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Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) and associations with attention-deficit/hyperactivity disorder and autism spectrum disorder in children

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ABSTRACT

Background: Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) may be a risk factor for neurodevelopmental deficits and disorders, but evidence is inconsistent.

Objectives: We investigated whether prenatal exposure to PFAS were associated with childhood diagnosis of attention-deficit/hyperactivity disorder (ADHD) or autism spectrum disorder (ASD).

Methods: This study was based on the Norwegian Mother, Father and Child Cohort Study and included n = 821 ADHD cases, n = 400 ASD cases and n = 980 controls. Diagnostic cases were identified by linkage with the Norwegian Patient Registry. In addition, we used data from the Medical Birth Registry of Norway. The study included the following PFAS measured in maternal plasma sampled mid-pregnancy: Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptanesulfonic acid (PFHpS), and perfluorooctane sulfonate (PFOS). Relationships between individual PFAS and ADHD or ASD diagnoses were examined using multivariable adjusted logistic regression models. We also tested for possible non-linear exposure-outcome associations. Further, we investigated the PFAS mixture associations with ASD and ADHD diagnoses using a quantile-based g-computation approach.

Results: Odds of ASD was significantly elevated in PFOA quartile 2 [OR = 1.71 (95% CI: 1.20, 2.45)] compared to quartile 1, and PFOA appeared to have a non-linear, inverted U-shaped dose-response relationship with ASD. PFOA was also associated with increased odds of ADHD, mainly in quartile 2 [OR = 1.54 (95% CI: 1.16, 2.04)] compared to quartile 1, and displayed a non-linear relationship in the restricted cubic spline model. Several PFAS (PFUnDA, PFDA, and PFOS) were inversely associated with odds of ADHD and/or ASD. Some of the associations were modified by child sex and maternal education. The overall PFAS mixture was inversely associated with ASD [OR = 0.76 (95% CI: 0.64, 0.90)] as well as the carboxylate mixture [OR = 0.79 (95% CI: 0.68, 0.93)] and the sulfonate mixture [OR = 0.84 (95% CI: 0.73, 0.96)].

Conclusion: Prenatal exposure to PFOA was associated with increased risk of ASD and ADHD in children. For some PFAS, as well as their mixtures, there were inverse associations with ASD and/or ADHD. However, the inverse associations reported herein should not be interpreted as protective effects, but rather that there could be some unresolved confounding for these relationships. The epidemiologic literature linking PFAS exposures with neurodevelopmental outcomes is still inconclusive, suggesting the need for more research to elucidate the neurotoxicological potential of PFAS during early development.

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) refer to a large group of synthetic compounds developed for use in various industrial processes and in a multitude of different products such as firefighting foam, textiles, cooking pans, and food packaging (Buck et al., 2011; Kissa, 2001). Due to extensive production and usage of PFAS after being produced in the late 1940s, it is now a globally spread environmental contaminant group (Wang et al., 2017a). Because of its high persistence in the environment, PFAS bioaccumulate in organisms (including humans) and in food chains (EFSA, 2018). Fish and seafood are the most important sources of exposure for human populations (Brantsæter et al., 2013; Haug et al., 2010; Papadopoulou et al., 2019). Due to phase-out by major producers as well as international restrictions, levels of some PFAS, particularly perfluorooctane sulfonate (PFOS), have declined in the environment and in humans during the last ten to 15 years (EFSA, 2018; Land et al., 2018; Mariussen, 2012). However, there has been an increase in production and emission of perfluoroalkyl carboxylic acids (PFCAs) and some precursors from China and other countries in Asia (Wang et al., 2014). There are uncertainties about the health effects of these new replacement compounds that are currently on the rise (Sunderland et al., 2019; Wang et al., 2017a).

Although PFAS such as PFOS and perfluorooctanoic acid (PFOA) have been shown to adversely affect several aspects of human health such as immune function, endocrine systems, and liver function (EFSA, 2018; Liew et al., 2018), there is still limited knowledge concerning potential adverse effects on intrauterine brain development (Liew et al., 2018; Vrijheid et al., 2016). The fetus is especially vulnerable to toxic chemicals, including PFAS, which are transferred from mother to fetus via the placenta and enter the fetal brain via an undeveloped blood-brain barrier and may disrupt the finely tuned brain developmental processes and timing (Grandjean and Landrigan, 2014; Gützkow et al., 2012; Kato et al., 2014). Results from animal and *in vitro* studies suggest that PFAS are developmental neurotoxicants, affecting several neurochemical targets in the developing brain (Johansson et al., 2009; Mariussen, 2012; Slotkin et al., 2008; Viberg et al., 2013). Additionally, PFAS have endocrine-disruptive abilities and can affect the maternal and fetal thyroid or sex steroid hormones, which are important for a normal development of the fetal nervous system and brain (De Cock et al., 2012; Mariussen, 2012; Tran and Miyake, 2017). Furthermore, humans are not only exposed to one chemical at a time, but multiple compounds. Chemical mixtures can induce different or stronger health effects than single chemicals. In mixtures, chemicals, may interact additively, or even synergistically or antagonistically (Henn et al., 2014; Rauh and Margolis, 2016). Accordingly, *in utero* exposure to multiple PFAS may disrupt normal brain development and thereby increase risk of neurocognitive deficits, behavioral problems, and neurodevelopmental disorders such as autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD).

ADHD and ASD interfere with normal functioning and learning during childhood and adolescence (Antshel et al., 2016; Kern et al., 2015). Childhood ASD has a prevalence between 1% and 2% in the Nordic countries and in the United States (Hansen et al., 2015; Idring et al., 2015; Lyall et al., 2017; Surén et al., 2012). It comprises heterogeneous disorders that are characterized by persistent deficits in social communication and social interaction, in addition to restricted and repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013). ADHD is one of the most common neurodevelopmental disorders characterized by inattention, impulsivity, and hyperactivity (American Psychiatric Association, 2013) and associated with a range of sub-optimal long-term outcomes, including low educational levels and low life satisfaction (Rauh and Margolis, 2016). Globally, ADHD affects approximately 3–4% of children (Polanczyk et al., 2007). Both ADHD and ASD are more prevalent in boys compared to girls (Nussbaum, 2012; Polanczyk et al., 2007; Werling and Geschwind, 2013). The etiologies of these disorders are multifactorial in which heredity is thought to play a major role (Faraone et al., 2005; Kern et al., 2015; Sandin et al., 2014; Thapar et al., 2017). However, environmental risk factors, including *in utero* toxicant exposure, may interact with genetic factors (Nuttall, 2017; Sandin et al., 2014; Thapar et al., 2013). Children with ADHD and ASD share several overlapping genetic and psychopathological traits indicating that there may be common risk factors or etiological mechanisms at play (Kern et al., 2015; Martin et al., 2018). As toxicant exposure in pregnant women and their fetuses are potentially modifiable, it is of high importance to investigate their contribution to the risks of these disorders.

Several studies have reported higher levels of some PFAS among pregnant women with higher socioeconomic status (SES), indicated by higher education and income (Brantsæter et al., 2013; Montazeri et al., 2019; Tyrrell et al., 2013). This is probably to some degree related to the higher consumption of fish and seafood among those with higher educational levels (e.g. Touvier et al., 2010). In addition, maternal education has been associated with ASD and ADHD in children (Lung et al., 2018; Torvik et al., 2020). There are studies reporting differential relationships by child sex, which could be a consequence of altered prenatal sex steroid levels, and/or sex-specific neurodevelopmental vulnerabilities (Baron-Cohen et al., 2019; Wang et al., 2017b; Werling and Geschwind, 2013). Although the experimental literature has demonstrated PFAS' potential for developmental neurotoxicity in the human brain (Mariussen, 2012), the epidemiological literature has been mixed, with some studies finding increased risks of adverse outcomes, whereas others found none or even report inverse associations (Forns et al., 2020; Liew et al., 2018; Rappazzo et al., 2017; Vrijheid et al., 2016). There is a lack of investigation of prenatal exposure to PFAS and their mixtures, as well as investigating the risk of childhood ADHD or ASD diagnoses as outcomes. Even if child sex and factors related to parental SES (e.g. maternal education) often are included as covariates in studies of PFAS and neurodevelopmental outcomes, very few investigate if these relationships differ by these important variables.

Against this background, the overall aim of the present study was to investigate the associations between prenatal exposure to seven PFAS, individually and as mixtures, and childhood diagnosis of ADHD and ASD. An additional goal was to explore whether these relationships were modified by child sex or maternal education (as a proxy for SES).

2. Methods

2.1. Study design and participants

The present study is based on data from the Norwegian Mother, Father and Child Cohort Study (MoBa) and the Medical Birth Registry of Norway (MBRN) with linkage to the Norwegian Patient Registry (NPR) (Bakken et al., 2020). MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Participants were recruited from all over Norway from 1999 to 2008. The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers (Magnus et al., 2016). Differences between those who consented vs non-consented are described elsewhere (Biele et al., 2019; Nilsen et al., 2009). The current study is based on version 12 of the quality-assured data files released for research in January 2019. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is now based on regulations related to the Norwegian Health Registry Act. The current study was approved by The Regional Committee for Medical and Health Research Ethics (ref. no. 2012/985–1). The NPR has approved the linkage between NPR and MoBa, identifying ADHD and ASD diagnostic cases. The NPR is a national health care registry that receives patient data on diagnoses reported from all hospitals and specialized health care services in Norway. The registry contains diagnoses for in-

and outpatients recorded from 2008 onward (Bakken et al., 2020). The diagnostic codes reported to the NPR are according to the International Statistical Classification of Diseases, 10th Revision (ICD-10). The MBRN is a national health registry containing information about all births in Norway. Blood samples were obtained from both parents at the free routine ultrasound examination around gestational week 18 and from mothers and children (umbilical cord) at birth.

2.2. Cases and controls

Based on available data, information on diagnosis of ADHD and ASD among children born in 2002 or later were obtained from the NPR. ADHD is a term used in the DSM system, while hyperkinetic disorder is the term used in ICD-10 (Thapar and Cooper, 2016). In the present study, we will use the term ADHD. For ADHD, we selected cases if they had at least two registrations of "hyperkinetic disorder" according to ICD-10 codes F90, F90.0, F90.1, F90.8 or F90.9 (World Health Organization, 1993). We required two registrations in order to exclude erroneous or false diagnoses (Surén et al., 2018). The ICD-10 criteria for hyperkinetic disorder are "early onset; a combination of overactive, poorly modulated behavior with marked inattention and lack of persistent task involvement; and pervasiveness over situations and persistence over time of these behavioral characteristics" (World Health Organization, 1993). Hyperkinetic disorder requires the combination of inattentive and hyperactive symptoms and is thus similar to the ADHD combined subtype in the DSM system (Thapar and Cooper, 2016). We selected cases of ASD if they had registrations of "pervasive developmental disorders" according to ICD-10 codes F84.0, F84.1, F84.5, F84.8 or F84.9 (World Health Organization, 1993). We could include all cases because the positive predictive value was high (Surén et al., 2012). For both ADHD and ASD, girls were oversampled among cases if possible. We retrieved a random sample of controls from MoBa that were frequency-matched to case categories on sex and birth year. In the present study, we included children that were: singletons, alive at 2 years of age (controls only), born in 2002 or later, had available record from the MBRN and available MoBa questionnaire 1, had no registration of Down's syndrome or of serious malformation in MBRN, and had available maternal blood samples (Fig. 1). The present study included 821 ADHD cases, 400 ASD cases, 980 controls, and their mothers (Fig. 1).

2.3. Exposures

We used maternal plasma sampled in gestational week 18 to measure PFAS concentrations. Details about the sampling procedure and handling and storage in the MoBa biobank is described in detail elsewhere (Paltiel et al., 2014). Nineteen PFAS were determined in maternal plasma (Table S1), using liquid chromatography-triple quadruple mass spectrometry (LC-MS/MS) as described previously (Haug et al., 2009). This method has been thoroughly validated and used for determination of more than 5000 serum/plasma samples so far, including approximately 2000 samples from MoBa (Singer et al., 2018). Only PFAS with levels above limit of quantification (LOQ) in >80% of the plasma samples were included in the present study; PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS) and PFOS. Internal quality control samples and procedure blanks were analyzed along with each batch of samples to ensure high quality of the determinations throughout the project. Case and control status were randomized to batch and blinded to the analysist. More detailed information on limits of detection (LOD) and limits of quantification (LOQ) can be found in Table S1.

2.4. Covariates and other variables

Potential covariates were obtained from the MBRN and MoBa questionnaires completed during pregnancy and up to child's age three years. The MoBa study also included a food frequency questionnaire (FFQ) completed at 22 weeks' gestation, which was designed to capture the average dietary intake during the first four to five months of pregnancy, providing good validity for estimates of foods and nutrients (Brantsæter et al., 2008). Potential adjustment variables were selected *a priori* based on existing literature using a directed acyclic graph (DAG) approach (Greenland et al., 1999; Textor et al., 2011). We considered

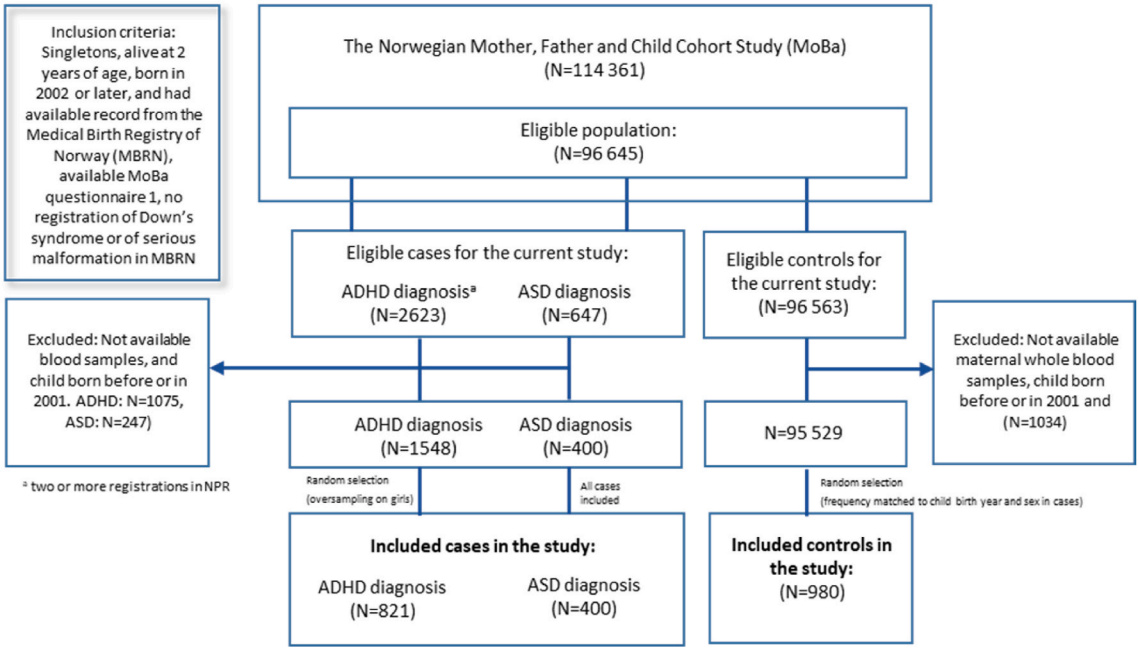

Fig. 1. Flow chart of recruitment of cases and controls in in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); Medical Birth Registry of Norway (MBRN); The Norwegian Mother, Father and Child Cohort Study (MoBa).

child sex, birth weight, and small for gestational age (SGA); maternal age at delivery, education, parity, pre-pregnancy body mass index (BMI, kg/m2), self-reported smoking and alcohol intake during pregnancy, as well as FFQ-based estimates of seafood (g/day), and dietary iodine intake (μg/day). Figures illustrating the assumed causal structures are shown in supplementary material (Figs. S1 and S2). The final adjustment set included: maternal age, seafood intake, education, parity, and child sex and birth year. We additionally adjusted for maternal ADHD symptoms with ADHD diagnosis as the outcome, measured by the Adult ADHD Self-Report Scale (ASRS screener) in the questionnaire at child age three years (Kessler et al., 2007).

2.5. Statistical analysis

PFAS concentrations were natural log-transformed to approximate normal distributions. Among the seven PFAS included here, four of them had missing values due to levels below LOQ (PFNA, PFDA, PFUnDA, and PFHpS). In addition, some of the covariates had missing values. To replace missing data, we ran multiple imputation by chained equations, separately for the ADHD sample (cases and controls) and the ASD sample (cases and controls). We generated 50 datasets with the exposure and outcome variables, covariates, and auxiliary variables (Rubin, 1976; Sterne et al., 2009) using the mi ice command in Stata (Royston, 2009). We employed the method for interval-censored data and specified upper and lower limit for imputed results for PFAS as limit of detection (LOD) and zero, respectively (Royston, 2009). The pooling procedure utilized in the present article was mi estimate (Stata Press, 2017). In the ADHD imputation model we included the following (% missing): PFOA (0), PFNA (0.1), PFDA (3), PFUnDA (1.2), PFHxS (0), PFHpS (1.4), PFOS (0), maternal age (0), maternal ADHD symptoms (41.6), maternal education (2.9), parity (0) maternal seafood intake (13.2), child sex (0), child birth year (0), length of gestation (0.3), child birth weight (0), maternal smoking (0.1), maternal iodine intake (13.2), and maternal folate intake (8.8).

In the ASD imputation model we included the following (% missing): PFOA (0), PFNA (0.1), PFDA (1.4), PFUnDA (0.4), PFHxS (0), PFHpS (1.1), PFOS (0), maternal age (0), maternal education (2.6), parity (0) maternal seafood intake (10.5), child sex (0), child birth year (0), length of gestation (0.2), child birth weight (0), maternal smoking (0), maternal iodine intake (10.5), and maternal folate intake (7.5).

As a first approach, we performed logistic regression analyses separately for ADHD and ASD diagnoses and levels of individual PFAS categorized into quartiles, with the lowest quartile as the reference group. Quartile regression models were chosen in order to investigate dose-response relationships. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). We also explored effect measure modification (Wald test; significance at $p < 0.10$) by child sex and maternal education (as a measure of SES). We additionally, conducted a sensitivity analysis in the quartile models, where we excluded mothers who were overweight or obese.

As many PFAS were tested individually in quartile plots, the number of tests performed is fairly high ($n = 21$ for each outcome), thus inflating the probability of type 1 error. Therefore, we also evaluated the results with 99.7% CIs and $p < 0.003$ for the quartile analyses. This would correspond to Šidák correction to control for familywise error rate (false discoveries or type 1 errors) for $k = 21$ number of tests calculated by 100 $(1\text{-}\alpha)^{1/k}$ % confidence intervals with $\alpha = 0.05$.

Secondly, we modeled the associations between PFAS and ASD and ADHD with restricted cubic splines with knots at 10th, 50th, and 90th percentiles. We tested if this model described the data better than a basic logistic regression model, using likelihood ratio tests (LRT; significance for non-linearity at $p < 0.05$). These analyses were performed in one of the imputed data sets. Prior to these analyses, PFAS outliers were replaced (less or equal to the 1st percentile and greater or equal to the 99th percentile) by the values above or below the 1st and 99th percentile (Liao et al., 2016). As a sensitivity analysis, we compared splines with and without outliers and in complete case analyses.

Finally, in order to address the inter-correlations among PFAS chemicals, we analyzed the joint effect of the PFAS mixture on ASD and ADHD diagnoses separately. The effects of individual chemicals may be small and thus more challenging to identify. This makes it difficult to predict the joint (total) effect of the mixture based on modelling of individual PFAS. For the mixture analyses we used a quantile-based g-computation approach (Keil et al., 2020; Keil, 2020). This novel method, combining weighted quantile sum regression and g-computation, was developed to assess the effect of mixtures, giving estimates of the simultaneous effect on the outcome of an increase of all exposures in the mixture by one quantile (Keil et al., 2020). In our study, the quantile was set to one quartile increase in log-PFAS concentrations. We investigated three different mixtures *a priori* based on PFAS structural groups: a mixture containing all seven PFAS, a mixture containing carboxylates (PFOA, PFNA, PFDA, PFUnDA), and a mixture containing sulfonates (PFHxS, PFHpS, PFOS). Results are presented as ORs with 95% CIs associated with one quartile increase in all compounds of the respective mixtures. We also calculated weights; indicating the relative contribution of each PFAS to the associated outcome estimate, for each mixture. The weights are useful to identify compounds contributing most to the mixture effect and the direction of association for each compound.

Most statistical analyses were performed in Stata version 15 (StataCorp, 2019). In addition, we used R version 3.6.2 (R Core Team, 2018) with the "foreign" (R Core Team, 2020), "Amelia" (Honaker et al., 2019), "psych" (Revelle, 2020), "readstata13" (Garbuszus and Jeworutzki, 2018), "qgcomp" (Keil, 2020), "ggplot2" (Wickham et al., 2020), and "tidyverse" (Wickham, 2019) packages. The imputed and adjusted results for the logistic regression models are presented in the main text, while complete case analyses are presented in the supplementary material (Figs. S3–S6 and Table S2). Also, the PFAS quartile boundaries and corresponding ORs with 95% CIs from the quartile models are presented in supplementary material (Table S3).

3. Results

3.1. Study sample characteristics and PFAS distribution

The study sample characteristics are displayed in Table 1. Mothers of both ASD and ADHD cases were slightly younger than mothers of controls. The majority of mothers had higher education (university/college) among controls and ASD cases, and lower education (less than university/college) among the ADHD cases. Most of the mothers of controls and ADHD cases were multiparous, whereas most of the mothers of ASD cases were primiparous. Mothers of ADHD cases were more likely to have reported smoking during pregnancy than mothers of controls and ASD cases.

Table 2 shows the distribution of maternal blood concentrations of PFAS in our sample, including the mean, median and interquartile range of maternal PFAS concentrations during pregnancy. Three of the PFAS (PFOA, PFHxS and PFOS) were above LOQ in all measurements. The spearman correlations among the PFAS for the whole study population (cases and controls) are displayed in Table 3. The strongest correlations were between PFOS and PFHpS ($r = 0.81$), PFDA and PFUnDA ($r = 0.74$), PFDA and PFNA ($r = 0.73$), PFHxS and PFHpS ($r = 0.69$), PFOA and PFHpS ($r = 0.65$), PFOA and PFOS ($r = 0.61$), and PFOS and PFHxS ($r = 0.61$).

3.2. Associations between maternal PFAS concentrations and odds of ADHD in children

The quartile models showed an elevated risk for ADHD in children across all quartiles of PFOA [Q2: OR = 1.54 (95% CI: 1.16, 2.04); Q3: OR = 1.41 (95% CI: 1.05, 1.89); Q4: OR = 1.38 (95% CI: 1.01, 1.89)] compared to quartile 1 (reference) and with a decreasing monotonic trend (Fig. 2, Table S3). The second quartile of PFOA remained with

Table 1
Characteristics of study population in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Characteristic	MoBa Controls Mean ± SD or n (%)	NPR ADHD Cases Mean ± SD or n (%)	NPR ASD Cases Mean ± SD or n (%)	*p*
Total N	980	821	400	
Maternal ADHD sum score	2.10 ± 0.56	2.27 ± 0.70	2.23 ± 0.58	0.017
Missing (*n*)	400	321	171	
Maternal education				0.000
< University/college	319 (33.4)	429 (53.9)	164 (42.1)	
University/college	636 (66.6)	367 (46.1)	226 (57.9)	
Missing (*n*)	25	25	10	
Maternal age (years)	30.1 ± 4.45	28.9 ± 4.98	29.5 ± 4.92	0.001
Missing (*n*)	0	0	0	
Parity				0.000
0	420 (42.9)	394 (48.0)	231 (57.8)	
1 or more	560 (57.1)	427 (52.0)	169 (42.2)	
Missing (*n*)	0	0	0	
Maternal total seafood intake (g/day)	36.9 ± 21.8	36.8 ± 26.7	36.6 ± 25.1	0.564
Missing (*n*)	117	112	27	
Child sex				0.001
Girl	306 (31.2)	231 (28.1)	62 (15.5)	
Boy	674 (68.8)	590 (71.9)	338 (84.5)	
Missing (*n*)	0	0	0	
Child year of birth				0.000
2002	240 (24.5)	152 (18.5)	35 (8.8)	
2003	146 (14.9)	196 (23.9)	70 (17.5)	
2004	172 (17.6)	173 (21.1)	55 (13.8)	
2005	228 (23.3)	144 (17.5)	71 (17.8)	
2006	77 (7.9)	83 (10.1)	69 (17.2)	
2007	64 (6.5)	61 (7.4)	55 (13.7)	
2008	48 (4.9)	12 (1.5)	38 (9.5)	
2009	5 (0.5)	–	7 (1.7)	
Missing (*n*)	0	0	0	
Smoking during pregnancy				0.000
No	854 (87.1)	617 (75.2)	335 (83.7)	
Yes	126 (12.9)	203 (24.8)	65 (16.3)	
Missing (*n*)	0	1	0	
Alcohol during pregnancy				0.044
No	638 (67.9)	579 (73.0)	272 (70.5)	
Yes	301 (32.1)	214 (27.0)	114 (29.5)	
Missing (*n*)	41	28	14	
Maternal marital status				0.000
Married/cohabitant	952 (97.1)	751 (91.5)	380 (95.0)	
Other (single, divorced, widow)	28 (2.9	70 (8.5)	20 (5.0)	
Missing (*n*)	0	0	0	
Maternal folate supplement				0.640
No	384 (39.2)	334 (40.7)	129 (32.3)	
Yes[a]	596 (60.8)	487 (59.3)	271 (67.7)	
Missing (*n*)	0	0	0	

Note.
[a] Any folate supplements between 4wk before and 8 wk after conception. p-values are based on chi-square test where case groups are combined. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); Norwegian Patient Registry (NPR); standard deviation (SD); The Norwegian Mother, Father and Child Cohort Study (MoBa).

99.7% CIs (Table S4). The restricted cubic splines suggested evidence of non-linearity for PFOA and ADHD, with a linear increase of risk with increasing PFOA levels and flattening out at a plateau at the highest levels of PFOA (Fig. 3). A similar non-linear pattern was observed for PFOS and ADHD (Fig. 3).

We identified negative associations with ADHD (lowered risk) in the third and fourth quartiles of PFUnDA [Q3: OR = 0.58 (95% CI: 0.43, 0.77); Q4: OR = 0.49 (95% CI: 0.36, 0.66)] (Fig. 2, Table S3). These associations remained with 99.7% CIs (Table S4). There was evidence of effect measure modification by maternal education for the second quartile of PFUnDA (p interaction = 0.02), where those with lower education had higher ORs [OR = 1.05 (95% CI: 0.71, 1.55)] and those with higher education had lower ORs [OR = 0.56 (95% CI: 0.37, 0.83)] (Table S5).

For PFDA, there was a negative association with ADHD in the fourth quartile [Q4: OR = 0.61 (95% CI: 0.46, 0.81)] (Fig. 2, Table S3). This association remained with 99.7% CIs (Table S4). There were no significant effect measure modifications by child sex in the associations between prenatal exposure to PFAS and ADHD in offspring. The sensitivity analysis, where we excluded mothers who were overweight or obese were excluded, did not differ considerably from the main models (Table S6).

3.3. Associations between maternal PFAS concentrations and odds of ASD in children

The quartile models showed an elevated risk for ASD in children in quartile 2 of PFOA [OR = 1.71 (95% CI: 1.20, 2.45)] compared to quartile 1 (reference) and with a decreasing monotonic trend in the next two quartiles (Fig. 4, Table S3). This association persisted with 99.7% CIs (Table S4). The restricted cubic spline suggested a non-linear dose-response relationship between PFOA and odds of ASD, with an inverse U-shape (Fig. 3).

For PFOA, there was evidence of effect measure modification by child sex in quartile 3 (p interaction = 0.05), such that the positive relationship in this quartile was greater for boys [OR = 1.47 (95% CI: 0.97, 2.23)] than for girls [OR = 0.56 (95% CI: 0.23, 1.39)] (Table S7). There was also evidence of effect measure modification by maternal education in quartile 2 of PFOA (p interaction = 0.01), with higher ORs for children of mothers with higher education [OR = 2.58 (95% CI: 1.61, 4.12)] compared to those with lower education [OR = 0.97 (95% CI: 0.56, 1.68)] (Table S5).

We further identified negative associations with ASD (lowered risk) in the third and fourth quartiles of PFDA [Q3: OR = 0.63 (95% CI: 0.45, 0.89); Q4: OR = 0.60 (95% CI: 0.42, 0.86)] and PFUnDA [Q3: OR = 0.59 (95% CI: 0.41, 0.85); Q4: OR = 0.57 (95% CI: 0.39, 0.82)] (Fig. 4, Table S3). For PFUnDA, there was also effect measure modification with maternal education in the second quartile (p interaction = 0.02), where children of mothers with lower education had higher ORs [OR = 1.42 (95% CI: 0.86, 2.34)] compared to those with higher education [OR = 0.64 (95% CI: 0.40, 1.02)] (Table S5).

There was a negative association between PFOS and ASD in quartile 3 [OR = 0.63 (95% CI: 0.44, 0.91) (Fig. 4, Table S3). We observed effect measure modification by child sex in the fourth quartile of PFOS (p interaction = 0.02), with elevated odds for girls [OR = 1.80 (95% CI: 0.79, 4.11)] and lowered odds boys [OR = 0.62 (95% CI: 0.40, 0.95)] (Table S7). There was also effect measure modification by maternal education in the second quartile of PFOS (p interaction = 0.09), where children of mothers with higher education had higher ORs [OR = 1.20 (95% CI: 0.79, 1.84)] compared to those with lower education [OR = 0.65 (95% CI: 0.37, 1.15)] (Table S5).

We observed effect measure modification by child sex for PFHpS, where the girls were driving the relationship in the third quartile (p interaction = 0.09), with higher ORs for girls [OR = 1.53 (95% CI: 0.69, 3.40)] compared to boys [OR = 0.73 (95% CI: 0.49, 1.08)] (Table S7). There was also evidence of effect measure modification by child sex in the second quartile of PFHxS (p interaction = 0.06), where the girls were driving the relationship [OR = 1.80 (95% CI: 0.78, 4.11)], while the boys had lower ORs [OR = 0.76 (95% CI: 0.52, 1.11)] (Table S7). We also identified effect measure modification by maternal education in the fourth quartile of PFHxS (p interaction = 0.04), where those with lower education had higher ORs [OR = 1.12 (95% CI: 0.63, 1.98)] compared to those with higher education [OR = 0.52 (95% CI: 0.33, 0.83)] (Table S5). The sensitivity analysis, where we excluded mothers who

Table 2
PFAS distribution (ng/mL) in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

Metal	Case/								
Control	N	% > LOQ	Mean (SD)	Min	25%	50%	75%	Max	
PFOA	Control	980	100	2.31 (1.19)	0.37	1.42	2.12	2.95	16.1
	ADHD case	821	100	2.39 (1.06)	0.47	1.65	2.23	2.99	7.01
	ASD case	400	100	2.46 (3.46)	0.28	1.60	2.11	2.86	67.8
PFNA	Control	979	99.9	0.40 (0.19)	0.05	0.28	0.37	0.49	1.78
	ADHD case	821	100	0.39 (0.24)	0.09	0.27	0.35	0.45	4.40
	ASD case	400	100	0.42 (0.20)	0.10	0.29	0.38	0.49	1.64
PFDA	Control	963	98.3	0.20 (0.10)	0.02	0.13	0.18	0.24	1.05
	ADHD case	778	94.8	0.19 (0.11)	0.05	0.12	0.17	0.22	1.47
	ASD case	399	99.8	0.19 (0.15)	0.01	0.12	0.16	0.23	2.09
PFUnDA	Control	975	99.5	0.26 (0.13)	0.05	0.17	0.24	0.32	0.96
	ADHD case	797	97.1	0.23 (0.12)	0.05	0.14	0.20	0.28	1.02
	ASD case	399	99.8	0.24 (0.15)	0.01	0.14	0.21	0.30	1.10
PFHxS	Control	980	100	0.79 (0.73)	0.15	0.46	0.64	0.91	12.1
	ADHD case	821	100	0.87 (1.18)	0.12	0.47	0.63	0.87	15.2
	ASD case	400	100	0.81 (0.94)	0.10	0.45	0.61	0.87	9.33
PFHpS	Control	969	98.9	0.19 (0.27)	0.02	0.12	0.16	0.22	7.90
	ADHD case	804	97.9	0.19 (0.10)	0.02	0.12	0.17	0.23	1.67
	ASD case	398	99.5	0.17 (0.08)	0.01	0.11	0.15	0.21	0.64
PFOS	Control	980	100	14.2 (9.45)	1.85	9.29	12.9	16.9	203
	ADHD case	821	100	14.1 (6.68)	2.96	9.66	13.0	17.0	89.2
	ASD case	400	100	12.42 (5.83)	1.21	8.10	11.5	16.2	33.5

Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); limit of quantification (LOQ); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS); standard deviation (SD).

Table 3
Spearman correlations between PFAS (ng/mL) in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	1.00						
PFNA	0.55	1.00					
PFDA	0.39	0.73	1.00				
PFUnDA	0.23	0.56	0.74	1.00			
PFHxS	0.57	0.47	0.36	0.36	1.00		
PFHpS	0.65	0.44	0.34	0.30	0.69	1.00	
PFOS	0.61	0.41	0.39	0.41	0.61	0.81	1.00

Abbreviations: Perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

were overweight or obese, did not differ considerably from the main models (Table S6).

3.4. Total mixture effect by quantile-based g-computation

The PFAS demonstrated strong inter-correlations (Table 3), making it difficult to ascertain chemical-specific effects. We therefore used quantile-based g-computation to investigate the joint (additive) effects of PFAS on ASD and ADHD. We observed a negative (inverse) association, such that a one-quantile increase in the overall PFAS mixture was associated with lower odds of ASD [OR = 0.76 (95% CI: 0.64, 0.90)] (Table 4 and Fig. S7). However individual PFAS constituents had both negative and positive influences on the outcome. By weight (in decreasing order), PFNA, PFOA, and PFOS had the highest positive influence in the total PFAS mixture estimate on ASD, while PFDA, PFUnDA, PFHpS, and PFHxS had the highest negative influence (Fig. S7). The carboxylate and sulfonate mixtures were negatively associated with ASD [OR = 0.79 (95% CI: 0.68, 0.93) and OR = 0.84 (95% CI: 0.73, 0.96), respectively] (Table 4, Figs. S8 and S9). By weight, PFNA in the carboxylate mixture had a positive influence while PFDA, PFUnDA, and PFOA (in decreasing order) had a negative influence on ASD estimates (Fig. S8). In the sulfonate mixture, PFHxS, PFHpS, and PFOS (in decreasing order) were negatively associated with ASD (Fig. S9). None of the PFAS mixtures were significantly associated with risk of ADHD (Table 4, Figs. S7–S9). The sensitivity analysis where we ran the quantile-based g-computation analyses with complete cases, did not deviate from the imputed results (Table S2).

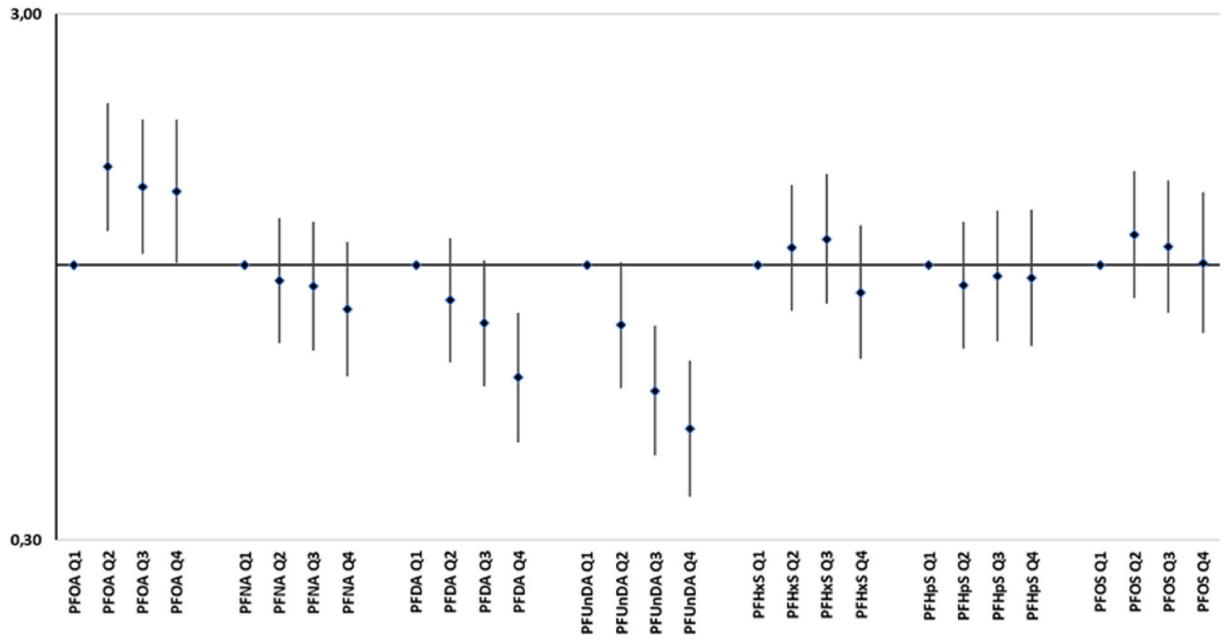

Fig. 2. Odds ratios and 95% confidence intervals of logistic regression models predicting attention-deficit/hyperactivity disorder from quartile categories of each PFAS in a nested case–control study of attention-deficit/hyperactivity disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n = 1801). Note: Logistic regression with multiple imputation. The PFAS were log transformed. The odds ratio and 95% confidence intervals for each PFAS quartile are represented on the vertical axis (the reference level is the first quartile). Each regression model was adjusted for maternal ADHD symptoms, age, education, parity, seafood intake, child sex, and child birth year. Abbreviations: Perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

4. Discussion

In this large prospective population-based study, we found increased risk of ASD and ADHD diagnoses in children prenatally exposed to PFOA, however associations were not monotonic, with the strongest association found at mid-range (2nd quartile) of exposure. PFOA exhibited non-linear dose-response relationships with odds of ASD and ADHD. Additionally, PFOS displayed a similar non-linear pattern with ADHD. We also found several negative (inverse) associations between individual PFAS and ASD and ADHD. Furthermore, there were negative associations between the total, carboxylate, and sulfonate mixtures with odds of childhood ASD.

4.1. Associations between prenatal PFOA and ASD and ADHD in children

Although PFOA is one of the most studied PFAS, there is still insufficient evidence for associations between prenatal exposure and neurodevelopment, particularly regarding ASD (EFSA, 2018; Liew et al., 2018). In the current study we found almost two times higher odds of ASD in children in the second quartile PFOA exposure group (1.47–2.11 ng/ml) compared to the lowest exposure group (0.28–1.47 ng/ml), which also persisted after adjusting for multiple comparisons. In addition, there appeared to be an inverse U-shaped pattern between PFOA and ASD diagnosis in children, with highest risk in the mid-range levels of PFOA. Our findings are in line with a study from the USA that reported positive associations between prenatal PFOA exposure and ASD diagnosis in children (Oh et al., 2020). Another study from the USA reported higher odds of ASD diagnosis in children in the highest prenatal exposure group of PFHxS, but not for PFOA (Shin et al., 2020). Furthermore, research from the Faroe Islands reported a positive association between PFOA measured in blood at child age five years with autism symptoms at seven years of age (Oulhote et al., 2016). The Faroese study did, however, not find any significant associations between prenatal PFAS concentrations (including PFOA) and autism symptoms (Oulhote et al., 2016). Some studies have reported null findings or inverse associations for prenatal exposure to various PFAS, including PFOA, and ASD diagnoses or related symptoms in children (Braun et al., 2014; Liew et al., 2015a; Long et al., 2019; Lyall et al., 2018).

The C8 Science Panel is among the most known PFOA studies. Among the various health effects following PFOA-contaminated drinking water in Mid-Ohio Valley communities, West-Virginia (USA) they investigated children's neurodevelopmental outcomes in relation to estimated fetal exposure and concurrent childhood PFOA levels measured in blood (Steenland et al., 2020). Altogether, they found no evidence of adverse effects of PFOA exposure on child ADHD (diagnosis and symptoms) or cognitive functions (Steenland et al., 2020). Likewise, several studies have also reported null findings between prenatal exposure to PFAS and ADHD diagnosis or symptoms (Carrizosa et al., 2021; Fei and Olsen, 2011; Ode et al., 2014; Quaak et al., 2016; Strøm et al., 2014; Vuong et al., 2018a) and a recent meta-analysis showed little support for an association between early-life exposure to PFOS or PFOA and ADHD (Forns et al., 2020). In contrast, the results herein indicated increased risk of ADHD in children prenatally exposed to mid-range PFOA levels (1.52–2.17 ng/ml) compared to the lowest exposure group (0.37–1.51 ng/ml), which also persisted after adjusting for multiple comparisons. And there are other studies that support our findings. A study based on the Danish National Birth Cohort found higher risk of ADHD diagnosis in children exposed prenatally to the highest levels of PFOA compared to the lowest exposure levels (although they questioned the consistency across results) (Liew et al., 2015a). Another study based on pooled data from Poland, Ukraine, and Greenland found an association between the highest levels of prenatal PFOA exposure (compared to the lowest levels) and increased levels of hyperactivity in children of five to nine years of age (Høyer et al., 2015). Furthermore, a study from the Faroe Islands reported that postnatal (but not prenatal) exposure to several PFAS was associated with an increase in behavioral difficulties which consisted of hyperactivity problems, inattention, conduct problems, and peer relationship problems among seven-year-olds (Oulhote et al., 2016). A recent study found associations

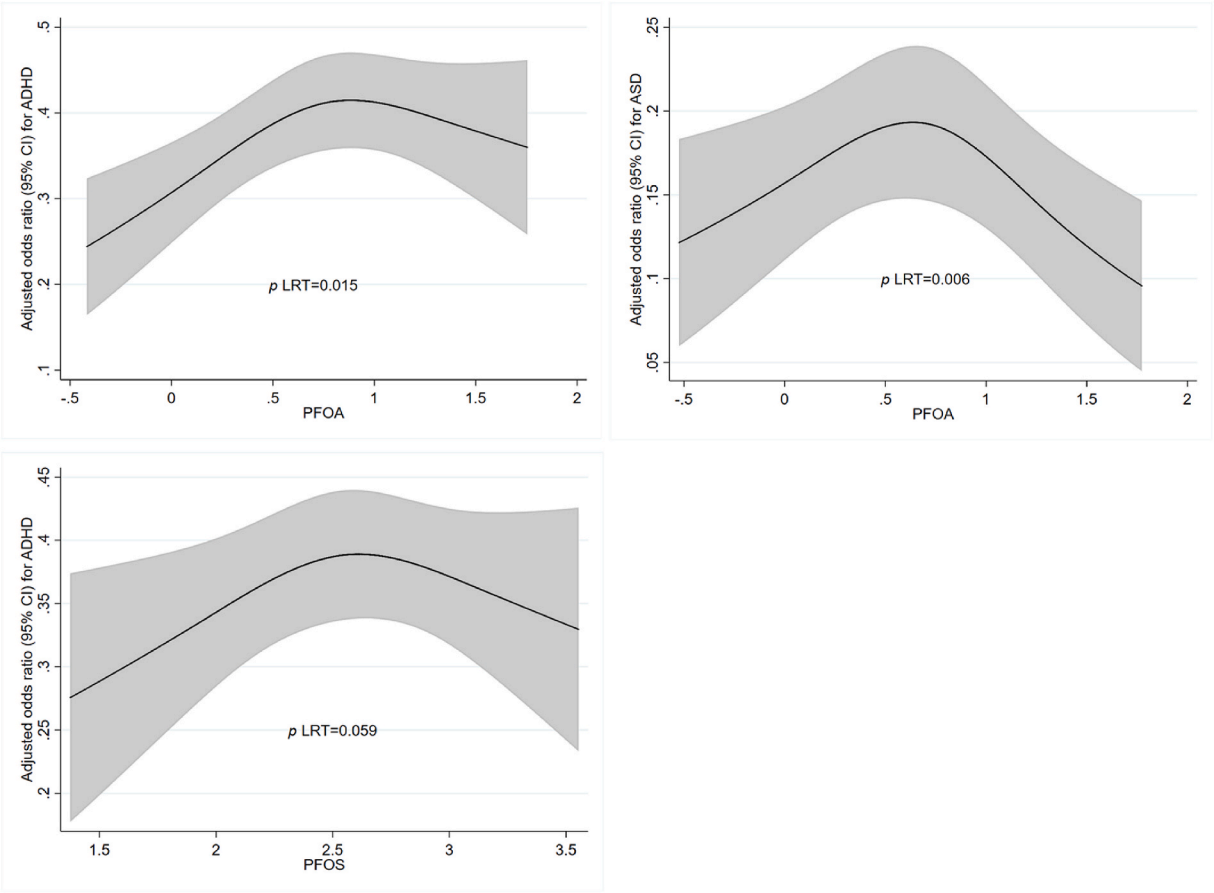

Fig. 3. Logistic regression models predicting attention-deficit/hyperactivity disorder and autism spectrum disorder from restricted cubic splines of PFOA and PFOS in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009. Note: Three knot positions at 10th, 50th and 90th percentiles of PFOA and PFOS. Solid lines represent estimated odds ratios, and the grey areas illustrate 95% confidence intervals. P-values were calculated with likelihood ratio test, comparing a model with a log-linear exposure to a restricted cubic spline exposure. PFOA and PFOS were log transformed, outliers less or equal to the 1st percentile and greater or equal to the 99th percentile were replaced with the value above/below 1st and 99th percentile and missing data imputed. The model was adjusted for maternal ADHD symptoms (ADHD diagnosis only), age, education, parity, seafood intake, child sex, and child birth year. Abbreviations: Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); confidence intervals (CI); likelihood ratio test (LRT); perfluorooctanoic acid (PFOA); perfluorooctane sulfonate (PFOS).

between prenatal exposure to PFHxS, PFOS, and PFNA and increased externalizing behavior and additionally increased symptoms of ADHD with the two latter PFAS (Vuong et al., 2021). Additionally, a study reported an association between externalizing behavior (including hyperactivity/inattention) and maternal levels of PFNA, but not with the other measured PFAS (Luo et al., 2020). Reasons for the inconsistencies across studies could be attributed to differences in timing of PFAS measurements (e.g. mid-pregnancy vs newborn cord blood) as well as exposure levels. Differences could also be due to varying sample sizes and different outcome measurements.

In the present study we observed that prenatal PFOA exposure increased risk of both ADHD and ASD diagnosis with non-linear dose-response-relationships. This may suggest that PFOA is a common risk factor for both disorders, perhaps affecting shared neurochemical and neurodevelopmental pathways (Kern et al., 2015). Interestingly, we reported in a previous study based on MoBa data that increasing prenatal PFOA exposure was related to poorer nonverbal working memory (Skogheim et al., 2020). Deficits in working memory is a common executive function impairment often found in children with ADHD and ASD (Geurts et al., 2004; Thapar and Cooper, 2016). We thus speculate if PFOA could affect neurobehavioral domains mutual for ASD and ADHD, such as working memory. Associations between prenatal or childhood PFAS exposure and executive functions, including working memory, have been reported in other studies as well (Vuong et al., 2016, 2019), while other studies report mixed (Vuong et al., 2018b) or null findings (Carrizosa et al., 2021; Stein et al., 2013). Why there were only positive (adverse) associations between PFOA and ASD and ADHD in the quartile analyses, can only be speculated upon. One potential suggestion could be that PFOA is, together with PFOS, among the PFAS with the highest concentration levels in humans. Furthermore, PFOS showed a similar non-linear dose-response pattern with ADHD as PFOA. The few differences in the overall findings for ASD and ADHD, is also up for speculation. It could be due to differential vulnerability to PFAS during fetal development that is linked to specific features of the respective etiologies of ASD and ADHD. Also, the lower validity of ADHD diagnoses compared to ASD diagnoses (Surén et al., 2014, 2018) may have contributed.

4.2. *Inverse associations between prenatal exposure to some PFAS and ASD and ADHD in children*

In the present study, we observed some counter-intuitive associations between prenatal exposure to PFAS and ASD and ADHD diagnosis in children. Although there is no plausible biological reason for these inverse associations, this phenomenon has also been reported in previous research (e.g. Braun et al., 2014; Lien et al., 2016; Liew et al., 2015a; Long et al., 2019; Lyall et al., 2018; Stein et al., 2013; Vuong et al., 2018a). It has been speculated that the "live birth bias" might explain

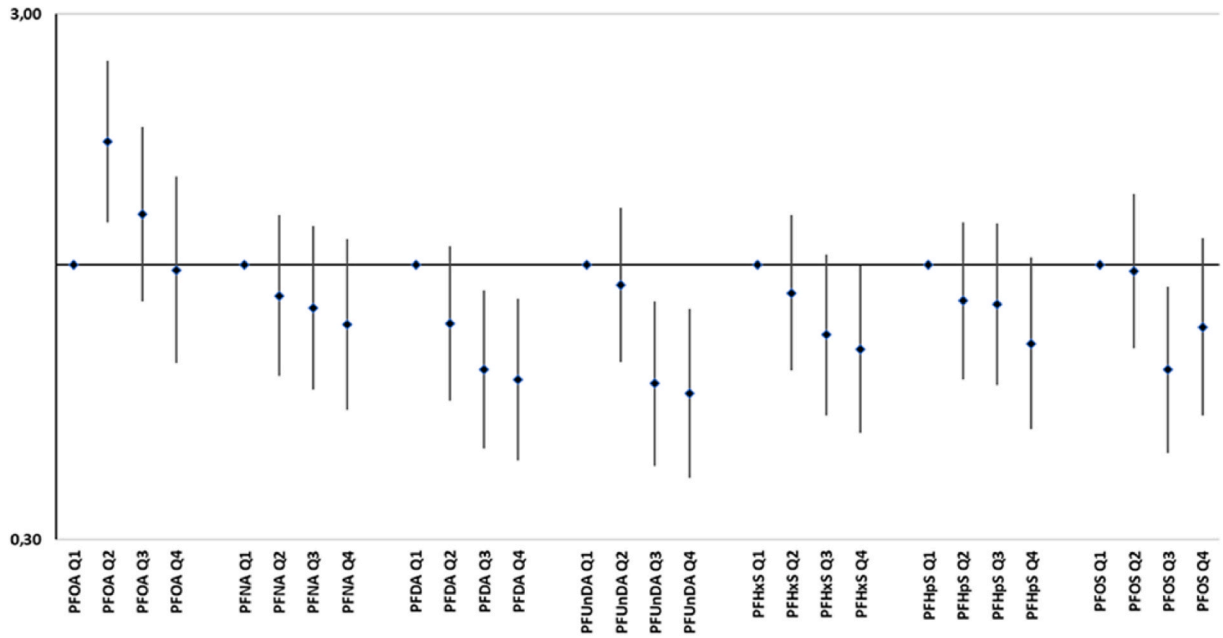

Fig. 4. Odds ratios and 95% confidence intervals of logistic regression models predicting autism spectrum disorder from quartile categories of each PFAS in a nested case–control study of autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009 (n = 1380). Note: Logistic regression with multiple imputation. The PFAS were log transformed. The odds ratio and 95% confidence intervals for each PFAS quartile are represented on the vertical axis (the reference level is the first quartile). Each regression model was adjusted for maternal age, education, parity, seafood intake, child sex, and child birth year. Abbreviations: Perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorohexane sulfonate (PFHxS); perfluoroheptanesulfonic acid (PFHpS); perfluorooctane sulfonate (PFOS).

Table 4
Odds ratios and 95% confidence intervals for quantile-based g-computation approach of PFAS on attention-deficit/hyperactivity disorder and autism spectrum disorder in a nested case–control study of attention-deficit/hyperactivity disorder and autism spectrum disorder in The Norwegian Mother, Father and Child Cohort Study (MoBa), 2002–2009.

PFAS mix	ADHD and controls		ASD and controls	
	OR	95% CI	OR	95% CI
Total mix	0.93	0.82, 1.07	0.76	0.64, 0.90
Carboxylates	0.94	0.83, 1.06	0.79	0.68, 0.93
Sulfonates	0.85	0.88, 1.11	0.84	0.73, 0.96

Note: The models were adjusted for maternal age, education, parity, seafood intake, child sex and child birth year. For child ADHD, maternal ADHD symptoms was also included as a covariate. Attention-deficit/hyperactivity disorder (ADHD); autism spectrum disorder (ASD); confidence intervals (CI); odds ratio (OR).

these inverse associations between prenatal PFAS exposure and neurodevelopmental outcomes observed in children (Liew et al., 2015b). This entails that bias is introduced when one studies the effects of prenatal exposure to environmental factors and later health outcomes only in live births (Liew et al., 2015b). If the contaminant being investigated can contribute to termination of the pregnancy, the number of exposed live born children will be reduced, which can influence the surviving children in this group and thus the results, introducing seemingly protective effects. There are, however, few epidemiologic studies that have investigated miscarriage and PFAS exposure and differences in type of PFAS compound. One study found that PFNA and PFDA were associated with increased odds of miscarriage (Jensen et al., 2015), which to some extent can be compared with the negative associations among carboxylates (PFDA and PFUnDA) in the present study. A more recent study reported increased risk of miscarriage with PFOA and PFHpS and PFAS mixtures (Liew et al., 2020). Finding an increased risk with a PFAS mixture, may complicate prediction of which single PFAS compounds may be confounded by live birth bias.

Others have also proposed that PFAS can activate the proliferator-activated receptors (PPARs) alpha and gamma, which is known to have neuroprotective and central-nervous-system anti-inflammatory properties, and have been implicated in treatment of neurodegenerative diseases such as Alzheimer's disease (Quaak et al., 2016; Stein et al., 2013). As many PFAS have seafood consumption as the main dietary source for humans (Haug et al., 2010), it may be that the inverse associations reflect beneficial consumption of polyunsaturated fatty acids and micronutrients from eating seafood rather than the possibly adverse effects from PFAS. Although we adjusted for self-reported seafood intake in the present study, the adjustment may not have been sufficient. This could also be due to unmeasured confounding, as we were not able to account for several (other) sources of PFAS, such as exposure via consumer products.

4.3. *Sex-specific effects and socioeconomic factors*

Experimental animal studies have suggested that there are sex differences in both exposure and capacity to metabolize and eliminate PFOS and other PFAS, although this has not been consistently found in human studies (Agency for Toxic Substances and Disease Registry, 2021; Lau et al., 2007; Mariussen, 2012). There may also be sex-differences in the vulnerability for adverse neurodevelopmental effects, linked to PFAS's ability to disrupt sex steroid hormone levels and functioning which are involved in sex differentiation of the fetal brain (Kjeldsen and Bonefeld-Jørgensen, 2013; Mariussen, 2012). An *in vitro* study showed that both single PFAS and as a mixture, have the potential to interfere with sex steroid hormone receptors (androgens and estrogens) (Kjeldsen and Bonefeld-Jørgensen, 2013). Epidemiological studies have also reported associations between sex hormones and PFAS, both prenatal, childhood, and adolescent exposures (Itoh et al., 2016; Lopez-Espinosa et al., 2016; Tsai et al., 2015).

A recent meta-analysis showed that early life-exposure to PFAS and associations with ADHD was more prominent among girls compared to boys (Forns et al., 2020). In the present study, we observed effect measure modification by child sex in several associations between PFAS

and ASD. For PFOA, it was the boys who accounted for the positive association, with higher odds of ASD compared to girls. For the remaining associations with PFOS, PFHxS, and PFHpS, increased odds ratios for ASD were mainly found in girls, while odds for ASD were decreased in boys. This could indicate that overall, the girls had a higher risk of ASD with higher PFAS levels. Likewise, a study from Norway reported a positive association between early-life exposure to PFOS and ADHD diagnosis, with stronger associations among girls compared to boys (Lenters et al., 2019). Furthermore, a study from the Faroe Islands, reported consistently positive scores on behavioral problems and autistic screening scores among girls compared to boys with postnatal PFAS exposure (Oulhote et al., 2016). While there is scarce research on prenatal PFAS and ASD, two studies investigated sex differences and found an opposite pattern; with higher risk among boys exposed prenatally to PFOS and PFNA, respectively (Braun et al., 2014; Shin et al., 2020).

PFAS exposure levels have been associated with SES, such as education, income, and employment (Brantsæter et al., 2013; Montazeri et al., 2019; Tyrrell et al., 2013). In addition, ADHD in children is associated with mothers who have lower education (Torvik et al., 2020), while for ASD, results are mixed with some reporting associations with higher maternal education and some with lower (Lung et al., 2018). In the present study, we observed that several associations between PFAS and ASD diagnosis were modified by maternal education, however inconsistencies regarding the direction complicates interpretation. For PFOA and PFOS, there were higher odds for the child having an ASD diagnosis among those with mothers who had higher education (college or higher). This is in line with a study on determinants of PFAS, where they also used data from MoBa, where they found that levels of PFOA and PFOS increased with educational level and household income, respectively (Brantsæter et al., 2013). A similar finding was reported in a study on environmental contaminants and SES, showing higher concentrations of PFAS among employed pregnant women (Montazeri et al., 2019). In the present study we found the opposite pattern for PFUnDA, PFNA, and PFHxS, with higher odds of ASD diagnosis among children whose mothers had lower education. For ADHD, education appeared to be a modifier only in relation to PFUnDA exposure, as we found higher odds of ADHD among children whose mothers had lower education. Our findings for ADHD and PFUnDA, are in line with a study where stratified analyses indicated higher odds of ADHD with early-life exposure to PFAS among offspring of mothers with lower education (Forns et al., 2020).

4.4. Mixtures

The human fetus is exposed to a range of highly inter-correlated PFAS and other toxicants that can interfere in combination with brain development (Mariussen, 2012; Quaak et al., 2016; Vrijheid et al., 2016). There are, however, still only a few studies that have investigated prenatal exposure to the total mixture of PFAS and neurodevelopmental outcomes (Vrijheid et al., 2016). In the present study, the total mixture of PFAS as well as the carboxylate and the sulfonate mixtures were negatively associated with ASD. Although we would expect an overall positive (adverse) effect from prenatal exposure to PFAS, the general pattern in the present study consisted of many negative associations between individual PFAS and ASD. Similarly, research from the USA found associations between increasing levels of PFOA and lower autistic behavior scores in a multi-pollutant model with several types of chemicals, including PFOS, PFNA, and PFHxS (Braun et al., 2014). Likewise, a Danish study using principal component analysis to reduce the number of toxicant exposures including PFAS, found negative (inverse) associations between a component with PFAS (PFOS, PFOA, PFOSA) and ASD diagnosis (Long et al., 2019). In contrast, a study from the Faroe Islands, reported positive associations between maternal levels of PFOA and PFOS and behavioral problems in children at age seven in a multi-pollutant model (Oulhote et al., 2019). In addition, a Norwegian study found associations between early-life exposure to PFOS and ADHD diagnoses, but not with the other PFAS, using a multi-pollutant model that was also adjusted for other chemicals (Lenters et al., 2019).

4.5. Public health implications and regulations

Although the reported PFAS levels in the present study were lower than what has been reported in other studies that investigated neurodevelopment (e.g. Liew et al., 2015a; Oulhote et al., 2016; Stein et al., 2013), results from a study comparing PFAS levels in several European cohort studies showed that the PFAS levels in a subsample from MoBa were equal to or higher than in the other cohorts (Haug et al., 2018). The PFAS levels in that MoBa subsample (Haug et al., 2018) were similar to the levels in the present MoBa-based study population. Most of the knowledge about PFAS levels in Europe is based on cohort studies dating back in time, meaning that we do not know the present-day levels and what the population is exposed to with regards to novel PFAS. Although the production of some PFAS, such as PFOS and PFOA, has declined, several new replacement compounds are on the rise, with unknown health effects (Sunderland et al., 2019; Wang et al., 2017a). Therefore, it is of high importance to perform studies measuring the present levels of PFAS exposure and other environmental toxicants in pregnant women and assess potential adverse effects on health and development in children.

The results from epidemiological research on prenatal PFAS exposure and neurodevelopment are inconsistent, with many studies showing null findings. However, different methods and measures to assess neurodevelopmental outcomes complicate comparison across studies. Even so, there are other health outcomes with more consistent findings on adverse effects following PFAS exposure, such as fetal growth and immune function (Liew et al., 2018; Vrijheid et al., 2016). We urge for more research to investigate potential neurotoxicological effects of PFAS and their mixtures on human brain development, both epidemiological and experimental, to elucidate mechanistic underpinnings and assess risk of adverse neurodevelopment associated with *in utero* exposure. The paradoxical findings of seemingly improved neurodevelopmental outcome measures with PFAS exposure that is regularly reported in epidemiological studies, including the present study, also need to be resolved.

4.6. Limitations and strengths

Despite our efforts to oversample girls, our study included fewer girls than boys, especially in the ASD case group. Although all analyses were adjusted for child sex, the estimates for girls were less precise and less reliable than for boys and this may have influenced the effect measure modification analyses. It is therefore possible that the small numbers of girl cases across quartiles is resulting in the appearance of heterogeneity in estimates by child sex. Another potential limitation is that we could not account for variation in maternal glomerular filtration rate, which may be a source of residual bias. In our sample, there was no information of overlap between ASD and ADHD cases, as coding according to ICD-10 does not allow comorbid primary diagnoses (F84 and F90). However, this does not exclude the possibility of overlap regarding symptoms, but only diagnostic codes were available for our research. Another potential limitation concerns the clinical basis for the ADHD NPR registrations and the possibility that alternative diagnoses should have been considered (Surén et al., 2018). Limitations also include potential self-selection bias. Participating women in MoBa were generally older, had higher educational level, and reported less smoking compared with the general population (Nilsen et al., 2009). However, our study may be less prone to self-selection bias, as the mothers only had to complete the first questionnaire and because we nested case groups based on registries that are mandatory in Norway.

Our study also has several strengths. We had a large sample size of mother-child pairs, meaning that we could investigate potential effect measure modification by child sex and maternal education as well as non-monotonic relationships, in addition to the main association analyses. Another strength was that this study was nested within a

prospective birth cohort, meaning that our analyses benefitted from a large number of relevant covariates to account for residual confounding pathways. Furthermore, we examined several PFAS, both individually and as mixtures. To our knowledge, only one other study has investigated prenatal PFAS exposure and compared ADHD and ASD diagnosis in children in the same study (Liew et al., 2015a). Particularly studying ASD is a strength, as there is a lack of research on prenatal PFAS exposure and ASD in children. Furthermore, the validity of the ASD diagnoses in NPR was found to be very high in a study involving participants in MoBa (Surén et al., 2012).

5. Conclusion

Results from the present study showed elevated risk of both ASD and ADHD in children prenatally exposed to mid-range levels of PFOA and that this relationship was non-linear in both case groups. For ASD, the increased risk with PFOA was mainly found for boys. For other carboxylates, sulfonates, and their mixtures, there appeared to be inverse associations with ASD. However, the inverse associations reported herein should not be interpreted as protective effects, but rather that there could be some unresolved confounding for these relationships. Also, SES appeared to modify some of the relationships. Overall, the literature linking PFAS exposures with neurodevelopmental outcomes is still inconsistent, suggesting the need for more research to elucidate the neurotoxicological potential of PFAS, both epidemiological and experimental.

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Ethics

The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is now based on regulations related to the Norwegian Health Registry Act. The current study was approved by The Regional Committee for Medical and Health Research Ethics (ref. no. 2012/985–1). The NPR has approved the linkage between NPR and MoBa, identifying ADHD and ASD diagnostic cases.

Declaration of competing interest

The 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111692.

Availability of data and material

The consent given by the participants does not open for storage of data on an individual level in repositories or journals. Researchers who want access to data sets for replication should submit an application to datatilgang@fhi.no. Access to data sets requires approval from The Regional Committees for medical and health research ethics in Norway and a formal contract with MoBa.

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