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Childhood exposure to per- and polyfluoroalkyl substances and neurodevelopment in the CHARGE case-control study

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Abstract

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Author's contributions

JO, HS, IH, and DHB conceived the study, and IH and DHB oversaw its coordination. JO conducted data analyses and drafted the initial manuscript. HS, RJS, JBS, IH, and DHB helped oversee the study. KK quantified PFAS in child serum samples. SAB conducted preliminary data analyses. All authors reviewed 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 (IRB) for the State of California and the University of California-Davis (UC-Davis). Participants provided written informed consent before collection of any data.

Consent for publication

Not applicable.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

Background: Per- and polyfluoroalkyl substances (PFAS) are shown to have neurotoxic effects on animals, but epidemiological evidence for associations between childhood PFAS exposure and neurodevelopment is inconclusive. We examined if childhood PFAS concentrations are associated with a diagnosis of autism spectrum disorder (ASD), developmental delay (DD), and other early concerns (OEC) in development.

Methods: We included 551 children 2 to 5 years old from the CHildhood Autism Risks from Genetics and Environment (CHARGE) case-control study. Children were clinically diagnosed and classified as having ASD, DD, OEC, and typical development (TD). Fourteen PFAS were quantified in child serum samples collected when diagnostic assessments were performed. We used multinomial logistic regression models to investigate the cross-sectional associations of individual PFAS concentrations with neurodevelopmental outcomes and weighted quantile sum (WQS) regression models with repeated holdout validation to investigate the associations with PFAS mixtures.

Results: Childhood perfluorooctanoic acid (PFOA) was associated with increased odds of ASD (odds ratio [OR] per ln ng/mL increase: 1.99, 95% confidence interval [CI]: 1.20, 3.29) and DD (OR: 2.16, 95% CI: 1.21, 3.84) versus TD. Perfluoroheptanoic acid (PFHpA) was associated with increased odds of ASD (OR: 1.61, 95% CI: 1.21, 2.13). However, perfluroundecanoic acid (PFUnDA) was associated with decreased odds of ASD (OR: 0.43, 95% CI: 0.26, 0.69). From mixture analyses, the WQS index was associated with increased odds of ASD (average OR: 1.57, 5^{th} and 95^{th} percentile: 1.16, 2.13). Child's sex and homeownership modified associations of perfluorodecanoic acid (PFDA) with DD and ASD, respectively.

Conclusions: In this case-control study, childhood PFOA, PFHpA, and a PFAS mixture was associated with increased odds of ASD, while PFUnDA was associated with decreased odds of ASD. Because we used concurrent measurements of PFAS, our results do not imply causal relationships and thus need to be interpreted with caution.

1. Background

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic, fluorinated chemicals that have been used in a variety of consumer products, including non-stick cookware, food packaging materials, fabric and carpet treatments, and firefighting foams, since the 1950s (ATSDR 2021). Infants and young children are exposed to PFAS through ingestion of breast milk, contaminated food and water, and house dust and soil (Fromme et al. 2010; Fromme et al. 2009; Kärrman et al. 2007; Trudel et al. 2008; Winkens et al. 2017; Wu and Kannan 2019). Due to their widespread exposure and persistence, several long-chain PFAS, including perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexane-1-sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA), are frequently detected in the blood of children aged from 0 to 17 years (Duffek et al. 2020; Gump et al. 2011; Koponen et al. 2018; Schecter et al. 2012; Ye et al. 2018; Zhang et al. 2018b).

Early-life exposure to PFAS is shown to cause neurotoxic effects and behavioral changes in laboratory animals (Mariussen 2012). From repeated studies, male mice that were neonatally exposed to PFOA and PFOS showed persistent disturbances in spontaneous behaviors,

such as reduced habituation and increased hyperactivity, in their adulthood (Hallgren et al. 2015; Johansson et al. 2008). They also failed to respond normally to nicotine injections, suggesting adverse effects of PFOA and PFOS on the developing cholinergic system (Johansson et al. 2008). PFOS exposure disturbed the cholinergic system in the brain of male neonatal mice by inducing reduction of transcriptional levels of several genes involved in acetylcholine signaling and metabolism after 24 hours (Hallgren et al. 2015). Neonatal exposure to PFOA and PFOS altered protein levels that are critical for normal brain development through their involvement in neuronal growth and survival and synaptogenesis in the neonatal brain (Johansson et al. 2009). Similar to PFOA and PFOS , PFHxS exposure to both male and female mice in the neonatal period was associated with alterations in spontaneous behaviors and habituation as well as functions of the cholinergic system in adulthood (Viberg et al. 2013).

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction and communication skills and restricted, repetitive, and stereotypic behaviors and interests (American Psychiatric Association 2013). The prevalence of ASD in the United States (U.S.) has increased over the last decades, from 1 in 68 in 2010 to 1 in 44 children in 2018 (Baio 2014; Maenner et al. 2021). Exposure to a single compound or mixtures of environmental chemicals, especially during prenatal and early postnatal periods, can contribute to the development of ASD by disrupting thyroid functions which are critical for child brain development (Braun 2017; Hertz-Picciotto et al. 2018; Williams 2008). There is evidence that prenatal and early childhood exposures to PFAS interfere with thyroid hormone levels of newborns and children at age 1-5, respectively (Ballesteros et al. 2017; Lopez-Espinosa et al. 2012). Epidemiological studies showed null (Liew et al. 2014), adverse (Braun et al. 2014; Long et al. 2019; Lyall et al. 2018), or favorable (Oh et al. 2021a; Shin et al. 2020) associations between ASD risk and prenatal PFAS exposure, yet no previous study examined an association of ASD with PFAS exposure during postnatal periods. Early- to mid-childhood exposure to PFAS has been well studied for neurodevelopmental outcomes such as cognition, behaviors, and neuropsychological functioning, but the results are inconclusive, showing adverse (Harris et al. 2021; Harris et al. 2018; Oulhote et al. 2016; Vuong et al. 2021) or favorable (Stein et al. 2013; Vuong et al. 2019; Zhang et al. 2018a) associations.

Given the long half-lives of PFAS on the order of years (Bartell et al. 2010; Olsen et al. 2007; Wong et al. 2014), PFAS measured in 2- to 5-year-old children represent their exposures during the first few years of life, when the brain is still plastic, and its development is ongoing. The objective of this study was to elucidate if individual PFAS serum concentrations representing a cross-sectional look at ages 2-5 years, for at a set of compounds with half-lives of years, were associated with child neurodevelopmental outcomes. These outcomes are: ASD, developmental delay (DD), and other early concerns (OEC), as well as cognitive and adaptive functions. Child blood samples were collected on the same day that the child neurodevelopmental assessment was performed. We also examined the associations between exposure to a PFAS mixture and child neurodevelopment.

2. Methods

2.1. Study population

Children included in this study were participants in the Childhood Autism Risks from Genetics and Environment (CHARGE) study, a population-based case-control study (Hertz-Picciotto et al. 2006). Children who received services for ASD and DD were identified and recruited through Regional Centers that contract with the California Department of Developmental Services to provide services for individuals with developmental conditions. The general population controls were recruited through California birth files, frequency-matched to the ASD cases on child's sex and age and Regional Center catchment area. Because ASD is approximately four times more prevalent in males, the study sought to recruit a similar ratio among controls: 80% males and 20% females. All children meeting the following eligibility criteria were included: a) aged between 2 and 5 years at study enrollment, b) born in California, c) living with at least one biological parent who speaks English or Spanish, and d) residing in one of the CHARGE study catchment areas. Study protocols were approved by the institutional review boards of the State of California and the University of California (UC), and written informed consent was obtained from the parents prior to collection of any data. Detailed information on study design, study population, recruitment, eligibility criteria, and data and sample collection is described elsewhere (Hertz-Picciotto et al. 2006). Though launched in 2003, the CHARGE study started collecting serum in 2009. Among the 889 CHARGE children who were enrolled between 2009 and 2017, we included 551 children who provided serum samples with sufficient volume for PFAS quantification.

2.2. Child neurodevelopmental assessment

Children 2 to 5 years old visited the UC Davis Medical Investigations of Neurodevelopmental Disorders (MIND) Institute for neurodevelopmental assessment.

Parents of children with ASD were administered the Autism Diagnostic Interview-Revised (ADI-R) (Le Couteur et al. 2003; Lord et al. 1997; Lord et al. 1994), and children were assessed using the Autism Diagnostic Observation Schedules-Generic (ADOS-G) (Lord et al. 2000) by clinical personnel who had achieved reliability on their respective instruments. Children with DD or recruited from the general population controls were screened for ASD symptoms using the Social Communication Questionnaire (SCQ) (Rutter et al. 2003). Those children whose SCQ score was above 14 were further administered the ADI-R and ADOS-G to assess for ASD diagnosis. For all children included in this study, cognitive functions were assessed using the Mullen Scales of Early Learning (MSEL) (Mullen 1997), and adaptive functions were assessed using the Vineland Adaptive Behavior Scales (VABS) (Sparrow et al. 1984). The MSEL scores on four subscales (Fine Motor, Visual Reception, Receptive Language, Expressive Language), which are combined to generate an Early Learning Composite (Composite) score. The VABS scores on four subscales (Socialization, Daily Living Skills, Motor Skills, Communication), are also combined to yield an Adaptive Behavior Composite (Composite) score. Composite and subscale scores for both MSEL and VABS are standardized with a normative mean of 100 and a standard deviation (SD)

of 15. Higher MSEL and VABS scores indicate better cognitive and adaptive functions, respectively.

A final diagnosis of ASD was assigned when the child (1) met criteria on either the social or communication domains of the ADI-R and were within two points of meeting criteria in the other domain and (2) scored above the social and communication total cutoff for ASD on the ADOS module 1 or 2 (Risi et al. 2006). A diagnosis of DD was defined as having either one of the MSEL or VABS scores, but not both, less than 1.5 SD below the mean, having scores on the other instrument less than 2 SD below the mean, and scoring above 14 on the SCQ (Hertz-Picciotto et al. 2006). Among the children who did not meet the criteria for either ASD or DD in this study, children who previously received services for ASD or DD were classified as OEC, while children recruited from the general population controls who did not meet criteria for either ASD or DD were classified as typical development (TD). As a result, 551 children were categorized as ASD (n = 190), DD (n = 103), OEC (n = 78), and TD (n = 180).

2.3. Serum sample collection and PFAS quantification

Child blood samples were collected at the end of the neurodevelopmental assessment study visit. The mean child age at the sample collection was 3.4 years (range = 2 to 5 years). Whole blood samples were centrifuged, and the separated serum was transferred to a vial and frozen at −80 °C until analysis. For PFAS quantification, aliquots of the serum samples were shipped to the New York State Department of Health's Wadsworth Center's Human Health Exposure Analysis Resource Targeted Analysis Laboratory. The analytical method for 14 PFAS in child serum was previously described (Honda et al. 2018). Briefly, 0.25 mL of serum samples were aliquoted into 15 mL polypropylene tubes and spiked with 5 ng of ^{13}C-labeled internal standard mixture and 0.7 mL of 1% ammonium formate (w/v) in methanol. The mixture was centrifuged for 5 min at 5000 rpm, and the supernatant was collected and passed through hybrid-solid-phase extraction cartridge (Phospholipid, 30 mg, 1 cc, Sigma-Aldrich, St. Louis, MO, USA). The cartridges were conditioned with 1 mL of methanol containing 1% ammonium formate (w/v). The samples were eluted through the cartridge and analyzed using an AB SCIEX™ 5500 electrospray triple quadrupole mass spectrometer (SCIEX, Framingham, MA, USA), interfaced with a Nexera X2 LC-30AD series high-performance liquid chromatography (Shimadzu, Kyoto, Japan). Details of the LC parameters and mass spectrometric conditions are described elsewhere (Honda et al. 2018).

Fourteen PFAS quantified included: PFOA, PFOS, PFHxS, PFNA, perfluorodecanoic acid (PFDA), perfluoro-n-pentanoic acid (PFPeA), perfluoroheptanoic acid (PFHpA), perfluoroundecanoic acid (PFUnDA), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluorododecanoic acid (PFDoDA), N-methyl perfluorooctane sulfonamido acetic acid (MeFOSAA), ethyl perfluorooctane sulfonamido acetic acid (EtFOSAA), and perfluorooctanesulfonamide (PFOSA). For quality control, five replicates of procedural blanks and quality control samples (i.e., water spiked with native standards at 5 ng for all analytes and internal standards) were processed for each batch of 100 samples. In addition, two replicates of Standard Reference Material (SRM1957 and SRM1958, NIST, Gaithersburg, MD, USA), spiked with internal standards, containing

certified values for PFOA, PFOS, PFHxS, PFNA, PFDA, PFHpA, and PFUnDA for SRM1957 and PFOA, PFOS, PFHxS, and PFNA for SRM1958 were processed. The limit of detection (LOD) was 0.02 ng/mL for PFOS, PFHxS, PFNA, PFDA, PFHpA, PFUnDA, PFBS, MeFOSAA, EtFOSAA, and PFOSA, and 0.05 ng/mL for PFOA, PFPeA, PFHxA, and PFDoDA. We used instrument-observed values for PFAS concentrations below the LOD, rather than imputing them with a single value, to avoid biased estimates (Richardson and Ciampi 2003; Schisterman et al. 2006).

2.4. Statistical analysis

Participant characteristics were compared by neurodevelopmental outcomes (ASD, DD, OEC, TD) using the chi-squared test for categorical variables or the Kruskal-Wallis test for continuous variables. MSEL and VABS Composite scores were compared by participant characteristics using the Wilcoxon rank-sum test or the Kruskal-Wallis test. Child serum PFAS concentrations were compared by neurodevelopmental outcomes using the Kruskal-Wallis test and by participant characteristics using the Wilcoxon rank-sum test or the Kruskal-Wallis test. Spearman's correlation coefficients (r_{sp}) were computed among PFAS concentrations. Among 14 PFAS quantified, 9 PFAS that were detected in greater than 74% of the serum samples were used in the regression analyses. Because instrument-observed values were used, some of the PFAS concentrations below the LOD contained negative values. Therefore, the minimum value of each PFAS concentration and a value of 0.01 were added to all PFAS concentrations to avoid negative and zero values (Bennett et al. 2022), which were then natural log (ln)-transformed to normalize the right-skewed distributions.

Multinomial logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for associations between child serum PFAS concentrations and ASD, DD, and OEC, compared to TD. A false discovery rate (FDR) *p*-value was corrected per neurodevelopmental outcome to account for multiple comparisons. For the associations with continuous MSEL and VABS scores, multiple linear regression models were used to estimate regression coefficients (βs) and 95% CIs for Composite and each subscale. Among potential confounders *a priori* identified from a directed acyclic graph (Figure S1) (Hernán et al. 2004), those covariates that were associated with both exposures and outcomes ($p < 0.20$) were adjusted in the regression models. The covariates included in the final models were CHARGE frequency matching factors (child's sex [female, male] and age at sampling [in years], recruitment regional center); sampling year (2009-2010, 2011-2013, 2014-2017); gestational age at delivery (< 37, ≥ 37 weeks), maternal factors (birthplace [U.S., non-U.S.], parity [1, 2, > 2], breastfeeding duration [never, ≤ 3, > 3 months], race/ethnicity [non-Hispanic white, Hispanic, Black/Asian/ multiracial], and homeownership [non-owner, owner] as an indicator of socioeconomic status [SES]). To achieve the most parsimonious model, maximum education in household, maternal age at delivery, and health insurance type at delivery, all of which represent or reflect SES, were excluded from the final models because homeownership was most strongly associated with outcomes. As four covariates had approximately 2% to 4% of the missing observations, we imputed them in a multiple imputation model by chained equations, which included all exposures, outcomes, and the selected covariates (White et al. 2011). We generated 20 imputed datasets and pooled the estimates across datasets (Graham et al. 2007; Rubin 2004). For sensitivity analysis, we ran

the multinomial logistic regression and multiple linear regression models without imputing missing covariates.

To examine the associations of mixtures of nine PFAS with child neurodevelopment, we performed weighted quantile sum (WQS) regression analyses with repeated holdout validation, accounting for multicollinearity and nonlinear relationships (Carrico et al. 2015; Lazarevic et al. 2019). After randomly splitting the data into training (40%) and validation (60%) sets, an average of empirical weights of each PFAS across the 100 bootstrap samples was estimated and applied to quartiles of PFAS concentrations to yield a WQS index, represented by the total body burden. We then examined associations of the WQS index with neurodevelopmental outcomes using the multinomial logistic regression models and with MSEL and VABS Composite scores using multiple linear regression models, respectively. We adjusted for the same covariate set as before, but without multiple imputation. The process of splitting participants into training and validation sets was repeated 100 times, resulting in 100 effect estimates. We constrained the inference in a positive direction for neurodevelopmental outcomes and in a negative direction for cognitive and adaptive functions to test the hypothesis that a PFAS mixture was associated with increased odds of ASD, DD, and OEC and poorer performance on the MSEL and VABS, i.e., lower Composite scores. When at least 95% of the holdouts resulted in increased odds, per diagnosis group, chemical weight distributions were interpreted. Chemicals with 50% of repetitions above a threshold of 0.11 (1/number of PFAS detected greater than 74% of samples = 1/9) were defined as being possible contributors (Bennett et al. 2022; Carrico et al. 2015).

For associations between individual PFAS concentrations and each of the outcomes, we investigated effect modification by child's sex, homeownership (as a proxy of SES), and breastfeeding duration, based on suggestive evidence from previous studies (Forns et al. 2020; Oh et al. 2021b; Oulhote et al. 2016; Skogheim et al. 2021; Stein et al. 2014; Vuong et al. 2019; 2021). We compared the regression coefficients in the stratified analyses and evaluated a p-value for the interaction term between each PFAS concentration and each potential effect modifier. We also stratified the multiple linear regression analyses for MSEL and VABS scores by neurodevelopmental outcomes: ASD ($n = 190$) and non-ASD ($n = 361$). To assess the confounding effect of breastfeeding duration, we ran the final multinomial logistic regression and multiple linear regression models without adjusting for breastfeeding duration. Statistical analyses were conducted using R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria), including R packages "mice", "nnet", and "gWQS" (Renzetti et al. 2021; Ripley et al. 2016; Van Buuren and Groothuis-Oudshoorn 2011). Associations with $p < 0.05$ were considered as statistically significant, and those with $p < 0.10$ after FDR correction as borderline significant.

3. Results

3.1. Participant characteristics by neurodevelopmental outcomes

This study population consisted of 190 ASD, 103 DD, 78 OEC, and 180 TD children (Table 1). Seventeen pairs of twins and 9 families with 2 siblings enrolled in the study were included. Among 551 children, approximately 74% were males and 82% were born full-term. Because children with ASD and TD were frequency-matched on child's sex, they

were more likely to be male than those with DD and OEC. The goal was to age-match within 6 months, but children with ASD were older than children with TD. Children with ASD tended to be the firstborn child and recruited in later years compared to those with TD, DD, or OEC. Mothers of children with TD were more likely to be non-Hispanic white, own a home, be born in the U.S., and have insurance at delivery and also more likely to have breastfed longer than 3 months compared to those of children with other diagnoses. There was no difference in demographic characteristics between the CHARGE mother-child pairs included and excluded from this study (Table S1).

Children with TD had higher mean MSEL and VABS Composite scores than those with ASD (107 vs. 63 for MSEL and 98 vs. 62 for VABS) (Table S2). By definition, the TD group had higher scores than the DD group for MSEL (107 vs. 55) and VABS (98 vs. 59). Composite scores differed by several participant characteristics (Table 1). MSEL and VABS Composite scores were higher in children who were recruited in earlier years and born at or after 37 weeks of pregnancy and whose mother took prenatal vitamins during the first month of pregnancy, was non-Hispanic white, held a bachelor's or higher degree, owned a home, was born in the U.S., had insurance at delivery, and breastfed their child longer than 3 months.

3.2. Child serum PFAS concentrations

PFOA, PFOS, PFHxS, and PFNA were detected in 100% of the child serum samples, and PFDA, PFPeA, PFHpA, PFUnDA, and MeFOSAA were detected in 99.6, 99.3, 96.0, 74.6, and 81.1% of the samples, respectively (Table 2). The detection frequencies of PFBS, PFHxA, PFDoDA, EtFOSAA, and PFOSA were less than 30%. Median concentrations of several PFAS were different by the neurodevelopmental outcomes. Children with TD had higher medians of PFOA, PFOS, PFHxS, PFNA, and PFUnDA compared to those with ASD, higher medians of PFDA and PFUnDA than those with ASD and OEC, and higher median PFHpA than those with DD and OEC. On the contrary, children with DD and OEC had higher median PFPeA compared to those with TD. Among 9 PFAS detected in greater than 74% of the samples, PFAS compounds were weakly to moderately correlated with each other (r_{sp} = 0.15 to 0.69), except for PFPeA (r_{sp} = −0.02 to 0.14) (Figure S2). There were differences in median PFAS concentrations by participant characteristics (Table S3). Most PFAS concentrations tended to be higher in children recruited in earlier years and born at or after 37 weeks of pregnancy and whose mother was normal or underweight at pre-pregnancy, non-Hispanic white, and had longer breastfeeding duration.

3.3. Associations of child serum PFAS concentrations with child neurodevelopmental outcomes and MSEL and VABS scores

After adjusting for selected covariates, child serum PFOA concentrations were associated with increased odds of ASD (OR per ln ng/mL increase: 1.99, 95% CI: 1.20, 3.29) and DD (OR: 2.16, 95% CI: 1.21, 3.84) compared to TD (Table 3). PFHpA was associated with increased odds of ASD (OR: 1.61, 95% CI: 1.21, 2.13). On the other hand, PFUnDA was associated with decreased odds of ASD (OR: 0.43, 95% CI: 0.26, 0.69) compared to TD. After FDR correction, the associations of ASD with PFOA, PFHpA, and PFUnDA remained significant, while those of DD with PFOA became borderline significant.

When MSEL and VABS scores were used as outcomes, PFOA was consistently associated with decreased Composite and subscale scores (β per ln ng/mL increase: −6.82 to −4.91 for MSEL; β: −4.51 to −2.45 for VABS) (Figure 1). PFPeA similarly was associated with decreased MSEL Composite (β: −4.29, 95% CI: −8.42, −0.17) and Fine Motor scores (β: −4.73, 95% CI: −8.58, −0.88) and lower VABS Composite and subscale scores (β: −3.39 to −2.77), except for Motor Skills. A third PFAS, PFHpA, was consistently associated with lower MSEL and VABS Composite and subscale scores (β: −5.13 to −3.28 for MSEL; β: −2.86 to −1.92 for VABS). In contrast, PFUnDA was associated with increased MSEL and VABS Composite and subscale scores (β: 4.61 to 7.34 for MSEL; β: 3.51 to 4.95 for VABS). In sensitivity analyses performing the regression analyses without imputing missing covariates, the results did not change (Table S4 and Figure S3).

From repeated holdout WQS regression analyses, the PFAS index constrained in the positive direction was associated with increased odds of ASD (average OR: 1.57, 5^{th} and 95^{th} percentile: 1.16, 2.13) (Table 4). The PFAS index in the negative direction was associated with decreased MSEL (average β: −3.45, 5^{th} and 95^{th} percentile: −6.44, −0.47) and VABS Composite scores (average β: −3.34, 5^{th} and 95^{th} percentile: −6.37, −0.31). For the associations of PFAS mixtures with ASD as well as MSEL and VABS Composite scores, PFHpA, PFPeA, and PFOA were common possible contributors (Figure S4).

Effect modification by child's sex was observed for the association between PFDA and DD, with increased odds among females (OR: 2.44, 95% CI: 0.80, 7.50) and decreased odds among males (OR: 0.56, 95% CI: 0.28, 1.15; *p*-value for interaction = 0.02) (Table S5). The associations of PFDA with MSEL and VABS Composite scores were also modified, showing adverse associations among females (β: −6.7 for MSEL, −2.3 for VABS) and favorable associations among males (β: 3.9 for MSEL, 3.3 for VABS; *p*-values for interaction < 0.04) (Table S6). We also observed that the associations of PFDA with ASD as well as MSEL and VABS Composite scores were modified by homeownership (*p*-values for interaction < 0.04) (Table S7 and Table S8). Among homeowners, PFDA was associated with decreased odds of ASD (OR: 0.50, 95% CI: 0.26, 0.96) and higher MSEL and VABS scores (β: 4.3 for MSEL, 4.1 for VABS), whereas among those not owning a home, odds of ASD were increased (OR: 1.64, 95% CI: 0.71, 3.78), and those of cognitive and adaptive skills were reduced (β: −3.8 for MSEL, −2.3 for VABS). The associations of PFOA, PFOS, and PFUnDA with MSEL Composite scores were also modified by homeownership, with favorable associations among homeowners (*p*-values for interaction < 0.07). There was no consistent effect modification by breastfeeding duration (never or ≤ 3 months versus > 3 months) (Table S9 and Table S10).

When stratifying the associations between PFAS and MSEL and VABS scores by children with and without ASD, most of the associations were not statistically significant in both sub-groups (Figure S5). However, among children with ASD, MSEL and VABS scores tended to be poorer with increasing exposures to PFHpA and better with increasing exposures to PFUnDA. Among children without ASD (i.e., DD, OEC, and TD), MSEL and VABS scores indicated poorer skills with increasing exposures to PFOA and PFPeA and better skills with increasing exposures to PFUnDA. When performing the analyses without adjusting for the breastfeeding duration, the associations between PFOA and increased odds of ASD (OR:

1.15, 95% CI: 0.74, 1.79) and DD (OR: 1.26, 95% CI: 0.75, 2.12) as well as decreased MSEL (β: −2.05 to 0.91) and VABS (β: −1.27 to 0.47) scores moved toward the null and were no longer statistically significant (Table S11 and Figure S6). The associations for PFPeA, PFHpA, and PFUnDA for neurodevelopmental outcomes and MSEL and VABS scores remained similar.

4. Discussion

In this case-control study, we examined if PFAS concentrations in child serum samples collected at 2 to 5 years of age were associated with increased odds of ASD, DD, and OEC as well as with reduced cognitive and adaptive abilities, assessed using the MSEL and VABS, respectively. Childhood PFOA was associated with increased odds of ASD and DD, and PFHpA was associated with increased odds of ASD. PFOA and PFHpA were also associated with decreased scores on cognitive and adaptive functions, and additionally, PFPeA was associated with lower adaptive function scores. However, PFUnDA was associated with decreased odds of ASD and DD and higher scores on cognitive and adaptive abilities. A PFAS mixture, highly weighted by PFPeA and PFHpA, was associated with increased odds of ASD. A PFAS mixture, which was weighted with PFOA, PFPeA, PFHpA, and MeFOSAA, was associated with poorer cognitive and adaptive functions. Child's sex modified the associations of PFDA with odds of DD and MSEL and VABS scores, showing that among females, increasing exposure tended to be towards an association with higher odds of the DD diagnosis and poorer scores whereas males with decreased odds and better scores. Furthermore, the associations of PFDA with odds of ASD and MSEL and VABS scores were modified by homeownership, showing that homeowners tended to have decreased odds of neurodevelopmental diagnoses and higher skills, whereas non-owners tended to have increased odds of diagnoses and poorer skills.

The case-control design of this study did not allow a causal interpretation for the associations observed between PFAS and the outcomes considered based on the fact that the neurodevelopmental assessment and the blood sample collection were performed on the same day. Most of the children had already been diagnosed prior to the assessment. In fact, the cross-sectional data collection opens the possibility of reverse causation if the different dietary and behavior patterns of the children with ASD or DD could have influenced the child's PFAS levels (Shin et al. 2020). On the other hand, PFAS quantified at ages 2-5 represent exposures beginning in at least as far back as the early postnatal period and may extend even into the prenatal period, due to their long half-lives: estimated at 2.1-10.1 years for PFOA, 3.3-27 for PFOS, 4.7-35 years for PFHxS, and 2.5-4.3 years for PFNA (ATSDR 2021). PFAS in child's serum or plasma showed moderate to strong correlations between 3 months and 2 years (r_{sp} = 0.82 for PFOA, 0.76 for PFOS, 0.82 for PFHxS, 0.68 for PFNA, 0.46 for PFDA) (van Beijsterveldt et al. 2022) and between 1.5 and 5 years (r_{sp} = 0.57 for PFOA, 0.69 for PFOS, 0.75 for PFHxS, 0.49 for PFNA, 0.41 for PFDA) (Blomberg et al. 2021), and weak to moderate correlations of birth (i.e., cord serum) with 1.5 years (r_{sp} = 0.35 to 0.44), 3 years (Pearson's correlation coefficients [r_p] = −0.19 to 0.46), and 5 years (r_{sp} = 0.21 to 0.42) (Blomberg et al. 2021; Kingsley et al. 2018). Therefore, our findings warrant further investigations regarding effects of PFAS exposures during the first few years of life on child neurodevelopment.

We observed that increased odds of an ASD diagnosis and cognitive and adaptive functions were adversely associated with PFOA and PFHpA but favorably associated with PFUnDA. The mixed associations depending on the PFAS compound may be partly explained by their different exposure sources. In this study, child concentrations of PFUnDA were relatively weakly correlated with those of PFOA (r_{sp} = 0.36) and PFHpA (r_{sp} = 0.21), compared to the correlations between PFOA and PFHpA (r_{sp} = 0.62), implicating slightly different exposure sources of PFUnDA. PFUnDA has a longer carbon chain (C 11), compared to PFOA (C 8) and PFHpA (C 5) with a higher bioaccumulation factor (Chen et al. 2018; Liu et al. 2019). In studies conducted in Europe and Asia, the dietary intake of PFUnDA was predominated by the consumption of fish products due to the bioaccumulative property, while that of PFOA and PFHpA originated from various food categories, such as dairy, fruits, and vegetable products, (Eriksson et al. 2013; Fujii et al. 2015; Heo et al. 2014; Johansson et al. 2014; Klenow et al. 2013; Vestergren et al. 2012). Many long-chain perfluorocarboxylates can also originate from hepatic biotransformation of fluorotelomer alcohols, which are frequently used in paper products and textiles (Sinclair et al. 2007). In this study population, diet and fish consumption data were collected but not adjusted for because changes in the diet questionnaire in the CHARGE study midway through this study period (i.e., in 2011) presented obstacles to the use of these data. However, among children who had fish consumption data from either the old or new questionnaire (n = 416), the percentage of those who consumed fish before 2 years was higher in the TD group (54%), compared to ASD (31%) and DD (44%) groups. In an earlier report from the CHARGE Study, children with ASD were shown to be far less likely to consume fish, including tuna, other ocean fish, or freshwater fish, than the children with TD (Hertz-Picciotto et al. 2010). Therefore, the residual confounding by diet, including fish consumption, may have affected the mixed associations between PFOA or PFHpA and PFUnDA.

Breastfeeding is a primary exposure pathway to PFAS for infants (Zheng et al. 2021). The duration of breastfeeding was positively associated with PFAS concentrations in 2- to 5-year-old children (Gyllenhammar et al. 2018; Kingsley et al. 2018; Mogensen et al. 2015; Papadopoulou et al. 2016). In this study population, we also observed that most of the PFAS concentrations were higher in the children with longer breastfeeding duration, showing positive correlations (range = 0.14 to 0.43), except for PFPeA and MeFOSAA (Table S3 and Table S12). Due to the highest correlations between PFOA and breastfeeding duration, we observed that the associations between childhood PFOA and increased odds of ASD and DD and decreased MSEL and VABS scores became nonsignificant after excluding breastfeeding duration from the models. Breastfeeding, a major determinant of PFAS concentrations in early childhood, is also associated with better child neurodevelopment, including ASD and cognitive development (Bar et al. 2016), and hence, should be considered a likely confounder of the relationship between childhood PFAS and neurodevelopment.

Previous epidemiological studies focused on the associations of the risk of ASD or DD with prenatal PFAS exposure rather than childhood PFAS exposure as pregnancy is often thought to be the most sensitive window for brain development (Buck Louis et al. 2019). Still, developmental exposures to neurotoxicants during the first few years of life have been emphasized in association with child neurodevelopment (Hertz-Picciotto et al. 2018; Lyall et al. 2014; Selevan et al. 2000; Volk et al. 2013). However, epidemiologic evidence on

childhood PFAS exposure and neurodevelopmental scores, assessing cognitive functioning and behavioral difficulties at various ages, is inconclusive. For example, in the mid-Ohio-Valley population who were highly exposed to PFOA, PFOA in child serum samples collected at age 2-8 was associated with higher IQ and better visual-spatial processing at 6-12 years of age (Stein et al. 2013). A Cincinnati study that collected child serum samples at ages 3 and 8 years observed that PFOA, PFOS, and PFNA were associated with better reading skills at 5 and 8 years of age (Zhang et al. 2018a). In the same population, PFNA at age 3 was associated with higher IQ and perceptual reasoning and less attention problems at 8 years of age, while concurrent PFNA was associated with poorer activity of daily living at age 8 (Vuong et al. 2019; 2021).

Numerous other studies observed associations similar to those of the current study: A Boston-area birth cohort study observed that PFOA, PFOS, and PFHxS at 6-10 were associated cross-sectionally with lower visual-motor abilities and greater parent-reported behavioral difficulties (Harris et al. 2021; Harris et al. 2018). In the Faroese population, PFOA, PFNA, and PFDA at 5 years were associated with parent-reported behavioral difficulties at 7 years of age (Oulhote et al. 2016). Only the Faroese birth cohort investigated the joint effects of a chemical mixture, combining PFAS, mercury, and polychlorinated biphenyls, at age 5 on cognitive and behavioral functions at 7 years of age (Oulhote et al. 2019). They observed that the mixture concentrations were associated with higher scores of parent-reported behavioral difficulties, which was consistent with our findings. Some of the variations across studies could be due to the sources of PFAS, for instance, fish consumption can be quite high in countries where most inhabitants live near coasts, and is associated with improved cognitive abilities.

Our study provided additional evidence on sexually dimorphic associations of childhood PFDA with neurodevelopment. We observed increased odds of DD and decreased MSEL and VABS scores among females, while decreased odds of DD and increased MSEL and VABS scores among males. As this study enrolled fewer female children (n = 145) than male children (n = 406), stratified analyses of neurodevelopmental outcomes by child's sex should be interpreted cautiously. Still, it is noteworthy that we observed consistent sex-specific associations not only for the odds of DD but also for MSEL and VABS scores. The underlying mechanism remains unclear, but it was suggested that PFAS may disrupt functions of sex hormones, having sexually dimorphic effects on neurodevelopment (Kjeldsen and Bonefeld-Jørgensen 2013). Similar to our findings, a previous study reported that concurrent PFOS, PFHxS, and PFNA were adversely associated with autism screening and externalizing problems among females at 7 years of age, but not or favorably associated among males (Oulhote et al. 2016). Another study also observed adverse associations between PFOA at age 2-8 and mother-reported executive functions and behavioral assessment among females and favorable associations among males at 6-12 years of age (Stein et al. 2014). However, in the Cincinnati birth cohort, concurrent PFOS was associated with better IQ and cognitive functions only among 8-year-old males (Vuong et al. 2019), and PFOA and PFNA at age 3 and 8 were associated with greater externalizing and internalizing problems, aggression, and depression among males, compared to females (Vuong et al. 2021). Other studies did not find evidence of heterogeneity of PFAS

associations with neurodevelopment by child's sex (Harris et al. 2021; Harris et al. 2018; Stein et al. 2013).

Homeownership, as a proxy of SES, modified associations of PFDA with ASD and cognitive and adaptive scores and those of PFOA and PFUnDA with cognitive scores, showing favorable associations among homeowners and adverse associations among those who did not own a home. In this study population, child PFAS concentrations and neurodevelopmental outcomes as well as cognitive and adaptive scores differed by SES. For example, serum concentrations of most PFAS were higher among children whose parents owned a home, had higher educational attainment (i.e., Bachelor's degree or higher), and had an insurance at delivery (Table S3). These children also accounted for a lower percentage of ASD, DD, and OEC groups, compared to the TD group, and had higher scores for cognitive and adaptive functions (Table 1). In line with our findings, several studies also observed the effect modification of associations between early-life PFAS and neurodevelopmental outcomes by maternal education, another measure of SES. In a Norwegian cohort, a diagnosis of ASD was associated with higher prenatal maternal PFOA and PFOS and lower PFHxS and PFUnDA among children whose mother had higher education (Skogheim et al. 2021). In a meta-analysis of European population-based studies, higher odds of attention deficit hyperactivity disorder were associated with early-life exposure to PFOA and PFOS among children born to low-educated mothers (Forns et al. 2020). However, because the evidence on effect modification by SES is limited and mixed, further studies are needed to confirm our findings.

This study has several limitations. As mentioned above, PFAS were quantified in child serum samples that were collected at the end of the visit for neurodevelopmental assessment. Therefore, our findings based on concurrent exposure measurements should not be interpreted as a causal relationship. Moreover, the estimates from the sub-group analyses were unstable due to the small subsample size, thus the results should be interpreted cautiously. On the other hand, a major strength of this study is the clinical confirmation of ASD, DD, and OEC diagnoses and the assessment of cognitive and adaptive functions using established reliable assessment tools. We accounted for an extensive list of potential confounders, including demographic, maternal, and perinatal characteristics and breastfeeding duration, contributing to the scientific rigor of this analysis. Furthermore, because 9 PFAS compounds were widely detected (> 74%) in the study samples, we could evaluate associations of PFAS mixtures with neurodevelopment. We also found of associations with adverse neurodevelopmental outcomes in relation to two short-chain PFAS (i.e., PFHpA and PFPeA) for which neurotoxic effects have not been well- studied. Because epidemiological evidence was also limited partly due to their low detection rates, our findings warrant further studies of these two compounds in association with child neurodevelopment. This large study provided robust results identifying several PFAS compounds measured in early life that were associated with neurodevelopmental conditions as well as of cognitive and adaptive skills.

5. Conclusions

In this case-control study that quantified PFAS in child serum samples and assessed neurodevelopment at 2 to 5 years of age, PFOA was associated with increased odds of ASD and DD, whereas PFUnDA with decreased odds. PFHpA was associated with increased odds of ASD. Similarly, PFOA and PFHpA showed associations with poorer cognitive and adaptive skills, while PFUnDA showed associations with stronger skills. Higher concentrations of the PFAS mixture were associated with increased odds of ASD and decreased scores of cognitive and adaptive functions, with PFHpA, PFPeA, and PFOA as major contributors. There was evidence of effect modification of the associations between PFDA and neurodevelopment by child's sex and homeownership, which should be replicated in larger samples. Although a prenatal period may be the most susceptible window of PFAS exposure for neurodevelopment, this study suggests that early postnatal periods need further scrutiny.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Competing interests

RJS has received lodging for the Baby Siblings Research Consortium Meeting; travel and lodging for invited talks at the University of Sherbrooke, Sherbrooke, Québec, Canada; the University of California Santa Cruz. Santa Cruz, California (Lodging); Epigenomics 2016, Puerto Rico (Lodging); Neurotoxicity Society & International Neurotoxicology Association, Florianópolis, Brazil; RISE 2017 Second International Meeting on Environmental Health in Strasbourg. Strasbourg, France. RJS also received funding from Autism Speaks grant to develop an online autism environmental questionnaire and from Simons Foundation. Other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and material

Lab and epidemiological data are hosted at the Human Health Exposure Analysis Resources (HHEAR) Data Center Repository (https://hheardatacenter.mssm.edu/).

List of abbreviations

ADI-R	Autism Diagnostic Interview-Revised

ADOS-G	Autism Diagnostic Observation Schedules-Generic
ASD	autism spectrum disorder
CHARGE	CHildhood Autism Risks from Genetics and Environment
CI	confidence interval
Composite	Early Learning Composite/Adaptive Behavior Composite
DD	developmental delay
EtFOSAA	ethyl perfluorooctane sulfonamido acetic acid
ln	natural log
LOD	limit of detection
MeFOSAA	N-methyl perfluorooctane sulfonamido acetic acid
MIND	Medical Investigations of Neurodevelopmental Disorders
MSEL	Mullen Scales of Early Learning
OEC	other early concerns
OR	odds ratio
PFAS	per- and polyfluoroalkyl substances
PFBS	perfluorobutanesulfonic acid
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane-1-sulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PFOSA	perfluorooctanesulfonamide
PFPeA	perfluoro-n-pentanoic acid
PFUnDA	perfluoroundecanoic acid
r_{sp}	Spearman’s correlation coefficients
SCQ	Social Communication Questionnaire

SD	standard deviation
SES	socioeconomic status
TD	typical development
UC	University of California
U.S.	United States
VABS	Vineland Adaptive Behavior Scales
WQS	weighted quantile sum

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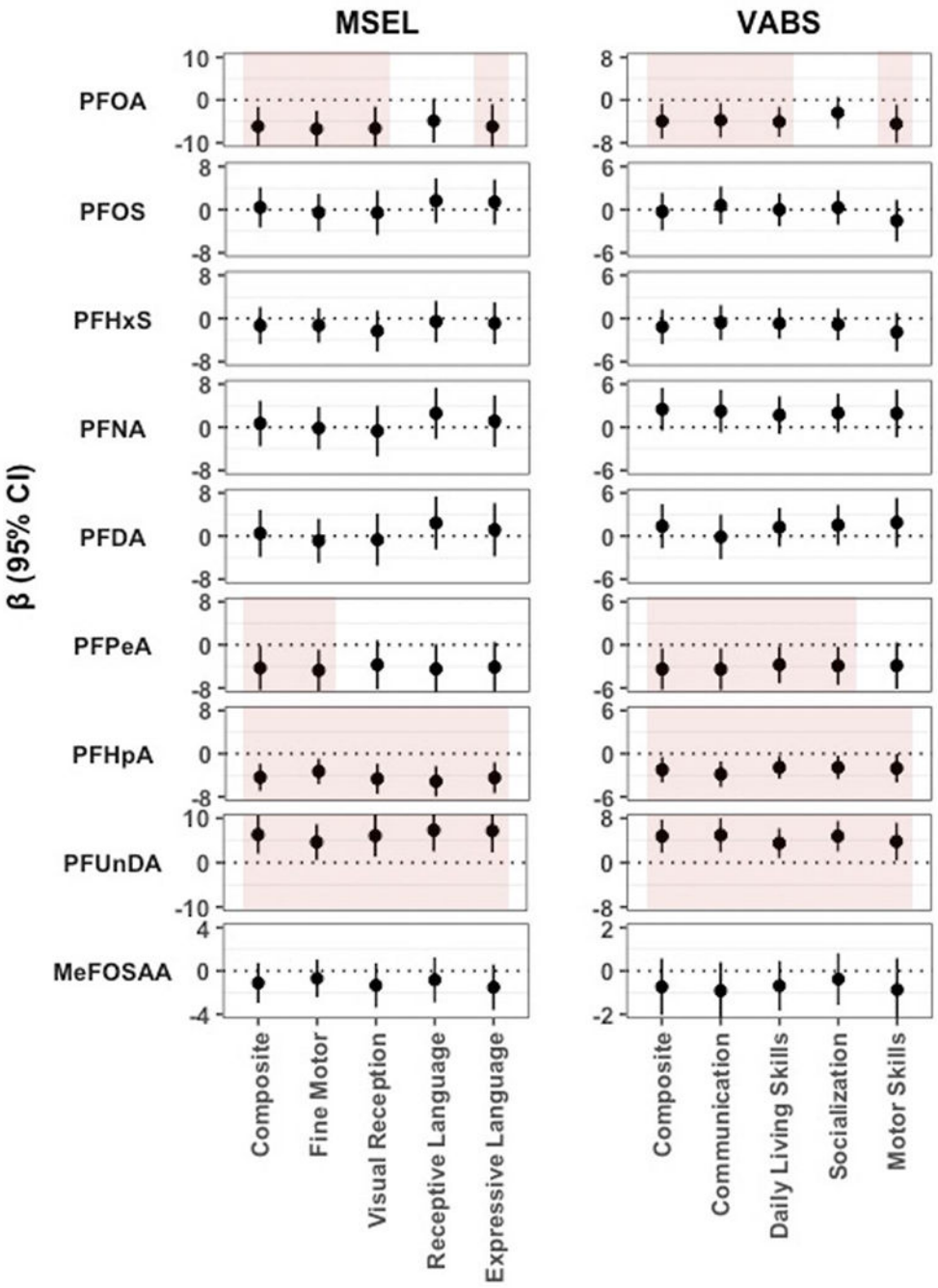

Figure 1.

Estimated mean differences (β) in MSEL and VABS Composite and subscale scores per ln ng/mL increase in child serum PFAS concentrations. Multiple linear regression models were adjusted for CHARGE frequency matching factors (child's sex and age at sampling, recruitment regional center), sampling year, gestational age at delivery, mother's birthplace, parity, mother's race/ethnicity, homeownership, and breastfeeding duration. Shaded areas indicate estimates with a *p*-value < 0.05.

Table 1.

Participant characteristics of 551 mother-child pairs by neurodevelopmental outcomes and MSEL and VABS Composite scores by characteristics in the CHARGE study.

Characteristic	All (*n* = 551) [a]	TD (*n* = 180)	ASD (*n* = 190)	DD (*n* = 103)	OEC (*n* = 78)	*p* [b]	MSEL Composite	*p* [c]	VABS Composite	*p* [c]
	Freq (%)	Freq (%)	Freq (%)	Freq (%)	Freq (%)		Mean (SD)		Mean (SD)	
Child sex						<0.001		0.147		0.052
Female	145 (26.3)	32 (17.8)	42 (22.1)	41 (39.8)	30 (38.5)		77 (31)		73 (21)	
Male	406 (73.7)	148 (82.2)	148 (77.9)	62 (60.2)	48 (61.5)		82 (28)		77 (20)	
Sampling year						<0.001		0.015		<0.001
2009 – 2010	180 (32.7)	65 (36.1)	48 (25.3)	33 (32.0)	34 (43.6)		84 (28)		81 (22)	
2011 – 2013	214 (38.8)	82 (45.6)	46 (24.2)	57 (55.3)	29 (37.2)		81 (29)		77 (20)	
2014 – 2017	157 (28.5)	33 (18.3)	96 (50.5)	13 (12.6)	15 (19.2)		75 (28)		69 (18)	
Gestational age at delivery						<0.001		0.074		0.045
< 37 weeks	86 (15.6)	14 (7.8)	22 (11.6)	27 (26.2)	23 (29.5)		76 (27)		71 (17)	
≥ 37 weeks	452 (82.0)	162 (90.0)	163 (85.8)	72 (69.9)	55 (70.5)		81 (29)		77 (21)	
Mother's age at delivery						0.179		0.103		0.072
< 30 years	247 (44.8)	77 (42.8)	94 (49.5)	43 (41.7)	33 (42.3)		78 (29)		75 (21)	
30 – 35 years	156 (28.3)	60 (33.3)	50 (26.3)	23 (22.3)	23 (29.5)		84 (28)		79 (21)	
≥ 35 years	148 (26.9)	43 (23.9)	46 (24.2)	37 (35.9)	22 (28.2)		80 (29)		74 (19)	
Parity						0.006		0.452		0.875
1	204 (37.0)	55 (30.6)	91 (47.9)	29 (28.2)	29 (37.2)		80 (28)		75 (19)	
2	209 (37.9)	73 (40.6)	67 (35.3)	40 (38.8)	29 (37.2)		79 (29)		76 (21)	
> 2	120 (21.8)	47 (26.1)	28 (14.7)	28 (27.2)	17 (21.8)		83 (28)		76 (21)	
Mother's pre-pregnancy BMI						0.985		0.562		0.574
Normal/underweight	286 (51.9)	97 (53.9)	100 (52.6)	51 (49.5)	38 (48.7)		81 (29)		76 (20)	
Overweight	120 (21.8)	40 (22.2)	41 (21.6)	23 (22.3)	16 (20.5)		80 (29)		76 (20)	
Obese	134 (24.3)	41 (22.8)	45 (23.7)	28 (27.2)	20 (25.6)		78 (28)		75 (21)	
Vitamin intake during the first month of pregnancy						0.150		0.026		0.007
No	159 (28.9)	50 (27.8)	65 (34.2)	29 (28.2)	15 (19.2)		77 (30)		73 (21)	
Yes	329 (59.7)	117 (65.0)	106 (55.8)	57 (55.3)	49 (62.8)		83 (28)		78 (21)	

Characteristic	All (n = 551) [a]	TD (n = 180)	ASD (n = 190)	DD (n = 103)	OEC (n = 78)	p [b]	MSEL Composite	p [c]	VABS Composite	p [c]
	Freq (%)	Freq (%)	Freq (%)	Freq (%)	Freq (%)		Mean (SD)		Mean (SD)	
Mother's race/ethnicity						<0.001		<0.001		<0.001
Non-Hispanic White	317 (57.5)	126 (70.0)	96 (50.5)	47 (45.6)	48 (61.5)		87 (28)		80 (21)	
Hispanic	133 (24.1)	28 (15.6)	45 (23.7)	39 (37.9)	21 (26.9)		70 (25)		70 (19)	
Black, Asian, multiracial	101 (18.3)	26 (14.4)	49 (25.8)	17 (16.5)	9 (11.5)		74 (29)		71 (18)	
Maximum education in household						0.041		<0.001		<0.001
Less than college credit	271 (49.2)	78 (43.3)	91 (47.9)	64 (62.1)	38 (48.7)		76 (27)		73 (19)	
Bachelor's degree	179 (32.5)	69 (38.3)	66 (35.7)	23 (22.3)	21 (26.9)		84 (29)		78 (21)	
Graduate or professional degree	101 (18.3)	33 (18.3)	33 (17.4)	16 (15.5)	19 (24.4)		86 (28)		80 (20)	
Homeownership						0.005		<0.001		<0.001
Non-owner	223 (40.5)	55 (30.6)	82 (43.2)	49 (47.6)	37 (47.4)		75 (27)		72 (19)	
Owner	304 (55.2)	118 (65.6)	102 (53.7)	47 (45.6)	37 (47.4)		85 (29)		79 (21)	
Mother's birthplace						0.049		<0.001		<0.001
U.S.	431 (78.2)	152 (84.4)	140 (73.7)	76 (73.8)	63 (80.8)		83 (28)		77 (21)	
Non-U.S.	120 (21.8)	28 (15.6)	50 (26.3)	27 (26.2)	15 (19.2)		72 (28)		70 (18)	
Health insurance type at delivery						0.007		<0.001		<0.001
No insurance	139 (25.2)	32 (17.8)	55 (28.9)	36 (35.0)	16 (20.5)		69 (26)		69 (19)	
Insurance	394 (71.5)	140 (77.8)	129 (67.9)	65 (63.1)	60 (76.9)		84 (28)		78 (20)	
Breastfeeding duration						<0.001		<0.001		<0.001
Never	42 (8.0)	9 (5.2)	16 (8.7)	12 (12.8)	5 (6.6)		73 (29)		69 (18)	
≤ 3 months	168 (31.9)	32 (18.4)	76 (41.5)	35 (37.2)	25 (32.9)		72 (28)		70 (19)	
> 3 months	317 (60.2)	133 (76.4)	91 (49.7)	47 (50.0)	46 (60.5)		87 (27)		80 (20)	
Recruitment regional center						0.264		0.251		0.217
Alta, Far Northern, Redwood Coast	305 (55.4)	101 (56.1)	108 (56.8)	58 (56.3)	38 (48.7)		81 (29)		76 (20)	
North Bay, East Bay, San Andreas, Golden Gate	104 (18.9)	41 (22.8)	27 (14.2)	18 (17.5)	18 (23.1)		83 (30)		78 (22)	
Valley Mountain, Central Valley, Kern	142 (25.8)	38 (21.1)	55 (28.9)	27 (26.2)	22 (28.2)		78 (27)		73 (19)	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)					
Child's age at sampling (years)	3.4 (0.8)	3.2 (0.8)	3.5 (0.7)	3.4 (0.7)	3.3 (0.8)	0.004				

[a] Missing (frequency, %): gestational age at delivery (13, 2%), parity (18, 3%), mother's pre-pregnancy BMI (11, 2%), vitamin intake during the first month of pregnancy (63, 11%), homeownership (24, 4%), health insurance type at delivery (18, 3%), breastfeeding duration (24, 4%)

[b] P-values from the chi-squared test for categorical variables or the Kruskal-Wallis test for continuous variables

[c] P-values from the Wilcoxon rank-sum test for binary variables or the Kruskal-Wallis test for other categorical variables

Table 2.

Distributions of PFAS concentrations in 551 child serum samples.

			50th (5th, 95th) percentiles (ng/mL)					*p*-value (vs. TD) [b]		
PFAS [a]	**LOD (ng/mL)**	**% detect**	**All (*n* = 551)**	**TD (*n* = 180)**	**ASD (*n* = 190)**	**DD (*n* = 103)**	**OEC (*n* = 78)**	**ASD**	**DD**	**OEC**
PFOA	0.05	100	2.36 (0.93, 6.77)	2.58 (1.14, 6.50)	2.20 (0.91, 6.30)	2.22 (0.90, 9.45)	2.13 (0.90, 6.31)	0.01	0.10	0.13
PFOS	0.02	100	2.45 (0.93, 11.0)	2.58 (1.13, 10.7)	2.01 (0.81, 8.01)	2.62 (0.86, 11.0)	2.90 (0.99, 13.6)	<0.01	0.35	0.99
PFHxS	0.02	100	0.64 (0.24, 3.41)	0.72 (0.28, 3.07)	0.59 (0.20, 3.05)	0.59 (0.23, 3.48)	0.71 (0.24, 4.24)	0.01	0.14	0.98
PFNA	0.02	100	0.85 (0.29, 2.60)	0.92 (0.41, 3.24)	0.71 (0.26, 2.49)	0.92 (0.33, 2.49)	0.93 (0.29, 2.42)	<0.01	0.99	0.33
PFDA	0.02	99.6	0.16 (0.07, 0.48)	0.17 (0.08, 0.50)	0.14 (0.06, 0.49)	0.15 (0.07, 0.44)	0.15 (0.07, 0.34)	<0.01	0.06	0.02
PFPeA	0.05	99.3	0.57 (0.22, 1.25)	0.56 (0.22, 1.14)	0.51 (0.20, 1.33)	0.62 (0.24, 1.44)	0.65 (0.22, 1.17)	0.67	0.02	0.04
PFHpA	0.02	96.0	0.20 (0.03, 0.87)	0.22 (0.04, 0.72)	0.23 (0.03, 1.00)	0.16 (0.02, 0.86)	0.18 (<LOD, 1.04)	0.65	0.04	0.02
PFUnDA	0.02	74.6	0.04 (<LOD, 0.15)	0.05 (<LOD, 0.16)	0.03 (<LOD, 0.13)	0.04 (<LOD, 0.13)	0.04 (<LOD, 0.18)	<0.01	0.06	0.03
PFBS	0.02	29.6	<LOD (<LOD, 0.07)	<LOD (<LOD, 0.06)	<LOD (<LOD, 0.10)	<LOD (<LOD, 0.06)	<LOD (<LOD, 0.06)	-	-	-
PFHxA	0.05	16.2	<LOD (<LOD, 0.28)	<LOD (<LOD, 0.24)	<LOD (<LOD, 0.43)	<LOD (<LOD, <LOD)	<LOD (<LOD, 0.15)	-	-	-
MeFOSAA	0.02	81.1	0.12 (<LOD, 1.64)	0.12 (<LOD, 1.45)	0.10 (<LOD, 1.56)	0.14 (<LOD, 2.26)	0.13 (<LOD, 2.14)	0.06	0.24	0.66
EtFOSAA	0.02	17.1	<LOD (<LOD, 0.07)	<LOD (<LOD, 0.07)	<LOD (<LOD, 0.06)	<LOD (<LOD, 0.07)	<LOD (<LOD, 0.10)	-	-	-

[a] PFDoDA and PFOSA, which were detected in 4.2% and 0.7% of the samples, respectively, were excluded from the table

[b] P-values from the Wilcoxon rank-sum test were not calculated for PFAS that were detected in less than 30% of the samples.

Table 3.

Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of ASD (n = 190), DD (n = 103), and OEC (n = 78) versus TD (n = 180) in association with child serum PFAS concentrations and p-values with and without FDR correction for multiple tests.

	ASD vs. TD			DD vs. TD			OEC vs. TD		
PFAS (ng/mL) [a]	OR [b] (95% CI)	Unadj. p-value	FDR corrected p-value	OR [b] (95% CI)	Unadj. p-value	FDR corrected p-value	OR [b] (95% CI)	Unadj. p-value	FDR corrected p-value
PFOA	**1.99 (1.20, 3.29)**	**0.007**	**0.022**	**2.16 (1.21, 3.84)**	**0.009**	**0.083**	1.15 (0.61, 2.18)	0.658	0.658
PFOS	0.89 (0.59, 1.35)	0.586	0.586	1.37 (0.89, 2.12)	0.152	0.228	1.39 (0.87, 2.22)	0.165	0.371
PFHxS	1.22 (0.84, 1.78)	0.297	0.383	1.52 (1.00, 2.32)	0.053	0.158	1.41 (0.92, 2.18)	0.115	0.345
PFNA	0.75 (0.48, 1.17)	0.203	0.365	0.88 (0.53, 1.46)	0.621	0.798	0.74 (0.42, 1.30)	0.299	0.449
PFDA	0.78 (0.49, 1.24)	0.298	0.383	0.95 (0.56, 1.62)	0.861	0.969	0.61 (0.33, 1.10)	0.100	0.345
PFPeA	1.48 (0.96, 2.28)	0.074	0.167	1.59 (0.93, 2.74)	0.092	0.208	1.15 (0.67, 1.97)	0.608	0.658
PFHpA	**1.61 (1.21, 2.13)**	**0.001**	**0.005**	1.00 (0.72, 1.39)	0.993	0.993	0.81 (0.58, 1.14)	0.234	0.420
PFUnDA	**0.43 (0.26, 0.69)**	**0.001**	**0.005**	0.59 (0.34, 1.00)	0.052	0.158	0.62 (0.35, 1.10)	0.102	0.345
MeFOSAA	1.08 (0.89, 1.31)	0.451	0.508	1.19 (0.95, 1.50)	0.135	0.228	1.11 (0.87, 1.42)	0.413	0.531

[a] Nine PFAS that were detected in > 74% of the samples were individually included in the multinomial regression models.

[b] Multinomial logistic regression models were adjusted for CHARGE frequency matching factors (child's sex and age at sampling, recruitment regional center), sampling year, gestational age at delivery, mother's birthplace, parity, mother's race/ethnicity, homeownership, and breastfeeding duration.

Table 4.

Associations between combined child PFAS exposure and neurodevelopmental outcomes (ASD, DD, OEC versus TD) and MSEL and VABS Composite scores from repeated holdout weighted quantile sum (WQS) regression.

Outcome [a]	Direction	OR or β [b]	Mean	Median	5th PCT	95th PCT
ASD vs. TD	Positive	OR	1.57	1.59	1.16	2.13
DD vs. TD	Positive	OR	1.22	1.23	0.81	1.85
OEC vs. TD	Positive	OR	1.02	1.03	0.68	1.53
MSEL Composite	Negative	β	−3.45	−3.55	−6.44	−0.47
VABS Composite	Negative	β	−3.34	−3.21	−6.37	−0.31

[a] Outcome (*n*) for repeated holdout WQS regression: ASD (174), TD (166), DD (87), OEC (73), MSEL Composite (499), VABS Composite (500)

[b] Regression models were adjusted for CHARGE frequency matching factors (child's sex and age at sampling, recruitment regional center), sampling year, gestational age at delivery, mother's birthplace, parity, mother's race/ethnicity, homeownership, and breastfeeding duration

Note: Weight distributions of individual PFAS from each 100 repetitions of WQS are presented in Figure S4.