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Prenatal Maternal Serum Concentrations of Per- and Polyfluoroalkyl Substances in Association with Autism Spectrum Disorder and Intellectual Disability

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Background: Emerging work has examined neurodevelopmental outcomes following prenatal exposure to per- and polyfluoroalkyl substances (PFAS), but few studies have assessed associations with autism spectrum disorder (ASD).

Objectives: Our objective was to estimate associations of maternal prenatal PFAS concentrations with ASD and intellectual disability (ID) in children.

Methods: Participants were from a population-based nested case–control study of children born from 2000 to 2003 in southern California, including children diagnosed with ASD ($n = 553$), ID without autism ($n = 189$), and general population (GP) controls ($n = 433$). Concentrations of eight PFAS from stored maternal sera collected at 15–19 wk gestational age were quantified and compared among study groups. We used logistic regression to obtain adjusted odds ratios for the association between prenatal PFAS concentrations (parameterized continuously and as quartiles) and ASD versus GP controls, and separately for ID versus GP controls.

Results: Geometric mean concentrations of most PFAS were lower in ASD and ID groups relative to GP controls. ASD was not significantly associated with prenatal concentrations of most PFAS, though significant inverse associations were found for perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) [adjusted ORs for the highest vs. lowest quartiles 0.62 (95% CI: 0.41, 0.93) and 0.64 (95% CI: 0.43, 0.97), respectively]. Results for ID were similar.

Conclusions: Results from this large case–control study with prospectively collected prenatal measurements do not support the hypothesis that prenatal exposure to PFAS is positively associated with ASD or ID. https://doi.org/10.1289/EHP1830

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are man-made chemicals in wide use since the 1950s for a variety of industrial and commercial purposes, including most commonly as a coating or as a water or stain repellant in carpets and other products (DeWitt 2015; ATSDR 2016). PFAS have been detected with some variability in countries around the world in both sea and drinking water sources (Fromme et al. 2009; Zhao et al. 2012). In the United States, more than 95% of participants in the National Health and Nutrition Examination Survey (NHANES) study (1999–2008) had detectable serum concentrations of a number of PFAS in their bloodstream (Kato et al. 2011). Results from an *in vitro* study using cells derived from rat adrenal medulla suggested that some PFAS may affect neuronal cell development and cell differentiation (Slotkin et al. 2008), whereas animal *in vivo* studies have linked prenatal exposure to high levels of PFAS with low birth weight and reductions in thyroid hormone levels (Lau et al. 2004), as well as with hyperactivity and reduced habituation in adult mice (Johansson et al. 2008). In humans, PFAS can cross the placenta (Kim et al. 2011; Gützkow et al. 2012) and have been detected in breast milk (Fromme et al. 2010). A possible association between PFAS and thyroid hormones, which are known to be important in brain development (Rovet 2014), has limited evidence from two recent, small studies examining cord blood PFAS and neonatal thyroid hormone levels (de Cock et al. 2014; Shah-Kulkarni et al. 2016). Thus, exposure to the developing child during susceptible time periods of critical brain development is of potential concern. However, research on prenatal exposure to PFAS and neurodevelopmental outcomes in children has been somewhat limited.

Autism spectrum disorder (ASD) is characterized by impairments in social interaction and communication and by the presence of restricted, stereotyped interests and behaviors (American Psychiatric Association 2013). Both ASD and intellectual disability (ID)—often comorbid with ASD—are complex neurodevelopmental disorders that have suspected prenatal environmental influences and evidence of early disruption of neurodevelopmental processes (Rodier 2000; Scorza and Cavalheiro 2011; Lyall et al. 2017a). Prior work has suggested that environmental chemicals, including other persistent organic pollutants, may influence these outcomes (Landrigan 2010; Cheslack-Postava et al. 2013; Eskenazi et al. 2013; Grandjean and Landrigan 2014; Lyall et al. 2017b). However, only one study to our knowledge has examined prenatal PFAS exposure in association with ASD diagnosis, a case–control investigation that did not find evidence for increased risk with the PFAS measured (Liew et al. 2015). Another study, a prospective cohort examining several PFAS, including perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoate (PFNA), and perfluorohexane sulfonate (PFHxS), in association with autistic behaviors (as measured by Social Responsiveness Scale scores), reported an inverse association with serum concentrations of PFOA (Braun et al. 2014). More broadly, prenatal exposure to PFAS has been associated with various behavioral domains that

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have applicability to impairments in ASD or ID. Specifically, cord blood PFOS levels have been linked with lower gross and fine motor test scores in 2-y-old children (Chen et al. 2013); PFOA measured in maternal serum has been associated with lower mental development scores in girls at 6 mo of age [though not in boys, and associations were not seen with other outcomes or PFAS (Goudarzi et al. 2016)]; and maternal serum PFOS has been associated with lower behavior regulation, metacognition, and global executive functioning scores in children at 5 and 8 y of age [though again, no associations were found with other PFAS measured (Vuong et al. 2016)].

Given the limited research in this area, as well as biological plausibility for an association, we examined maternal prenatal PFAS serum concentrations in association with offspring ASD as well as intellectual disability without ASD.

Methods

Subjects

Subjects were from the Early Markers for Autism (EMA) study, a population-based, nested case–control study described in detail elsewhere (Croen et al. 2008). EMA utilizes archived specimens from mother–baby pairs to examine early biomarkers and their relationship with ASD. Women were eligible for inclusion in EMA if they participated in the prenatal expanded alpha-fetoprotein screening program in Orange, San Diego, and Imperial Counties, California; delivered a live-born infant between July 2000 and September 2003; and had stored specimens from prenatal and neonatal screening. Three groups of children, 4.5–9 y of age at time of data linkages in 2007 and 2009, born to these women were selected: *a*) children with ASD, *b*) children with developmental delay or intellectual disability without ASD (ID) (originally classified as mental retardation of unknown etiology according to terminology consistent with DSM-IV), and *c*) general population controls (GP), with a goal of including 400 in each group. Children with ASD or ID were identified from the California Department of Developmental Services (DDS), which coordinates services for individuals with ASD and other developmental disabilities. All individuals with substantial disabilities (as defined in California Welfare and Institutions Code) that begin before the 18th birthday are eligible for DDS services. GP controls were randomly selected within strata of matching factors from the birth certificate files after excluding DDS clients and infant deaths (with the resulting group being the eligible GP control group). GP controls (originally oversampled from the eligible GP control group to account for potential nonavailability of biospecimens) were frequency matched to ASD cases by sex, and by their month and year of birth. The distribution of maternal race, ethnicity, immigration status, education, and health insurance status is similar in the total DDS population (source of ASD and ID individuals) as the total live-birth population (source of GP controls; see Table S1). It has been estimated that DDS captures $\sim 80\%$ of ASD cases in the state of California, with milder cases being more likely to be missed (Windham et al. 2011). The institutional review boards (IRBs) of the California Department of Public Health and Kaiser Permanente Northern California approved the study protocol and methods. Further, study activities were approved by the California State Committee for the Protection of Human Subjects. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subject research. For these analyses, we used EMA participants with prenatal PFAS measurements available and final diagnostic classification (further described below).

Diagnostic Verification

Following a protocol developed by the Metropolitan Atlanta Developmental Disabilities Surveillance Program (Yeargin-Allsopp et al. 2003), trained medical record abstractors reviewed and compiled diagnostic and clinical data from DDS Regional Center records for children receiving services for ASD or ID. Expert clinical review of abstracted data was then conducted by a developmental pediatrician to confirm the ASD diagnoses for this study using *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV-TR) criteria (American Psychiatric Association 1994), with quality control checks performed in 10% of records (by a separate pediatrician). The initial group selected for whom biospecimens were available consisted of 430 ASD cases, 439 frequency-matched controls, and 328 children with ID; following expert (e.g., ASD specialist) clinician review of records, 136 children (24% of the final ASD case group) were reclassified from ID to the ASD case group on the basis of meeting DSM-IV criteria for ASD. Conditional logistic regression was not used in order to include all individuals classified as ASD following clinician review of records; rather, matching factors were accounted for as covariates in all analyses. The developmental pediatrician involved in the study also reviewed abstracted data to classify ASD cases according to the presence or absence of comorbid ID based on composite (total) scores from standardized cognitive and functional assessments in DDS records, when available. Children were classified as having intellectual disability if all composite score(s) on these assessments were ≤ 70, as not having ID if any recorded composite score was >70), and as unknown ID status if there were no standardized scores available in the chart.

Specimen Collection

Maternal mid-pregnancy specimens were retrieved from the California Department of Public Health's prenatal screening specimen archive. Approximately 70% of women in California participate in prenatal screening, with samples stored as part of the California biobank from 7–10 diverse counties throughout the state. As part of routine prenatal expanded alpha-fetoprotein (XAFP) screening, approximately 200,000 maternal serum and blood cell–pellet specimens were collected at 15–19 wk gestation from pregnant women living in three southern California counties according to residence at delivery in 2000–2003. Specimens were stored by obstetrical care service providers in serum separator tubes that underwent XAFP testing within 7 d of collection at a central laboratory (median time = 3 d). Leftover specimens were stored at -20°C after 1–2 d under refrigeration. Consent forms for the XAFP screening program were distributed at the time of the blood collection. These forms stipulated that specimens and results from prenatal testing could be used for legitimate research purposes given appropriate IRB approval, which the EMA study received; thus, individual mothers were not recontacted for the purposes of this study.

Laboratory Analysis of Serum for PFAS

We measured eight PFAS using a modification of a published method (Kuklenyik et al. 2005) [current acronyms (Buck et al. 2011) followed by previously used acronyms as applies in parentheses: perfluorooctane sulfonamide (FOSA/PFOSA), 2-(*N*-ethylperfluorooctane sulfonamido) acetate (Et-FOSAA/Et-PFOSA-AcOH), 2-(*N*-methyl-perfluorooctane sulfonamido) acetate (Me-FOSAA/Me-PFOSA-AcOH), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), PFOA, perfluorononanoate (PFNA), and perfluorodecanoate (PFDA/PFDeA)]. Briefly, isotope-labeled internal standards were used for

quantification. To compensate for the lack of isotope-labeled internal standard for PFHxS and to account for potential matrix effects, we spiked the calibration standards into calf serum. Analyte concentrations were detected by negative-ion TurboIonSpray–tandem mass spectrometry on an API 4,000 mass spectrometer (Applied Biosystems, Foster City, CA). The limits of detection (LODs) were 0.08 ng/mL (PFNA), 0.1 ng/mL (PFOSA, Et-PFOSA-AcOH, PFHxS, PFOA), and 0.2 ng/mL (PFOS, Me-PFOSA-AcOH, PFDeA). Low-concentration quality control materials (QCs) and high-concentration QCs, prepared from a calf serum pool, were analyzed with the unknown samples and with reagent and serum blanks to ensure the accuracy and reliability of the data (Kuklenyik et al. 2005).

Statistical Methods

Univariate statistics were calculated to compare the ASD, ID, and GP groups by basic demographic characteristics. For each PFAS, the percentage of the study population with nondetectable concentrations (<LOD) was calculated. For further analysis, values below the LOD were replaced with the LOD divided by the square root of 2 (Ogden 2010). Median and geometric mean concentrations for all PFAS were then calculated and compared by diagnostic group and to concentrations seen in the National Health and Nutrition Examination Survey (NHANES) sampling years 2003–2004 (CDC 2015).

Logistic regression was used to obtain crude and adjusted odds ratios (ORs) of the association between prenatal PFAS serum concentrations and ASD, and separately ID, relative to GP controls. (Alternate diagnostic categorizations for outcomes were also considered; see secondary analyses below.) Analytes with the majority of the study population (>60%) with values less than LOD (PFDeA and PFOSA) were included in further analyses only as binary variables, comparing those individuals with detectable versus those with nondetectable concentrations, in order to avoid having results based primarily on substituted values. For the remaining PFAS, concentrations were examined both continuously and categorically in quartiles and using a binary variable for above versus below the median value. Quartiles were created based on the distribution of the PFAS in controls. Tests of trend across quartiles were conducted using a Wald-test for the comparison of the median value in each quartile. For comparisons of continuous PFAS concentrations in logistic regression models, concentrations were $\log_{10}$ transformed in order to reduce the influence of outliers. We also examined potential nonlinearity in the association between PFAS and neurodevelopmental outcomes using cubic splines (Durrleman and Simon 1989; Govindarajulu et al. 2007). The following settings were used in these models [with software freely available via https://www.hsph.harvard.edu/donna-spiegelman/software/lgtphcurv9/ and documented according to Li et al. (2011)]: number of knots = 4 (with automatic/unspecified placement) and stepwise selection of knot points (with $p < 0.05$ for entry into model and to stay in the model); these models were adjusted as in primary models.

Covariates were selected based on *a priori* knowledge of associations with ASD and PFAS, as well as evidence of associations with both serum concentrations and ASD in this study population. Final models included adjustment for the study matching factors (child sex, month and year of birth), as well as maternal age (continuous), country of maternal birth (United States, Mexico, or other), maternal weight at blood draw (in categories as shown in Table 1), maternal race/ethnicity (non-Hispanic white, Hispanic, Asian, other), parity (multi- vs. primiparous), and maternal education (less than high school education, high school degree, college, and graduate degree). Separate categories were used for missing covariate data (~1% or less of each study group for maternal education and race/ethnicity). Adjustment for low birth weight (as a binary variable, <2,500 g), preterm birth (as a binary variable, <37 wk), paternal age (as a continuous variable), and insurance status at delivery (as a categorical variable: government, private, or self and other) was considered, but because these variables were not associated with both the exposures and outcome in our data and did not change the estimate by >10%, they were not included in final models. Secondary models explored further adjustment for other factors (described below).

Table 1. Characteristics of the study population by diagnostic group.

Characteristic	ASD $n = 553$	ID $n = 189$	GP $n = 433$
Maternal age (mean ± SD)	29.9 ± 5.6	27.1 ± 6.3	28.7 ± 5.4
Paternal age (mean ± SD)	32.9 ± 6.3	30.4 ± 7.5	31.5 ± 6.1
Child birth year [*n* (%)]			
2000	102 (18)	45 (24)	81 (19)
2001	144 (26)	54 (29)	112 (26)
2002	220 (40)	70 (37)	174 (40)
2003	87 (16)	20 (11)	66 (15)
Multiparous [*n* (%)]	300 (54)	124 (66)	267 (62)
Maternal birth place [*n* (%)]			
United States	275 (50)	84 (44)	209 (48)
Mexico	132 (24)	87 (46)	132 (31)
Other	144 (26)	18 (10)	92 (21)
Maternal race/ethnicity [*n* (%)]			
Non-Hispanic white	192 (35)	35 (19)	144 (33)
Asian	83 (15)	9 (5)	47 (11)
Black, Pacific Islander, and other	48 (9)	13 (7)	36 (8)
Hispanic	225 (41)	131 (69)	203 (47)
Missing	5 (1)	1 (0.5)	3 (0.7)
Maternal education [*n* (%)]			
Less than high school	99 (18)	79 (42)	106 (24)
High school	119 (22)	50 (26)	117 (27)
Some college/college degree	226 (41)	47 (25)	147 (34)
Post-graduate	101 (18)	11 (6)	59 (13)
Missing	8 (1.5)	2 (1)	4 (1)
Maternal weight at blood draw (lbs) [*n* (%)]			
<127.5	145 (26)	34 (18)	114 (26)
127.5–≤145	139 (25)	42 (22)	117 (27)
>145–≤169	137 (25)	55 (29)	100 (23)
>169	132 (24)	58 (31)	102 (24)
Insurance status at delivery [*n* (%)]			
Self and other	19 (3)	7 (4)	18 (4)
Private insurance	289 (53)	52 (27)	215 (50)
Government program	241 (44)	130 (69)	200 (46)
Child sex [*n* (%)]			
Male	453 (82)	107 (57)	359 (83)
Female	100 (18)	82 (43)	74 (17)
Child birth county [*n* (%)]			
Imperial	17 (3)	17 (9)	15 (3)
Orange	222 (40)	58 (31)	232 (54)
San Diego	314 (57)	114 (60)	186 (43)
Child preterm (<37 wk) [*n* (%)]	67 (13)	38 (21)	46 (11)
Child low birth weight (<2,500 g) [*n* (%)]	44 (8)	40 (22)	23 (5)
Maternal serum PCB138/158[a] concentrations[b] [*n* (%)]			
Quartile 1 (<3.2)	83 (16)	50 (28)	101 (25)
Quartile 2 (3.2–<5.5)	119 (22)	62 (35)	101 (25)
Quartile 3 (5.5–<8.9)	135 (25)	27 (15)	103 (25)
Quartile 4 (≥8.9)	197 (37)	37 (21)	103 (25)
Maternal serum PBDE153 concentrations[a] [*n* (%)]			
Quartile 1 (<2.3)	179 (34)	53 (30)	95 (23)
Quartile 2 (2.3–<4.2)	116 (22)	48 (27)	103 (25)
Quartile 3 (4.2–<8.8)	134 (25)	41 (23)	107 (26)
Quartile 4 (≥8.8)	104 (20)	34 (19)	102 (25)

Note: Data were complete unless otherwise indicated. ASD, autism spectrum disorder; GP, general population controls; ID, intellectual disability (without ASD).
[a]Concentrations in ng/g lipid. These congeners were previously associated with ASD and ID in this study population (Lyall et al. 2017b, 2017c).
[b]Previously associated with ASD and ID in this study population (Lyall et al. 2017b).

Table 2. Prenatal serum PFAS concentrations (ng/mL) according to outcome group and corresponding estimates for pregnant U.S. women from the NHANES study.

Compound	LOD (ng/mL)	Percent >LOD	GP [GM (95% CI)] $n = 433$	ASD [GM (95% CI)] $n = 553$	p-Value ASD vs. GP	ID [GM (95% CI)] $n = 189$	p-Value ID vs. GP	NHANES GM[a]
Et-PFOSA-AcOH	0.1	93	0.71 (0.66, 0.77)	0.68 (0.63, 0.73)	0.36	0.75 (0.66, 0.85)	0.48	Not reported
Me-PFOSA-AcOH	0.2	99	1.16 (1.09, 1.24)	1.14 (1.07, 1.23)	0.81	1.11 (1.01, 1.23)	0.49	Not reported
PFDeA	0.2	21	0.17 (0.16, 0.18)	0.17 (0.16, 0.18)	0.39	0.16 (0.15, 0.16)	0.01	Not reported
PFHxS	0.1	99	1.33 (1.24, 1.44)	1.39 (1.29, 1.49)	0.46	1.35 (1.19, 1.54)	0.85	1.14
PFNA	0.08	99	0.59 (0.55, 0.62)	0.60 (0.57, 0.63)	0.56	0.47 (0.43, 0.51)	<0.0001	0.70
PFOA	0.1	99.9	3.67 (3.49, 3.86)	3.58 (3.41, 3.76)	0.46	3.28 (3.01, 3.58)	0.02	2.39
PFOS	0.2	99.9	17.9 (17.0, 18.7)	17.5 (16.8, 18.3)	0.52	15.9 (14.6, 17.3)	0.01	12.29
PFOSA	0.1	39	0.10 (0.10, 0.11)	0.11 (0.10, 0.11)	0.57	0.10 (0.09, 0.11)	0.83	Not reported

Note: Mean values were derived after replacing values <LOD with the LOD divided by the square root of 2. CI, confidence interval; Et-PFOSA-AcOH, 2-(*N*-ethylperfluorooctane sulfonamido) acetate; GM, geometric mean; LOD, limit of detection; Me-PFOSA-AcOH, 2-(*N*-methylperfluorooctane sulfonamido) acetate; NHANES, National Health and Nutrition Examination Survey 2003–2004; PFAS, per and polyfluoroalkyl substances; PFDeA, perfluorodecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFOSA, perfluorooctane sulfonamide.
[a]Estimates for pregnant women from NHANES 2003–2004 reporting cycle (Woodruff et al. 2011). Geometric means were not reported for PFAS with detection frequencies <60%.

Sensitivity and Secondary Analyses

Analyses were rerun on the subgroup of ASD cases for whom GP controls were matched (excluding the 136 children reclassified from ID to ASD). To examine potential differences in the PFAS-ASD association by child sex, ethnicity, and maternal education, we re-ran models stratified by these factors (as male or female, Hispanic or non-Hispanic, and ≤high school or >high school, respectively). We also examined whether associations between ASD and prenatal PFAS differed according to comorbid ID status by separately examining ASD with and without ID (e.g., splitting the ASD case group based on this cognitive status information), relative to GP controls. Finally, we explored whether other factors may confound the association by determining whether estimates changed >10% after adjustment for *a*) county of residence at prenatal screening, or *b*) quartiles of prenatal lipid-adjusted serum concentrations of specific endocrine-disrupting chemicals previously associated with ASD in this EMA study (Lyall et al. 2017b) [specifically, polychlorinated biphenyl (PCB) 138/158) and polybrominated diphenyl ether (PBDE) 153]. We also examined whether adjustment for a measure of genetic inheritance (Price et al. 2006), derived from principal components (PC) analysis of available genome-wide data, altered results. Details of GWAS (genome-wide association study) and ancestry analyses in EMA are further described in another publication (Traglia et al. 2017); briefly, genome-wide multidimensional scaling (MDS) analysis was carried out on high-quality markers genotyped in maternal cell-pellet samples stored from the XAFP and in newborn samples collected through newborn screening, using a pairwise distance genomic matrix and the multidimensional scale functions implemented in PLINK software (Purcell et al. 2007) (–cluster and –mds plot). The resulting maternal and fetal genetic matrices included 10 principal components that summarized the genetic distance between each maternal and neonatal sample. The top two PCs, which accounted for the majority of variation, were used in ancestry-adjusted models here. PC-adjusted models included other potential confounders described above, with the exception of race/ethnicity, which would be accounted for by the PCs. Adjustment for PCs was not conducted for the ID to GP comparisons because genetic information was available only for the ASD and GP groups.

Results

After excluding children without serum PFAS data (due to inadequate samples), these analyses included 553 children with ASD, 189 children with ID, and 433 GP control children. A total of 291 children with ASD had comorbid ID, 224 had ASD without ID, and 38 children with ASD did not have information on ID status in their records to make this determination. Differences in a number of demographic factors were noted between the three primary diagnostic groups (Table 1). Mothers of ASD cases had higher age relative to other groups, lower parity, and greater likelihood of having higher education. By nature of the study design (which did not match ID cases to other groups) and the skewed sex ratio in autism, there was a much higher proportion of male children among ASD cases and GP controls compared with the ID group. Both ASD cases and ID children were more likely to be born preterm and low birth weight than GP controls, though these differences were greater for ID versus GP comparisons.

Geometric mean serum PFAS concentrations were similar between ASD and GP groups, but were significantly lower in ID versus GP children for PFOS, PFOA, PFNA, and PFDeA (Table 2). However, GMs for PFDeA concentrations should be interpreted with caution because 79% of samples were <LOD. Serum concentrations of PFAS in GP controls of our study population were comparable to those reported from the 2003–2004 NHANES for pregnant women (Woodruff et al. 2011) and were also comparable to those reported for all females (though a number of PFAS in NHANES had nondetectable concentrations) (CDC 2015). Descriptive statistics for the PFAS distribution by study group are shown in Table S2: Study groups had comparable distributions and percentages <LOD. Pairwise correlations were generally positive and statistically significant, particularly for the five PFAS with ≥99% of samples >LOD (see Table S3). The most strongly correlated PFAS were PFDeA and PFNA ($r = 0.69$) and PFOA and PFOS ($r = 0.55$).

In general, associations of prenatal PFAS with both ASD and ID were null to inverse (Tables 3 and 4). After adjustment for demographic factors (Model 2), ORs moved away from the null for those ORs <1 and toward or past the null for ORs >1. PFOA was inversely associated with both ASD and ID. For ASD, Model 2 ORs were significant for all quartiles (Q) compared with the lowest [Q4 vs. Q1 OR for the highest vs. lowest quartile = 0.62; 95% confidence interval (CI): 0.41, 0.93], with a significant but nonmonotonic trend (p-trend = 0.05; Table 3) and a negative slope for $\log_{10}$ PFOA as a continuous variable (OR = 0.78; 95% CI: 0.60, 1.01; Table 4). Corresponding ORs for PFOA and ID were slightly stronger (Q4 vs. Q1 OR = 0.48; 05% CI: 0.26, 0.88 and $\log_{10}$ OR = 0.63; 95% CI: 0.43, 0.92) but, as for ASD, ORs did not increase monotonically p-trend = 0.06. PFOS also was inversely associated with both outcomes, with a significant monotonic trend for ASD (Q4 vs. Q1 OR = 0.64; 95% CI: 0.43, 0.97 p-trend = 0.03; $\log_{10}$ OR = 0.77; 95% CI: 0.58, 1.01), and similar or slightly stronger associations with ID (Q4

Table 3. Odds ratios (95% CIs) for ASD and ID (vs. GP controls) in association with prenatal PFAS serum concentrations in quartiles.

Concentration (ng/mL)	GP control (*n*)	ASD case (*n*)	ASD vs. GP			ID (*n*)	ID vs. GP		
			Model 1 OR (95% CI)[a]	Model 2 OR (95% CI)[b]	*p*-Trend[c]		Model 1 OR (95% CI)[a]	Model 2 OR (95% CI)[b]	*p*-Trend[c]
Et-PFOSA-AcOH									
Q1 (<0.4)	118	165	1.0 (referent)	1.0	0.24	54	1.0	1.0	0.57
Q2 (0.4–<0.7)	101	137	0.93 (0.65, 1.35)	0.92 (0.63, 1.34)		37	0.79 (0.46, 1.36)	0.77 (0.44, 1.36)	
Q3 (0.7–<1.2)	107	126	0.79 (0.53, 1.18)	0.78 (0.51, 1.17)		44	0.68 (0.38, 1.21)	0.66 (0.36, 1.22)	
Q4 (≥1.2)	106	125	0.77 (0.50, 1.18)	0.75 (0.48, 1.18)		54	0.80 (0.44, 1.47)	0.73 (0.39, 1.22)	
Me-PFOSA-AcOH									
Q1 (<0.7)	113	163	1.0	1.0	0.37	52	1.0	1.0	0.54
Q2 (0.7–<1.1)	104	114	0.76 (0.53, 1.08)	0.66 (0.45, 0.96)		47	0.88 (0.53, 1.45)	0.94 (0.55, 1.59)	
Q3 (1.1–<1.9)	109	141	0.89 (0.63, 1.27)	0.81 (0.55, 1.18)		50	0.94 (0.57, 1.55)	1.08 (0.62, 1.87	
Q4 (≥1.9)	106	135	0.88 (0.62, 1.25)	0.73 (0.49, 1.09)		40	0.66 (0.39, 1.13)	0.82 (0.45, 1.50)	
PFHxS									
Q1 (<0.8)	112	144	1.0	1.0	0.94	48	1.0	1.0	0.60
Q2 (0.8–<1.3)	114	145	0.99 (0.70, 1.40)	0.87 (0.60, 1.25)		57	1.27 (0.78, 2.07)	1.43 (0.86, 2.40)	
Q3 (1.3–<2.0)	99	111	0.87 (0.60, 1.26)	0.73 (0.49, 1.07)		34	0.82 (0.48, 1.41)	1.03 (0.58, 1.85)	
Q4 (≥2.0)	107	153	1.11 (0.79, 1.58)	0.93 (0.63, 1.37)		50	1.04 (0.63, 1.72)	1.30 (0.74, 2.29)	
PFNA									
Q1 (<0.4)	131	159	1.0	1.0	0.26	83	1.0	1.0	0.17
Q2 (0.4–<0.6)	119	150	1.04 (0.74, 1.45)	0.92 (0.65, 1.32)		60	0.77 (0.49, 1.19)	0.92 (0.58, 1.46)	
Q3 (0.6–<0.8)	84	102	1.00 (0.69, 1.45)	0.75 (0.50, 1.13)		22	0.40 (0.23, 0.71)	0.57 (0.30, 1.06)	
Q4 (≥0.8)	98	142	1.20 (0.84, 1.70)	0.78 (0.51, 1.19)		24	0.41 (0.24, 0.71)	0.68 (0.35, 1.31)	
PFOA									
Q1 (<2.8)	113	182	1.0	1.0	0.05	67	1.0	1.0	0.06
Q2 (2.8–<3.8)	110	113	0.63 (0.44, 0.90)	0.56 (0.39, 0.81)		40	0.50 (0.30, 0.82)	0.44 (0.26, 0.76)	
Q3 (3.8–<5.2)	103	119	0.72 (0.50, 1.02)	0.58 (0.40, 0.86)		47	0.71 (0.49, 1.15)	0.67 (0.39, 1.14)	
Q4 (≥5.2)	106	139	0.82 (0.58, 1.15)	0.62 (0.41, 0.93)		35	0.50 (0.30, 0.83)	0.48 (0.26, 0.88)	
PFOS									
Q1 (<13.5)	109	148	1.0	1.0	0.03	64	1.0	1.0	0.17
Q2 (13.5–<18.3)	107	141	0.97 (0.68, 1.38)	0.85 (0.58, 1.23)		39	0.58 (0.35, 0.97)	0.61 (0.36, 1.05)	
Q3 (18.3–<26.1)	108	139	0.95 (0.66, 1.35)	0.74 (0.50, 1.09)		49	0.68 (0.49, 1.11)	0.80 (0.46, 1.38)	
Q4 (≥26.1)	108	125	0.85 (0.59, 1.22)	0.64 (0.43, 0.97)		37	0.52 (0.36, 0.87)	0.59 (0.32, 1.09)	
PFAS categorized as binary variables due to low detection rates in the study population									
PFDeA									
Non-detect (<0.2)	342	413	1.0	1.0	NA	168	1.0	1.0	NA
Detect (≥0.2)	91	140	1.28 (0.94, 1.74)	0.99 (0.69, 1.42)		21	0.52 (0.31, 0.89)	0.89 (0.46, 1.71)	
PFOSA									
Non-detect (<0.1)	268	331	1.0	1.0	NA	117	1.0	1.0	NA
Detect (≥0.1)	165	222	1.12 (0.85, 1.47)	1.07 (0.80, 1.43)		72	0.88 (0.59, 1.30)	0.96 (0.63, 1.47)	

Note: NA, not applicable.
[a]Model 1 adjusted for matching factors (child sex, month of birth, year of birth).
[b]Model 2 adjusted for matching factors, parity (multiparous vs. primiparous), maternal age (continuous), race/ethnicity (non-Hispanic white, Asian, Hispanic, other), weight at sample collection (continuous), and maternal birth place (United States, Mexico, other).
[c]*p*-Trend for Wald chi-square test of median values across quartiles (Model 2).

vs. Q1 OR = 0.59; 95% CI: 0.32, 1.09 *p*-trend = 0.17; $\log_{10}$ OR = 0.67; 95% CI: 0.45, 0.98). In general, Et-PFOSA-AcOH, Me-PFOSA-AcOH, PFHxS, and PFNA were inversely associated with ASD and ID, though ORs were close to the null, and for PFHxS and ID were slightly above the null (Tables 3 and 4). ORs for PFDeA and PFOSA (dichotomized as >LOD or <LOD) were close to the null for both outcomes.

Due to the small number of individuals with PFAS concentrations in the upper tails of our distribution, cubic spline results should be interpreted with caution. Exploring potential nonlinearity in the relationship between PFAS and ASD using these methods, the test of nonlinear relationship was significant for Me-PFOSA-AcOH and PFOA ($p = 0.009$ and $p = 0.001$ respectively); however, the former did not demonstrate a significant association with the outcome. Tests for other analytes indicated the relationship between the exposure and outcome, if any, was linear ($p < 0.0001$ from the test of linearity). Examining graphs of these adjusted relationships (which,

Table 4. Odds ratios (95% CI) of ASD and ID (vs. GP controls) in association with (log-transformed) continuously-modeled concentrations of prenatal PFAS.

PFAS	ASD ($n = 553$)		ID ($n = 189$)	
	Model 1[a]	Model 2[b]	Model 1	Model 2
Et-PFOSA-AcOH	0.93 (0.65, 1.35)	0.90 (0.74, 1.10)	0.95 (0.73, 1.25)	0.93 (0.70, 1.24)
Me-PFOSA-AcOH	0.98 (0.82, 1.17)	0.91 (0.75, 1.11)	0.83 (0.63, 1.08)	0.92 (0.68, 1.25)
PFHxS	1.06 (0.91, 1.24)	0.99 (0.83, 1.17)	0.99 (0.79, 1.23)	1.11 (0.86, 1.42)
PFNA	1.07 (0.85, 1.34)	0.76 (0.57, 1.02)	0.48 (0.34, 0.67)	0.62 (0.42, 0.92)
PFOA	0.92 (0.74, 1.15)	0.78 (0.60, 1.01)	0.65 (0.47, 0.89)	0.63 (0.43, 0.92)
PFOS	0.92 (0.73, 1.17)	0.77 (0.58, 1.01)	0.63 (0.45, 0.87)	0.67 (0.45, 0.98)

Note: Table includes only those PFAS with majority (>60%) of study population with values above LOD. ASD, autism spectrum disorder; CI, confidence interval; Et-PFOSA-AcOH, 2-(*N*-ethylperfluorooctane sulfonamide) acetate; GP, general population; OR, odds ratio; Me-PFOSA-AcOH, 2-(*N*-methyl perfluorooctane sulfonamide) acetate; PFAS, per and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.
[a]Model 1 adjusted for matching factors (child sex, month of birth, year of birth). (Crude models yielded nearly identical estimates for ASD analysis and similar estimates for ID analyses.)
[b]Model 2 adjusted for matching factors as well as parity, and maternal age, race/ethnicity, weight at sample collection, and birth place. Additional adjustment for paternal age, low birthweight, or insurance status at delivery did not materially alter results.

again, should be interpreted with caution, particularly at the upper tails), confidence bounds were wide, and with the exception of PFOA, overlapped the null for the full range of exposure values (see Figure S1). These graphs, which allowed for flexibility in the relationship between PFAS and ASD, suggested consistently below-the-null estimates for Et-PFOSA-AcOH, PFNA, and PFOS (with confidence intervals overlapping the null). Although estimates above the null were seen for high concentrations of Me-PFOSA-AcOH, PFHxS, and PFOA, this was only (with the exception of PFHxS) when concentrations were higher than the 95th percentile reported in NHANES for females (CDC 2015) and was based on sparse data. Confidence intervals for these higher ORs were wide and included the null, demonstrating the high degree of uncertainty in the estimates. Cubic spline analyses were not conducted for PFDeA and PFOSA, owing to the large proportion of the study population with concentrations below limits of detection for these chemicals, which would require basing results primarily on imputed data.

Secondary and Sensitivity Analysis Results

Results were similar in secondary and sensitivity analyses conducted (see Tables S4–S7). Overall, ORs were similar for ASD with and without ID, though estimates for PFOS were stronger (e.g., further below the null) for the ASD with ID group than for ASD without ID, which tended toward the null (see Table S5). Stratified by sex (see Table S5), results were generally similar in females, though effect estimates were above the null for PFOSA and PFDeA examined as the binary detect versus nondetect variables. However, confidence intervals for these analytes, as well as others, were quite wide, owing to the smaller number of female cases and controls (see Table S5). Though there was some variability in estimates across strata defined as Hispanic or non-Hispanic ethnicity (see Table S6), clear patterns were not seen, and estimates were not strongly different across strata. When stratified by maternal education (see Table S7), associations of PFAS with ASD were further from the null among mothers with lower education as compared with those for higher education, whereas the reverse was true for associations with ID.

Discussion

Results from this large, population-based case–control study with prospectively collected maternal prenatal biospecimens did not suggest an increase in odds of ASD or ID following prenatal exposure to PFAS. Instead, within the range of serum PFAS concentrations in this study population, null, and for certain PFAS, unexpected inverse associations, were observed.

The inverse associations most consistently seen across analyses were for PFOA and PFOS, the PFAS detected at the highest concentrations in the general population (CDC 2015). These were also two of the top three analytes with largest variability in our study population and are among the PFAS that are more persistent (Sargent and Seffl 1970; ATSDR 2016), with a serum half-life ranging from 2.3 to 5.8 y (Olsen et al. 2012). The unexpected direction of results could be due to some unmeasured confounder(s) that is/are negatively associated with ASD and ID and positively associated with concentrations of these PFAS. Although finer adjustment for socioeconomic status (SES) may be one potential bias of concern, SES tends to have a different relationship with ASD versus ID in the United States (Rai et al. 2012; Thomas et al. 2012; Sun et al. 2014; Durkin et al. 2017) and is generally not positively associated with PFAS concentrations (Apelberg et al. 2007a; Lien et al. 2013). We adjusted for a number of demographic and other covariates (including proxies for SES-like insurance status and maternal birth place, education level, and race/ethnicity) and also considered multiple parameterizations of the exposure (further discussed below), which generally did not alter conclusions. Results were generally fairly consistent across stratified and secondary analyses, though our finding in secondary analyses of a potentially stronger inverse association with PFOS for ASD with ID than for ASD without ID deserves further study.

In prior work, exposure to certain PFAS—primarily PFOS—measured in prenatal maternal serum samples or cord blood in humans has been associated with factors related to ASD and ID, such as low birth weight (Apelberg et al. 2007b; Chen et al. 2012; Manzano-Salgado et al. 2017), motor delays (Chen et al. 2013), and poorer scores on an executive functioning assessment (Vuong et al. 2016). However, results are inconsistent. Some cross-sectional studies conducted in the United States have linked higher concentrations of certain PFAS measured in existing cases of children with ADHD, including one study of 48 cases that reported modest associations with PFOS, PFOA, and PFHxS but not PFNA (Hoffman et al. 2010). Another study, including 1,303 children with ADHD and 9,243 without in the C8 Health Project, reported an association for PFHxS but not PFOS or PFNA (Stein and Savitz 2011). A number of prospective studies, with exposure measurements representing the suspected critical windows of neurodevelopment relevant to these conditions, have not found associations with PFAS and neurodevelopmental outcomes. Specifically, two Norwegian studies with prenatal concentrations comparable to those in our study reported no associations between cord serum PFOS, PFOA, or PFNA with ADHD [a case–control study with 206 children in each outcome group (Ode et al. 2014)], or for maternal serum PFOA or PFOS with ADHD (27 cases), depression (104 cases), or scholastic achievement scores (Strom et al. 2014). In a subset of 320 participants of the C8 Health Project of Ohio, where participants had much higher than background PFAS levels due to contaminated drinking water, *in utero* PFOA exposure was estimated (though not directly measured prenatally), and overall, adverse associations with neurodevelopmental measures were not noted (Stein et al. 2013). A few inverse associations were found; children born to mothers with PFOA concentrations estimated at the highest quartile had fewer ADHD symptoms, as well as higher full scale IQ scores at 6–12 y of age, when compared with children born to mothers in the lowest quartile of PFOA, but no consistent patterns across measures were noted and the authors deemed these findings "sporadic" (Stein et al. 2013). We are aware of only two studies of prenatal PFAS in association with ASD or ASD related symptoms (Braun et al. 2014; Liew et al. 2015). In the first, in the HOME (Health Outcomes and Measures of the Environment) study of 175 Cincinnati-area U.S. women, fewer autistic behaviors (as measured by the Social Responsiveness Scale) were associated with higher prenatal PFOA concentrations, but no associations were noted with PFOS, PFNA, or PFHxS (Braun et al. 2014). In the second, a Danish study including 220 autism cases identified through the Danish Psychiatric Register and 550 controls, no association with prenatal PFOA or PFOS, or 14 other PFAS, was found (Liew et al. 2015). These studies also had PFAS levels within the range of our study, and generally overlapping with each other. Overall, findings across prospective analyses are generally consistent with our results for ASD and ID, with the suggestion that, within current, commonly experienced exposure, prenatal PFAS exposure does not increase odds of ID or ASD.

The inverse and null findings are contrary to what we had hypothesized given the endocrine-disrupting properties and

neurodevelopmental impacts of PFAS in animals. Differences in concentrations may underlie discrepancies between animal and toxicological reports and those from human observational studies. It is also worth noting that in our work and in other human studies, in relative comparisons, referent groups do not include those truly "unexposed," but rather, those with lower levels, given the ubiquity of these chemicals. Though results from our cubic spline analysis suggested the potential for increases in odds of ASD at the highest concentrations of MePFOSA, PFHxS, and PFOA, these levels did not represent concentrations observed in our study population or in the majority of NHANES participants (CDC 2015), and confidence intervals were wide and overlapped the null—highlighting the importance of interpreting these spline results cautiously. The C8 Health Project discussed above, where unusually high levels were reported given contaminated water, found a reduction in ADHD risk (Stein and Savitz 2011) and a generally null-to-modest improvement in neuropsychological test scores in association with higher PFOA (Stein et al. 2013), findings not supportive of neurodevelopmental decrements with higher levels of this PFAS. It is possible that exposure to certain PFAS—or to some unidentified strongly correlated factor—could be neuroprotective; however, a causal mechanism that would explain such a relationship is not clear at this time.

A strength of this study is the assessment of PFAS exposure during a critical window of neurodevelopment and suspected susceptibility period for ASD. However, certain limitations should be noted. We did not have the ability to examine PFAS exposure in other time periods, such as during breastfeeding, though we hypothesize the prenatal period to be of key relevance to ASD risk. Our study included only women from southern California, and included a high proportion of Hispanics, and therefore may not be representative of the broader population. However, PFAS concentrations in our EMA study population are within the range of those from the national sample in NHANES. ASD and ID diagnoses were not based on direct clinical assessment, but relied on information recorded in service agency records. However, prior validation studies have confirmed the validity of ASD cases within DDS, with fewer than 2% of those identified with ASD in the DDS not meeting criteria for ASD according to gold-standard assessments (Hertz-Picciotto et al. 2006). The use of a standard protocol and expert review of records within our study also likely further minimized any potential misclassification of final case status. Although selection bias is possible, controls were randomly selected from the same geographic and birth years as cases. DDS cases may not be fully representative of all ASD cases in the state of California because milder cases have been shown to be somewhat less likely to be captured through this system; however, demographic factors for ASD cases captured and not captured by DDS are comparable, according to an assessment of 757 DDS-identified cases of ASD born in 1994 and 1996 and $\sim$80,000 births in corresponding years in California (Windham et al. 2011). Further, comparing characteristics of women participating in prenatal screening to all women in study counties and birth years demonstrated similarity with respect to education level, race/ethnicity, and health insurance status (see Table S1) as well as maternal age, place of birth, and parity, (unpublished data), suggesting comparability of our source population to the total birth population. Though we adjusted for a number of factors, including demographics and certain proxies of socioeconomic status (SES), we cannot rule out potential residual confounding by SES factors not measured or fully captured here. An additional limitation to note is that the small number of females in our study, owing to the skewed sex ratio in autism, limited our ability to examine sex differences with a high degree of statistical power. Finally, genetic variation may influence the role that these chemicals play in risk of neurodevelopmental outcomes, and we did not have the ability to examine gene by environment interaction here.

Conclusions

Results from this study suggest that prenatal exposure to PFAS resulting in serum concentrations consistent with those reported for the general population do not increase odds of ASD or ID. Instead, we found evidence for inverse associations for those with the highest levels of PFOA and PFOS. Further insight may be gained by considering potential interactions or PFAS with other exposures or genes, examining effects in subgroups, and from work examining the impact of prenatal exposure to other endocrine-disrupting chemicals and persistent organic pollutants on offspring neurodevelopment.

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