scored

at 15 or above on the SCQ, both ADI-R and ADOS were administered to determine the final diagnosis for the CHARGE study classification. Final controls were typically developing (TD) children defined as those who scored at 70 or above on the MSEL *and* VABS, and 14 or below on the SCQ. After clinical assessments and application of diagnostic criteria of Risi et al. [77], 239 children and 214 children were classified into ASD and TD, respectively.

2.3. Serum sample collection

This present study lacked prenatal samples because of its case-control design. As surrogate in utero exposure, we used both model-based reconstructed estimates during pregnancy and concurrent (post-diagnosis) measurements of maternal serum PFAS concentrations. CHARGE collected blood samples from mothers at the time of study enrollment (when the child was 2–5 years of age). The mean age of the all children (both cases and controls) at the time of collection was just under four years (average ± Std. Dev.: 46.5 ± 9.5 months); the youngest and oldest children were 25 months and 61 months old, respectively. The mean ages of ASD and TD children, respectively, were 47.4 months (Std. Dev. = ± 9.6 months) and 45.4 months (Std. Dev. = ± 9.4 months). Whole blood was drawn into a red-top vacutainer, then centrifuged, and stored in an amber vial in a −80°C freezer within 24 hours. Later, the stored serum was aliquoted in polypropylene cryovials and then stored in a −80°C freezer until analysis.

2.4. Serum PFAS quantification

We shipped 0.5 mL serum aliquots to the Centers for Disease Control and Prevention (CDC) for analysis. At CDC, we quantified the serum concentrations of nine PFAS using online solid-phase extraction coupled to reversed-phase high-performance liquid chromatography-isotope dilution tandem mass spectrometry as described elsewhere [78]. The analytical measurements followed strict quality control/quality assurance protocols, including participation in quality assessment schemes to demonstrate the method accuracy and precision, as required by the Clinical Laboratory Improvement Act of 1988 (CLIA ‘88) certification. Furthermore, along with study samples, each analytical run included spiked quality control materials and reagent blanks. In addition to study samples, we analyzed 38 duplicates for quality assurance. Depending on the analyte and concentration, median relative standard deviations for 19 pairs of blind duplicates ranged from 0 to 6%, except for PFHxS (17%) and perfluorodecanoate (20%; PFDA, also known as PFDeA).

The nine PFAS quantified were: PFHxS, PFOS, PFOA, PFNA, PFDA, perfluoroundecanoate (PFUA), perfluorododecanoate (PFDOA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (Me-FOSAA, also known as Me-PFOSA-ACOH), and 2-(N-ethyl-perfluorooctane sulfonamido) acetate (Et-FOSAA, also known as Et-PFOSA-ACOH). The limit of detection (LOD) was 0.1 ng/mL (nanograms per milliliter) for all PFAS.

2.5. Retrospective exposure assessment

Because the measured maternal PFAS serum concentrations collected when the child was 2 to 5 years old may not capture true in utero exposure to PFAS, we reconstructed maternal PFAS serum concentrations during pregnancy using a simple pharmacokinetic model and the following assumptions. First, we assumed that sources of exposure were fairly invariant

between pregnancy and sample collection (e.g., cookware is not replaced frequently, changes in diet are slow, drinking water source was not changed) but that the degree of PFAS exposure varies over time due to the observed temporal trends of PFAS exposure in the general U.S. population [3, 4]. Second, we assumed that compared to placental and lactational transfers, the contributions of other exposure modifiers to the measured maternal serum concentrations at the time of collection are small. Third, we assumed that the rates of placental and lactational transfers, the contributions of other exposure modifiers to the measured concentrations, changes of PFAS exposure over time, maternal elimination half-lives of PFAS, and other physiologic parameters (e.g., volume of distribution) are non-differential with respect to case-control status.

We performed multiple regression on the measured maternal PFAS serum concentrations as a dependent variable, and then obtained regression coefficients (β) of the measured maternal PFAS concentrations for each exposure modifier (i.e., breastfeeding duration, number of livebirths after the delivery of the index (i.e., participating) child, sampling year). Then, we used the following equation after rearranging a simple pharmacokinetic model used in our previous study [79].

$$C_{i,j,pre} = C_{i,j,post} - \beta_{i,time} \cdot T_j - \beta_{i,BF} \cdot BF_j - \beta_{i,delivery} \cdot Delivery_j$$

where $C_{i,j,pre}$ is the reconstructed (or modeled) maternal serum concentration of individual compound i for mother j during pregnancy ("pre"), $C_{i,j,post}$ is the measured maternal serum concentration of individual compound i for mother j when their child was 2 to 5 years old ("post"), $\beta_{i,time}$ is the percent change of individual compound i per one year over the study period, T_j is the child's age of the mother j to account for the time difference between delivery and blood sample collection at the time of the child's visit, $\beta_{i,BF}$ is the percent change of individual compound i per month of breastfeeding, BF_j is the total breastfeeding duration of mother j for the index child and younger siblings before sample collection, $\beta_{i,delivery}$ is the percent change of individual compound i per one livebirth delivery, and $Delivery_j$ is the number of livebirths after the delivery of the index child for mother j. Among 450 mothers included in the current study, 128 mothers delivered one younger sibling of the index child and 11 mothers delivered two younger siblings of the index child. The mean breastfeeding duration of younger siblings was 8.2 months (Std. Dev. = ± 6.0 months) and the mean breastfeeding duration of the index child was 8.4 months (Std. Dev. = ± 8.4 months). The values of $\beta_{i,time}$, $\beta_{i,BF}$, and $\beta_{i,delivery}$ used in the above model are summarized in Table S2.

In order to evaluate the performance of the above model, we relied on prospectively collected specimens from another study, MARBLES (***M***arkers of ***A***utism ***R***isk in ***B***abies - ***L***earning ***E***arly ***S***igns), which enrolled women during pregnancy and followed them longitudinally [80]. These mothers provided blood serum samples both during pregnancy and when their child was 2 years old (n = 40 pairs). For the four PFAS detected in more than 95% of the samples (i.e., PFOA, PFOS, PFHxS, PFNA), Spearman's correlation coefficients between the measured and the modeled concentrations (both during pregnancy) were 0.63 for PFOA, 0.78 for PFOS, 0.75 for PFHxS, and 0.76 for PFNA (see Figure S2).

2.6. Statistical analysis

For congeners with ≥ 90% detection frequency, we assigned a value of LOD divided by the square root of 2 [81]. For congeners with < 90% detection frequency (i.e., PFDA, PFUA, and Me-FOSAA), we conducted multiple imputation using the 'mi impute chained' command in STATA to replace the values below the LOD [82, 83]. We generated 10 simulated complete datasets via imputation.

We computed univariate statistics to compare the ASD and TD groups by basic population characteristics. Among those with a final diagnosis of ASD or TD, we also compared the same univariate statistics of populations who were eligible for inclusion in this study and who were ineligible to examine differences in characteristics between two populations. We compared median serum PFAS concentrations of the two diagnostic groups. To examine the difference in PFAS exposure between our study participants (California mothers with a young child) and the U.S. general female population (12 years of age and older), we also compared median serum PFAS concentrations of our study population to those reported in the National Health and Nutrition Examination Survey (NHANES) restricted to the female population.

To prepare for logistic regression, we initially selected confounders *a priori* based on a directed acyclic graph (DAG) and the risk and protective factors for ASD obtained from a literature review (see Figure S3 for DAG). In addition to the selected confounders, we also adjusted for CHARGE case-control study frequency matching factors (i.e., child's age and sex, recruitment regional center). Covariates adjusted in the final model included child's age at the assessment (in months) and sex (boy, girl), child's birth year (2004–2006, 2007–2009, 2010–2013), recruitment regional center (Alta, North Bay, East Bay, Valley Mountain), parity (1, >1), gestational age at delivery (preterm, full term), maternal race/ethnicity (white, Hispanic, others), maternal birthplace (USA, Mexico, other), mother's age at delivery (<25, between 25 and 30, between 30 and 35, >35), maternal pre-pregnancy BMI (underweight/ normal weight, overweight, obese), periconceptional maternal vitamin intake (yes, no), breastfeeding duration (in months), and homeownership (yes, no) as an indicator of socioeconomic status. We adjusted for child's birth year because some of the PFAS concentrations showed upward or downward trends in the U.S. general population [3, 4] during our sample collection period (see Figure S4), and because the recruitment ratio for cases vs. controls tended to vary over the period as a result of artifacts related to variation in our access to updated State files (Table 1). To robustly control confounding by child's birth year, we grouped birth years (2004–2006, 2007–2009, 2010–2013) and then included the categorized variable in the model. Parents' education was not included given (1) that it was not associated with exposures to PFAS; (2) that much of the recent literature does not show it to be a predictor of ASD diagnoses, particularly since the American Academy of Pediatrics began to recommend universal screening for ASD; and (3) that our model already includes homeownership, which is associated with PFAS, as well as other variables that tend to correlate with SES, including maternal birthplace, race/ethnicity, prenatal vitamin intake, and maternal age. For two mothers with multiple children participating in the study, we adjusted for within-family correlations in the regression models by using clustered sandwich variance estimators (https://www.stata.com/manuals13/xtvce_options.pdf).

To examine the association of modeled prenatal maternal serum PFAS concentrations with the odds of ASD in the children, we fit two main logistic regression models to obtain adjusted odds ratios (OR) and 95% confidence interval (CI). The first main model includes the selected covariates and one individual PFAS compound in one model as a predictor (Model 1). The second main model includes the selected covariates and all seven PFAS (excluding Et-FOSAA and PFDOA) in one model as individual predictors (Model 2). For each of the two main models, we fit three different sub-models for each of the individual PFAS using 1) ln-transformed PFAS concentrations (to normalize skewed distributions of PFAS concentrations), which assumes the log of the OR increases exponentially in relation to the PFAS concentration; 2) PFAS concentrations with no transformation, which assumes the log of the OR increases linearly in relation to the PFAS concentration; and 3) categorized PFAS concentrations, which assumes a single OR for each category relative to the referent group. For four compounds detected in over 95% of the samples, these categories were based on quartiles of the distribution of PFAS concentrations in controls. For three compounds detected in 35% to 70% of the samples (i.e., PFUA, PFDA, Me-FOSAA), we dichotomized concentrations into detectable and non-detectable, and then conducted the same statistical analyses as with continuous concentrations. For Et-FOSAA and PFDOA with 1% and 3% detection frequency, respectively, we did not perform statistical analysis. We reported ORs for a one-unit increase in the ln-transformed PFAS concentrations (ng/mL) and for a one-unit increase in PFAS concentrations with no transformation (ng/mL).

We calculated Spearman's correlation coefficients (r_{sp}) among PFAS concentrations. To determine whether any single maternal PFAS was associated with child diagnosis of ASD, we first calculated variance inflation factors (VIFs) to check the degree of multicollinearity. After confirming that VIFs were low [84] and did not affect ORs and confidence intervals, we constructed a "multi-PFAS" model by including all seven PFAS (excluding Et-FOSAA and PFDOA) in one model as individual predictors [54]. To determine whether there are additive effects on odds of having a child with ASD from exposure to PFAS mixtures, we also fit a model using the molar sum of the seven PFAS (nmol/mL) in the model, instead of individual PFAS concentrations. As sensitivity analyses, we ran a series of additional models by further adjusting for maximum parental education (less than college degree, bachelor's degree, graduate degree) and maternal folic acid intake (μg/day) during the month before pregnancy and during the first month of pregnancy in the final model, or by excluding breastfeeding duration (in months) in the final model. We also ran the main models using the measured maternal PFAS serum concentrations collected when their child was 2 to 5 years old.

Previous work described biologic plausibility and evidence for interactions of other environmental chemicals with sex and (separately) with nutrient supplements [57, 85–91]. Thus, in our regression models fit to the full sample, we examined potential effect modification by child's sex (boy, girl) and by self-reported maternal vitamin intake during the month before pregnancy and the first month of pregnancy (yes, no). We compared stratified estimates and examined the p-value for the interaction product terms between individual PFAS concentrations and the potential effect modifier (i.e., PFA×sex or PFAS×prenatal vitamin intake).

An alpha of 0.05 was used as the criterion for statistical significance. All statistical analyses were performed using STATA/MP version 15.1 (StataCorp LLC, College Station, TX, USA) and the R programming language version 3.6.1. (R Core Team, 2019) through RStudio version 1.2.1355 (RStudio, Inc., 2010).

3. Results

3.1. Population characteristics

The male to female ratio among ASD children was 3.7 (Table 1). Among TD children, the sex ratio was high as a consequence of matching controls by the expected sex ratio, but differed because relatively more boys than girls had an available serum sample (4.8 among TD). The ASD case group differed from the TD control group on many characteristics. Fewer TD children were recruited in later study years; while more TD children were born from non-Hispanic white mothers (70% vs. 50% with an ASD diagnosis). Half the children with an ASD diagnosis were the firstborn child, compared to about 1/3rd of TD children. Mothers of children with an ASD diagnosis were less likely to own a home (54%), compared to mothers of TD children (69%). More ASD children were born premature, or had a mother born outside the USA and Mexico. They were also breastfed for a shorter duration (6.5 months) than TD children (10.5 months).

We assessed differences between the study sample and those excluded because they lacked a serum sample or had insufficient volume of serum. Except for child's diagnosis and birth year, there was no difference for other population characteristics between mother-child pairs who were eligible for inclusion in the current study (n = 453) and those who were otherwise eligible but lacked the needed serum (n = 99) (see Table S1).

3.2. Maternal PFAS serum concentrations

The detection frequency of PFOA, PFOS, PFHxS, and PFNA was 100%, 100%, 98%, and 95%, respectively; the other five PFAS were detected in fewer than 70% of the samples (Table 2). The highest median was observed for PFOS (3.20 ng/mL), followed by PFOA (1.07 ng/mL), PFHxS (0.50 ng/mL), and PFNA (0.50 ng/mL). Median concentrations for all PFAS were not different between the ASD and the TD groups (p-value > 0.05). When restricting our comparison to the same study period as NHANES (i.e., 2009–2010) and PFAS with equal to or over 95% of detection frequency (i.e., PFOA, PFOS, PFHxS, PFNA), median concentrations were approximately 50% lower than those in the U.S. general female population reported in NHANES [92]. Median maternal serum PFAS concentrations differed by several characteristics of mothers or children. Child's birth year, mother's race/ethnicity, parity, number of livebirths after delivery of the index child, maternal birthplace, and breastfeeding duration were associated with concentrations for at least two of the five PFAS detected in more than 65% of the samples (see Table S3). Pairwise correlations among PFAS were generally positive. For the four PFAS detected in more than 95% of the samples (i.e., PFOA, PFOS, PFHxS, PFNA), concentrations were moderately correlated with each other (r_{sp} = 0.40 to 0.69) (see Table S4).

3.3. Associations between modeled prenatal maternal PFAS concentrations and child diagnosis of ASD

After adjusting for the CHARGE case-control study frequency matching factors (child's age and sex, and regional recruitment center) and child's birth year, parity, homeownership, maternal birthplace, breastfeeding duration, and gestational age at delivery, maternal race/ethnicity, mother's age at delivery, periconceptional vitamin intake, and maternal pre-pregnancy BMI (Model 1, Table 3), modeled prenatal maternal PFHxS was associated with increased odds of child diagnosis of ASD (per natural-log nanogram per milliliter increase: OR = 1.36; 95% CI: 0.96, 1.93). When the seven PFAS detected in more than 35% of the samples (i.e., PFOA, PFOS, PFHxS, PFNA, PFDA, PFUA, Me-FOSAA) were simultaneously included in the model as individual predictors (Model 2), modeled prenatal maternal PFHxS was associated with increased odds of child diagnosis of ASD (OR = 1.81; 95% CI: 1.04, 3.13). When using PFAS concentrations with no transformation in the model, modeled prenatal maternal PFHxS and PFOS were associated with increased odds of child diagnosis of ASD (per nanogram per milliliter increase: OR = 1.46; 95% CI: 0.98, 2.18 for PFHxS, OR = 1.03; 95% CI: 0.99, 1.08 for PFOS). In Model 2, none of the maternal PFAS serum concentrations were associated with odds of having a child with ASD (all p-values > 0.05). When the molar sum of the seven PFAS was included in the model, associations of prenatal maternal PFAS with child diagnosis of ASD were null for both ln-transformed PFAS concentrations and those with no transformation. Additional adjustment for maximum parental education and maternal folic acid intake during the month before pregnancy and during the first month of pregnancy and exclusion of breastfeeding duration in the model led to similar results (see Table S5).

When analyses were stratified by child's sex, among boys (n = 365; TD = 177, ASD = 188), modeled prenatal maternal PFOS was associated with increased odds of child diagnosis of ASD in concentrations with no transformation (OR = 1.05; 95% CI: 1.00, 1.10, see Table S6). Among girls (n = 88; TD = 37, ASD = 51), prenatal maternal PFNA was associated with decreased odds of child ASD in both ln-transformed concentrations and those without transformation (see Table S6 for ORs and 95% CI; p-value for interaction = 0.03 for both exposure measures), however the confidence interval ratios were 7.0 and 35, respectively, indicating very low stability of this estimate. When stratified by maternal prenatal vitamin intake before pregnancy and during the first month of pregnancy, associations with child ASD were null for all maternal (ln-transformed) PFAS concentrations for both mothers who took and those who did not take prenatal vitamins, and p-values for interaction were larger than 0.35 for all PFAS. When analyzing with PFAS concentrations with no transformation, among mothers who took prenatal vitamins, modeled prenatal maternal PFOS was associated with increased odds of child diagnosis of ASD (per nanogram per milliliter increase: OR = 1.03; 95% CI: 0.97, 1.10), but p-values for interaction were larger than 0.60 for all PFAS.

When concentrations were categorized into quartiles and compared to the lowest quartile (reference category, see Table 4), the highest quartile of maternal PFHxS was associated with increased odds of child diagnosis of ASD after adjusting for all covariates in Model 1 (OR = 1.95; 95% CI: 1.02, 3.72). When all maternal PFAS were simultaneously included as

individual predictors in the model (Model 2), we observed stronger positive associations with child diagnosis of ASD in the highest quartile of maternal PFHxS (OR = 2.65; 95% CI: 1.09, 6.38), compared to Model 1. We observed a non-monotonic trend (p-trend = 0.06) for PFHxS in both Model 1 and Model 2. Associations for other PFAS concentrations were null. For PFDA, PFUA and Me-FOSAA that were dichotomized as detectable vs non-detectable, associations with child diagnosis of ASD were null. Excluding breastfeeding duration in the model led to similar results (Table S7)

3.4. Associations between measured postnatal maternal PFAS concentrations and child diagnosis of ASD

When repeating the analysis by using measured maternal serum concentrations collected when their child was 2 to 5 years old, postnatal maternal PFHxS was associated with increased odds of child ASD (per natural-log nanogram per milliliter increase: OR = 1.42; 95% CI: 1.00, 2.03 for Model 1, OR = 1.74; 95% CI: 0.99, 3.05 for Model 2 in Table S8) after adjusting for the same selected covariates. When using PFAS concentrations with no transformation in the model, modeled prenatal maternal PFOS was associated with increased odds of child diagnosis of ASD (per nanogram per milliliter increase: OR = 1.05; 95% CI: 0.97, 1.13). When compared to the lowest quartile (see Table S9), none of the maternal PFAS serum concentrations were associated with odds of having a child with ASD in both Model 1 and Model 2.

4. Discussion

As surrogate in utero exposure, we used both model-based reconstructed estimates and concurrent (post-diagnosis) measurements of maternal serum PFAS concentrations. We also fit three different models for each of the individual PFAS using 1) ln-transformed PFAS concentrations; 2) PFAS concentrations with no transformation; and 3) categorized PFAS concentrations. Results from the regression analyses supported our hypothesis that exposure to PFHxS and PFOS were higher for mothers whose children were diagnosed with ASD as compared to mothers whose children were unaffected. When modeled prenatal concentrations were categorized into quartiles, we observed child diagnosis of ASD to be associated with modeled prenatal maternal PFHxS concentrations in the highest quartile, compared to the lowest quartile. Results for other seven PFAS considered in this study did not meet the *a priori* selected cut-point of statistical significance. When analyses were stratified by child's sex, among girls, higher prenatal maternal PFNA was associated with decreased odds of child ASD. Among boys, higher prenatal maternal PFOS was associated with increased odds of child ASD. We did not observe heterogeneity in the associations of maternal PFAS concentrations with child diagnosis of ASD when analyses were stratified by periconceptional prenatal vitamin intake. In the "multi-PFAS" model, when using ln-transformed maternal serum PFAS concentrations, ASD diagnoses were increased with higher modeled prenatal maternal PFHxS. In addition, when concentrations were categorized into quartiles, ASD diagnoses were increased in the highest (vs. lowest) quartile of modeled prenatal maternal PFHxS, with a non-monotonic trend.

When using the measured maternal serum PFAS concentrations 2 to 5 years post-partum, we observed similar results with modeled prenatal serum concentrations. From the analysis with ln-transformed PFAS concentrations, we observed mothers of children with ASD to have higher measured postnatal maternal PFHxS. From the analysis with PFAS concentrations with no transformation, we observed mothers of children with ASD to have higher measured postnatal maternal PFOS. Among four compounds analyzed in quartiles, only PFOS showed a linear dose-response relationship (Figure S5). Thus, we observed that maternal PFOS was associated with increased odds of child ASD in linear models (Table 3, Table S5, Table S6, Table S8).

As a starting point to estimate in utero exposure to PFAS, we selected mother's rather than child's blood collected at enrollment for several reasons. First, the behaviors of TD children differ from those of ASD children. For example, those with ASD often choose to eat a much less variable diet, and have a restricted repertoire of behaviors or activities that they engage in. These could result in different exposures to the target compounds postnatally as opposed to exposures during the fetal and infant periods [93]. Second, large variability in child's growth and development between delivery and enrollment might result in highly variable PFAS clearance and accumulation in their body. Third, Kingsley et al. reported that the correlation coefficients between cord blood and 3 year-old child's serum samples were 0.21 for PFOS, 0.45 for PFOA, and 0.46 for PFHxS [31]. These low to moderate correlations may be at least partially a result of not taking into account breastfeeding or other exposure-related factors that would influence the child's blood PFAS levels during the three-year period after birth.

Two previous epidemiologic studies examined prenatal exposure to PFAS in association with ASD using maternal blood collected in early or mid-pregnancy [54, 55]. Liew et al. did not find evidence to suggest that prenatal PFAS exposure increased the risk of ASD in children [54], and Lyall et al. found higher PFOA and PFOS were associated with decreased risk of ASD [55]. Possible reasons for inconsistent results among ours and these other two studies include differences in measures of exposure, characteristics of study populations, and methods of identification or confirmation of ASD cases. First, compared to the modeled concentrations reconstructed from serum samples of mothers collected when their child was 2 to 5 years old used in the current study, Liew et al. used measured concentrations in maternal plasma collected mostly during the first trimester and Lyall et al. used maternal sera collected at 15 to 19-week gestational age (mid-pregnancy). Second, Liew et al. recruited pregnant mothers who enrolled during 1996–2002 in a Danish National Birth Cohort (DNBC), Lyall et al. recruited mothers who delivered a live-born infant during 2000–2003 in Southern California from a population-based case-control study, and the current study used mothers who enrolled in a population-based case-control study during 2009–2017 in Northern California when their child was 2 to 5 years old. Compared to the coefficient of variation (CV) of measured PFAS concentrations in the current study (CV for PFOA = 0.75, PFOS = 0.75, PFHxS = 0.85), CVs among the selected DNBC participants in Liew et al. were 0.47 for PFOA, 0.40 for PFOS, and 0.45 for PFHxS [2], suggesting that smaller variability in PFAS concentrations in the Danish population might result in reduced statistical power for the Liew et al. study. Third, the current study used clinically confirmed final diagnosis (case = 239; control = 214) and Liew et al. identified children who were

clinically diagnosed with ASD through the Danish Psychiatric Central Registry (case = 220; control = 550). By contrast, Lyall et al. did not rely on clinical diagnosis but identified children using information recorded in local developmental service agency records (case = 553; control = 433). Thus, the potential differences in diagnostic criteria between two countries and sources of ASD cases among three studies may affect the comparability of results. In addition to the above two studies, Long et al. [24] also examined prenatal exposure to PFAS in association with ASD using PFAS concentrations in amniotic fluid and found that higher maternal PFOS was associated with decreased risk of ASD. The results from Long et al. should be interpreted with caution because PFOS was detected in 46.6% of the samples.

In our study, duration of breastfeeding was four months longer among children with TD diagnosis than ASD children (see Table 1). The putative benefit of breastfeeding on reducing ASD risk has not yet been established. Some studies reported no association between breastfeeding and ASD risk [94–96]. Other studies found that mothers who breastfed longer were less likely to have children with ASD diagnosis or autistic traits [97–99]. However, the association could reflect reverse causation if infants who develop ASD were more likely to have gastrointestinal or other problems leading to earlier discontinuation of breastfeeding [100]. Further studies are needed to tease out any causal relationship and its direction. Nevertheless, because breastmilk is a known strong contributor to early life PFAS exposure, adjustment for breastfeeding was needed in order to control for confounding from postnatal exposures, as we sought to examine prenatal exposures as a risk factor for ASD.

Breastfeeding is an important elimination route of PFAS for lactating mothers and an exposure route for nursing infants [29, 30]. At least in part because of the elimination of PFAS via breastfeeding, median concentrations of PFOA, PFOS, PFHxS, and PFNA in our population were approximately 50% lower than those in the U.S. general female population that includes girls 12 years old and older. We also found that maternal concentrations for five mostly detected PFAS decreased at a rate ranging from 1% to 2% per month of breastfeeding (p-value < 0.02, see Table S2). Therefore, mothers who breastfeed longer would have lower PFAS concentrations while children who are breastfed longer likely have experienced higher postnatal exposure to PFAS.

In spite of the marked difference in breastfeeding duration and parity between two diagnostic groups (Table 1), median maternal concentrations for all PFAS were not different between two groups (p-value > 0.30, Table 2). This seemingly paradoxical finding is because a relatively large portion of mothers with TD children (73%) were recruited in early study years when PFAS concentrations were relatively high, compared to mothers with ASD children (43%). Note that PFOA, PFOS, PFHxS, and Me-FOSAA concentrations showed downward trends over our study period (see Figure S4). In the current study, breastfeeding information was missing for only 2% of the participants. However, because our case-control study relied on breastfeeding information collected at the time of study enrollment when the child was 2 to 5 years old, there is a potential for errors in recall, which might have been differential by child's diagnosis. Thus, our findings need to be interpreted with caution.

A major strength of this study is the clinically confirmed diagnostic classification (ASD, TD) based on gold standard diagnostic assessments in a case-control study which aims to uncover a broad array of factors contributing to autism and developmental delay [68]. Another major strength was the wide array of potential confounders measured. However, some limitations should be noted. Whereas our population-based sampling of both cases and controls would increase generalizability, the case-control design requires enrollment after the earliest ages for a reliable diagnosis of ASD (generally in the 3rd or 4th year of life); this in turn meant that no biospecimens are available during pregnancy or breastfeeding. To circumvent this substantial limitation, we developed a model to estimate the maternal PFAS concentrations during pregnancy. As with any model, its accuracy depends on the quality of the model inputs, and the degree to which the assumptions are supported, which may in fact vary by individual, or be influenced by case-control status of the child. Thus, the model may not accurately capture true in utero exposure to PFAS, and inaccuracies might be differential by diagnosis of the child, and thus our findings need to be interpreted with caution.

5. Conclusions

In this case-control study, we observed that both model-based reconstructed estimates and concurrent (post-diagnosis) measurements of maternal PFHxS and PFOS serum concentrations were associated with increased odds of child diagnosis of ASD. To effectively use resources available in a valid and well-characterized case-control study for additional investigations of prenatal exposure to PFAS in association with ASD, we reconstructed maternal PFAS concentrations during pregnancy using measured concentrations collected a few years after delivery. The stronger associations of maternal PFAS measurements taken concurrently when the child was observed in the clinic at ages 2–5 years suggest that postnatal exposures may have influenced the child's brain development. Given that the volume of PFAS exposure for breastfed children would be larger with longer breastfeeding duration, further studies using exposure measures prospectively collected from mothers and children during prenatal and postnatal periods are needed to confirm and extend these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

ADHD
attention deficit hyperactivity disorder

ADOS
Autism Diagnostic Observation Schedules

ASD
Autism spectrum disorder

BMI
body mass index

CDC
Centers for Disease Control and Prevention

CHARGE
*CH*ildhood *A*utism *R*isk from *G*enetics and *E*nvironment

CI
confidence interval

DAG
directed acyclic graph

DQ
developmental quotient

Et-FOSAA or Et-PFOSA-ACOH
2-(N-ethyl-perfluorooctane sulfonamido) acetate

LOD
limit of detection

Me-FOSAA or Me-PFOSA-ACOH
2-(N-methyl-perfluorooctane sulfonamido) acetate

MSEL
Mullen Scales of Early Learning

NHANES
National Health and Nutrition Examination Survey

OR
odds ratio

PFDA or PFDeA
perfluorodecanoate

PFDOA
perfluorododecanoate

PFHxS
perfluorohexane sulfonate

PFNA
perfluorononanoate

PFOA
perfluorooctanoate

PFOS
perfluorooctane sulfonate

PFUA
perfluoroundecanoate

SD
standard deviation

TD
typical development

VABS
Vineland Adaptive Behavior Scales

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- Nine PFAS were quantified in maternal serum samples collected when their child was 2 to 5 years old
- PFHxS and PFOS were associated with increased odds of child diagnosis of ASD
- Associations for other PFAS were not statistically significant
- Observed a linear dose-response relationship for PFOS

Table 1.

Characteristics of participants (n = 453 mother-child pairs) included in the present study.

Characteristics	Total	TD		ASD		ASD versus TD [a]
	n	*n*	%	*n*	%	
Child's sex						0.28
Girl	88	37	17%	51	21%	
Boy	365	177	83%	188	79%	
Birth year						<0.001
2004–2006	118	60	28%	58	24%	
2007–2009	181	111	52%	70	29%	
2010–2013	154	43	20%	111	46%	
Child's race/ethnicity						0.049
White (non-Hispanic)	218	116	54%	102	43%	
Hispanic	126	53	25%	73	31%	
Other [b]	109	45	21%	64	27%	
Mother's race/ethnicity						<0.001
White (non-Hispanic)	270	150	70%	120	50%	
Hispanic	113	43	20%	70	29%	
Other [b]	70	21	10%	49	21%	
Gestational age at delivery						0.009
≤ 37 weeks	45	13	6%	32	13%	
> 37 weeks	399	199	93%	200	84%	
Maternal pre-pregnancy BMI						0.55
Normal/underweight	240	118	55%	122	51%	
Overweight	104	48	22%	56	23%	
Obese	101	46	21%	55	23%	
Maximum parental education						0.78
Less than college degree	192	87	50%	120	56%	
Bachelor's degree	164	80	36%	70	30%	
Graduate or professional degree	97	47	14%	49	13%	
Prenatal vitamin intake during the month before pregnancy and in the first month of pregnancy						0.40
Yes	285	138	64%	147	62%	
No	130	62	29%	68	28%	
Homeownership						0.007
Yes	277	147	69%	130	54%	
No	159	60	28%	99	41%	
Maternal birthplace						0.014
In the USA	354	180	84%	174	73%	
In Mexico	30	11	5%	19	8%	
Outside USA or Mexico	69	23	11%	46	19%	
Parity						<0.001

Characteristics	Total	TD		ASD		ASD versus TD [a]
	n	*n*	%	*n*	%	
1	189	69	32%	120	50%	
>1	251	140	65%	111	46%	
Regional center/Region						0.008
Alta, Far Northern	242	120	56%	126	53%	
North Bay	64	39	18%	23	10%	
East Bay	32	14	7%	18	8%	
Valley Mountain	115	41	19%	72	30%	
Health Insurance type at delivery						0.28
Government Program/No Insurance	95	38	18%	57	24%	
Private Insurance	340	167	78%	173	72%	
		Mean	**SD**	**Mean**	**SD**	
Child's age at assessment (months)		45.4	9.4	47.4	9.6	0.01
Mother's age at delivery (years)		30.7	5.7	30.0	5.8	0.21
Breastfeeding duration (months)		10.5	8.7	6.5	7.6	<0.001
Folic acid intake (μg/day)		641.5	545.1	568.4	413.7	0.20

[a] P-value from the Pearson's chi-squared test for categorical variables and Mann-Whitney test for continuous variables.

[b] Includes Black, Asian, and multiracial.

- The missing values for gestational age at delivery, maternal pre-pregnancy BMI, prenatal vitamin use, homeownership, parity, health insurance type at delivery, and breastfeeding duration are about 4%, 4%, 17%, 7%, 5%, 8%, and 5%, respectively.

Abbreviation: autism spectrum disorder (ASD), body mass index (BMI), standard deviation (SD), typically development (TD).

Table 2.

Distribution of maternal PFAS serum concentrations (ng/mL) collected from 453 CHARGE mother-child pairs.

			All participants (*n* = 453)			TD (*n* = 214)			ASD (*n* = 239)			ASD versus TD [a]	2009–2010 median [b]	
			Percentiles			Percentiles			Percentiles					
	LOD (ng/mL)	% detect	5	50	95	5	50	95	5	50	95			NHANES median [c]
PFOA	0.1	100	0.37	1.07	3.43	0.37	1.15	3.43	0.37	1.07	3.40	0.65	1.47	2.69
PFOS	0.1	100	1.10	3.20	9.82	1.20	3.30	9.58	1.08	3.10	10.03	0.86	4.40	7.80
PFHxS	0.1	98	0.20	0.50	1.50	0.12	0.40	1.18	0.20	0.50	1.63	0.30	0.60	1.30
PFNA	0.1	95	<LOD	0.50	1.26	<LOD	0.50	1.28	<LOD	0.50	1.23	0.43	0.50	1.15
PFDA	0.1	68	<LOD	0.20	0.50	<LOD	0.20	0.50	<LOD	0.20	0.50	0.77	<LOD	0.30
PFUA	0.1	36	<LOD	<LOD	0.30	<LOD	<LOD	0.30	<LOD	<LOD	0.30	0.66	<LOD	0.20
PFDOA	0.1	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD
Me-FOSAA	0.1	46	<LOD	<LOD	0.60	<LOD	<LOD	0.50	<LOD	<LOD	0.70	0.54	0.20	0.20
Et-FOSAA	0.1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	-	<LOD	<LOD

[a] P-value from the Wilcoxon rank-sum test of the null hypothesis that a randomly chosen value from members of one group is as likely to be greater than as to be less than a randomly chosen value from members of the comparison group.

[b] Median concentrations of the mothers included in the current study who provided blood samples during 2009–2010 (*n* = 139; ASD = 60; TD = 79).

[c] Median concentrations of the female population (12 years of age and older) reported in the 2009–2010 NHANES (*n* =1,158) [2].

Abbreviation: autism spectrum disorder (ASD), limit of detection (LOD), perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), perfluorodecanoate (PFDA or PFDeA), perfluoroundecanoate (PFUA), perfluorododecanoate (PFDOA), 2-(N-ethyl-perfluorooctane sulfonamido) acetate (Et-FOSAA or Et-PFOSA-AcOH), 2-(N-methyl-perfluorooctane sulfonamido) acetate (Me-FOSAA or Me-PFOSA-AcOH), typical development (TD).

Table 3.

Adjusted odds ratios (OR; 95% CI) of child diagnosis of ASD (n = 239) versus TD (n = 214) in relation to the reconstructed maternal prenatal serum PFAS concentrations, with and without ln-transformation, the CHARGE Study, 2009–2017.

	ln-transformed PFAS		linear scale for PFAS	
Concentration (ng/mL) [a]	**Model 1 OR** [b] **(95% CI)**	**Model 2 OR** [c] **(95% CI)**	**Model 1 OR** [b] **(95% CI)**	**Model 2 OR** [c] **(95% CI)**
PFOA	0.94 (0.59, 1.49)	0.66 (0.32, 1.37)	1.01 (0.89, 1.14)	1.00 (0.81, 1.23)
PFOS	1.18 (0.77, 1.80)	0.90 (0.38, 2.12)	1.03 (0.99, 1.08)#	1.01 (0.92, 1.12)
PFHxS	1.36 (0.96, 1.93)#	1.81 (1.04, 3.13)*	1.46 (0.98, 2.18)#	1.49 (0.85, 2.61)
PFNA	0.90 (0.66, 1.23)	0.87 (0.56, 1.35)	0.81 (0.51, 1.26)	0.64 (0.31, 1.32)
PFDA	0.94 (0.52, 1.69)	1.59 (0.71, 3.56)	0.93 (0.23, 3.75)	0.95 (0.10, 8.40)
PFUA	0.61 (0.20, 1.90)	0.47 (0.10, 2.08)	1.45 (0.07, 28.0)	1.41 (0.05, 38.3)
Me-FOSAA	1.17 (0.71, 1.93)	1.11 (0.53, 2.31)	1.31 (0.62, 2.74)	1.10 (0.42, 2.86)
ΣPFAS [d]	1.29 (0.76, 2.20)	Not modeled	Not modeled	Not modeled

[a] PFDOA and Et-FOSAA were excluded in this table because fewer than 3% of the samples had detectable concentrations.

[b] Adjusted for CHARGE case-control study frequency matching factors (child's age and sex, regional center) and child's birth year, parity, gestational age at delivery, maternal race/ethnicity, maternal birthplace, mother's age at delivery, maternal pre-pregnancy body mass index, periconceptional maternal vitamin intake, homeownership, and breastfeeding duration.

[c] Additionally adjusted by including all seven PFAS (PFOA, PFOS, PFHxS, PFNA, PFDA, PFUA, and Me-FOSAA) simultaneously in the model as individual predictors.

[d] Molar sum of all seven PFAS (nmol/mL).

* p-value <0.05,

p-value < 0.10

Abbreviation: autism spectrum disorder (ASD), typical development (TD)

Table 4.

Adjusted odds ratios (OR; 95% CI) and trend tests of child diagnosis of ASD (n = 239) versus TD (n=214) in relation to quartile or binary categories of the reconstructed maternal prenatal serum PFAS concentrations, the CHARGE Study, 2009–2017.

Concentration (ng/mL) [a]	TD (*n*)	ASD (*n*)	Model 1 OR (95% CI) [b]	*p*-trend [c]	Model 2 OR (95% CI) [d]	*p*-trend [c]
PFOA						
Q1(< 1.59)	54	81	1.00 (reference)	0.54	1.00 (reference)	0.47
Q2(1.59 – < 2.33)	53	58	1.08 (0.57, 2.05)		0.89 (0.41, 1.94)	
Q3(2.33 – < 3.32)	54	46	0.70 (0.35, 1.37)		0.55 (0.23, 1.30)	
Q4(≥ 3.32)	53	54	1.02 (0.50, 2.07)		0.73 (0.27, 1.94)	
PFOS						
Q1(< 3.86)	54	72	1.00 (reference)	0.84	1.00 (reference)	0.99
Q2(3.86 – < 5.81)	54	56	1.04 (0.54, 1.99)		1.07 (0.52, 2.18)	
Q3(5.81 – < 9.11)	52	63	1.15 (0.61, 2.18)		1.04 (0.46, 2.33)	
Q4(≥ 9.11)	54	48	1.37 (0.66, 2.83)		1.08 (0.41, 2.83)	
PFHxS						
Q1(< 0.48)	58	73	1.00 (reference)	0.06#	1.00 (reference)	0.06#
Q2(0.48 – < 0.67)	53	42	0.82 (0.41, 1.64)		0.99 (0.45, 2.16)	
Q3(0.67 – < 0.96)	51	50	1.06 (0.54, 2.07)		1.41 (0.63, 3.15)	
Q4(≥ 0.96)	52	74	1.95 (1.02, 3.72)*		2.65 (1.09, 6.38)*	
PFNA						
Q1(< 0.49)	65	78	1.00 (reference)	0.98	1.00 (reference)	0.92
Q2(0.49 – < 0.68)	48	61	0.89 (0.49, 1.63)		0.84 (0.43, 1.64)	
Q3(0.68 – < 0.91)	52	54	0.90 (0.48, 1.67)		0.78 (0.38, 1.62)	
Q4(≥ 0.91)	49	46	0.93 (0.48, 1.78)		0.79 (0.34, 1.80)	
PFAS categorized as binary variables because of relatively low detection frequency (<70%) in the study population						
PFDA						
Non-detect (< 0.1)	71	74	1.0	NA	1.0	NA
Detect (≥ 0.1)	143	165	0.83 (0.51, 1.36)		0.91 (0.51, 1.62)	
PFUA						
Non-detect (< 0.1)	110	108	1.0	NA	1.0	NA
Detect (≥ 0.1)	102	135	0.76 (0.47, 1.21)		0.76 (0.43, 1.34)	
Me-FOSAA						
Non-detect (< 0.1)	100	92	1.0	NA	1.0	NA
Detect (≥ 0.1)	112	151	1.49 (0.93, 2.39)		1.45 (0.89, 2.38)	

[a] PFDOA and Et-FOSAA were excluded in this table because fewer than 3% of the samples had detectable concentrations.

[b] Adjusted for CHARGE case-control study frequency matching factors (child's age and sex, regional center) and child's birth year, parity, gestational age at delivery, maternal race/ethnicity, maternal birthplace, mother's age at delivery, maternal pre-pregnancy body mass index, periconceptional maternal vitamin intake, homeownership, and breastfeeding duration.

[c] p-Trend from the Wald chi-square test for a linear trend.

[d] Additionally adjusted by including all seven PFAS (PFOA, PFOS, PFHxS, PFNA, PFDA, PFUA, and Me-FOSAA) simultaneously in the model as individual predictors.

[*] p-value < 0.05,

[#] p-value < 0.10

Abbreviation: autism spectrum disorder (ASD), not applicable (NA), typical development (TD)