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Prenatal Exposure to Per- and Polyfluoroalkyl Substances and Facial Features at 5 Years of Age: A Study from the Danish National Birth Cohort

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Background: Per- and polyfluoroalkyl substances (PFAS) are widespread persistent pollutants. Evidence regarding neurodevelopmental effects of PFAS have been mixed. The relation between PFAS exposure and anatomical markers that have been suggested to correlate with fetal brain development have not been studied.

Objectives: We investigated the association between prenatal PFAS exposures and three craniofacial features in children measured at 5 years of age.

Methods: Measures of palpebral fissure length (PFL), philtrum groove, and upper-lip thickness were generated from standardized digital facial photographs from 656 children in the Danish National Birth Cohort. PFL was classified into two groups (shorter; normal), and the philtrum (grooved; smooth; normal) and upper-lip (thick; thin; normal) measures into three groups each. Six PFAS were measured in maternal plasma (median = 8 gestational wk). Multinomial logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for each facial feature using the normal group as the reference according to $\log_2$-PFAS concentration (in nanograms per milliliter) or PFAS tertiles, adjusting for potential confounders, including maternal alcohol intake and smoking. Stratified analyses by maternal alcohol intake or child's sex were performed.

Results: Prenatal exposure to each PFAS was associated with elevated odds for a shorter PFL, with the strongest association observed for perfluorodecanoic acid (PFDA; per doubling OR = 2.02; 95% CI: 1.11, 3.70). Some nonlinear associations were found for philtrum measures: the second tertile of PFDA and perfluorononanoic acid were associated with grooved philtrum, whereas the second tertile of perfluoroheptane sulfonate with smooth philtrum. The associations between PFAS exposure and a shorter PFL were stronger among mothers who consumed alcohol in the first trimester, some sex-specific associations were noted for philtrum and upper-lip measures.

Discussion: Prenatal PFAS exposures might influence fetal craniofacial development. A larger study is needed to replicate the potential modifying effects observed for alcohol exposure and to clarify whether associations of craniofacial markers observed reflect specific neurologic deficits. https://doi.org/10.1289/EHP9478

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic organofluorine compounds that are widespread and persistent in the environment across the world (Houde et al. 2006; Lau et al. 2007). Because of their chemically and thermally stable properties, PFAS have been used in a variety of commercial products since the 1940s, including clothing, carpets, and kitchenware (Houde et al. 2006). Dietary exposure and contaminations from drinking water, indoor air, and household environments are likely the major sources in humans (D'Eon and Mabury 2011). The half-lives for commonly used PFAS in humans can range from 4 to 8 y (Olsen et al. 2007). Most PFAS compounds can cross the placenta barrier and expose the fetus during development (Fei et al. 2007).

Developmental and neurotoxicity of PFAS have been suggested in experimental models (DeWitt 2015; Gaballah et al. 2020; Lau et al. 2006; Slotkin et al. 2008). Exposure to common PFAS during the developmental period, including perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA), has been shown to delay neuromotor maturation and alter normal behaviors in mice and zebra fish (Jantzen et al. 2016; Mariussen 2012; Ulhaq et al. 2013). Population-based studies assessing neurobehavioral and neuropsychological end points have reported mixed findings (Fei et al. 2008; Liew et al. 2015, 2018a; Lyall et al. 2018; Oulhote et al. 2016; Vuong et al. 2021). Although prenatal exposures to the most commonly studied PFAS—PFOA and PFOS—were not linked to behavioral problems in early childhood (Fei and Olsen 2011), positive associations of less studied PFAS, such as PFNA and PFDA, with child behavioral difficulties have been reported in recent studies (Hoyer et al. 2018; Luo et al. 2020; Oulhote et al. 2016; Vuong et al. 2021). Two large cross-sectional studies have suggested that childhood PFOA, PFNA, or perfluorohexane sulfonate (PFHxS) concentrations are associated with attention deficit/hyperactivity disorder (Hoffman et al. 2010; Stein and Savitz 2011), but these findings were not corroborated in cohort studies with prospectively collected data on PFAS exposure (Liew et al. 2015; Ode et al. 2014; Skogheim et al. 2020; Strøm et al. 2014). Several cohort studies have also reported null or inverse associations between multiple prenatal PFAS and childhood autism or autistic behaviors (Braun et al. 2014; Liew et al. 2015; Lyall et al. 2018). Prenatal PFAS exposures have been suggested to influence childhood executive functions (Gump et al. 2011; Vuong et al. 2018b), but no consistent associations were found for cognitive functions, such as intelligence quotient (IQ) or visual spatial abilities (Liew et al. 2018b; Oh et al. 2021; Spratlen et al. 2020; Vuong et al. 2018a, 2019).

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Some neurodevelopmental disorders have been shown to be accompanied by facial dysmorphism (Bostwick et al. 2017; Kaufman et al. 2010; Sifrim et al. 2016; Stephen et al. 2018), with a suggestion of possible shared common genetic and environmental influences on fetal craniofacial and brain development (Bostwick et al. 2017; Pode-Shakked et al. 2019; Simões-Costa and Bronner 2013). Facial features have also been integrated into clinical assessments of neurological disorders resulting from *in utero* environmental insults. For instance, facial features characterized using palpebral fissure length (PFL; the horizontal distance of the space of the opening between the eyelids), philtrum groove, and upper-lip thickness are part of the key diagnostic criteria for fetal alcohol syndrome (FAS), a condition characterized by growth deficiency and neurobehavioral abnormalities caused by maternal alcohol intake during pregnancy (Astley 2013). Variations of each of these features are not specific to FAS and some children born to mothers without alcohol intake also present with these facial features, suggesting the presence of other undocumented risk factors (Bostwick et al. 2017; Kesmodel et al. 2019). The presence of these facial features correlates with impaired intelligence (Astley 2013; Kesmodel et al. 2019), motor delay, learning ability, and language delay in childhood (Bostwick et al. 2017; Pode-Shakked et al. 2019; Stephen et al. 2018), raising the possibility of using these facial features as physical markers in epidemiological studies to screen for adverse effects of intrauterine environmental exposures on fetal development.

Although numerous epidemiological studies have investigated potential neurodevelopmental toxicity of PFAS exposures, to our knowledge, none has assessed craniofacial development. We conducted the present study to evaluate the associations between prenatal exposures to six types of PFAS and three specific facial features characterized at 5 years of age using a subcohort nested within the Danish National Birth Cohort (DNBC).

Methods

Study Participants

The analysis population is a subset of the DNBC, a national birth cohort study in Denmark that has enrolled 101,041 pregnancies through general practitioners at the first antenatal visit [at gestational week (GW) 6–12] during 1996–2002 (Olsen et al. 2001). Approximately 50% of all general practitioners in Denmark participated in the study, and 60% of the women invited agreed to participate. Four computer-assisted telephone interviews (twice during pregnancy and twice postpartum) were conducted, and two prenatal maternal blood samples were collected and stored (one each in the first and second trimesters).

The present study additionally used data from the Lifestyle During Pregnancy Study (LDPS), a follow-up study based on a stratified sampling strategy that oversampled women with low-to-moderate alcohol intake and binge drinking from the DNBC (Kesmodel et al. 2010). Exclusion criteria for the LDPS were nonsingleton birth, children with impaired hearing or vision to the extent that the test session could not be performed, and severe disabilities due to congenital defects. A total of 1,781 mother–child pairs (51.2%) among 3,478 invited agreed to participate in the LDPS between September 2003 and June 2008 when the children were 5 years of age (age range: 60–64 months). Children attended one of the four testing sites in Denmark (i.e., Copenhagen, Aarhus, Odense, and Aalborg) for a 3-h extensive neuropsychological function assessment (Kesmodel et al. 2010). Of the 1,781 children participating in the LDPS, 670 had at least one of the three facial features generated with good quality for statistical analyses (Kesmodel et al. 2019). The final analytical sample size included 656 children with prenatal PFAS exposure data available in this cohort generated from previous research (Liew et al. 2018b). The flow chart of sample selection for the present study can be found in Figure S1.

Written informed consent was obtained from all participants at recruitment. Study procedures have been approved by the Danish data inspectorate (journal no. 2016-051-000001, serial number 1297) and the institutional review boards at Yale University (2000024089).

PFAS Measurements

The analytic methods that were used to quantify maternal plasma PFAS concentrations included in the present study have been described elsewhere (Liew et al. 2018b; Meng et al. 2018). In brief, two maternal blood samples collected in the DNBC were sent by mail to Statens Serum Institute in Copenhagen, aliquoted and stored in freezers at -20°C or -80°C. Stored maternal plasma (0.1 mL) from the first maternal sample (median = 8 GW, interquartile range = 7–10 GW) were sent to the Department of Environmental Health, Aarhus University, for sample extraction and purification. PFAS concentrations were measured by liquid chromatography–tandem mass spectrometry. Plasma concentrations of 16 types of PFAS were measured and the present study focused on six types of PFAS that were quantified in >90% of the samples measured, including PFOS, PFOA, PFDA, PFNA, PFHxS, and perfluoroheptane sulfonate (PFHpS). The distribution and the lower limit of quantitation for these samples are presented in Table S1.

Facial Feature Measures and Neurodevelopment Assessment

The procedures used to generate facial feature measures in the LDPS have been described elsewhere (Kesmodel et al. 2019). Briefly, standardized digital facial photographs of the children were taken by research associates in accordance with the FAS Facial Photographic Analysis Software instructions (Astley 2016). The child needed to have a relaxed facial expression (no smile, lips gently closed, eyes fully open, with no eyeglasses), and the digital images had to have proper rotation, exposure, and focus. A 19.05-mm diameter round paper sticker was placed between the participant's eyebrows as an internal measure of scale. In some cases, not all facial features, but one or two, were measurable in the digital photograph taken. For example, if a child opened the eyes fully but smiled, the research associates might be able to quantify only the measure for PFL with high quality but not philtrum smoothness or upper-lip thickness.

The University of Washington FAS Facial Photographic Analysis Software was used to generate measurements of (dysmorphic) facial features, including the philtrum, the upper-lip, and PFL (Astley 2016). The Scandinavian PFL growth charts (Strömland et al. 1999) and University of Washington Lip-Philtrum Guide 1 (Astley 2016) were used in combination to characterize these outcomes for this Danish cohort. A brief illustration of these three facial features and a link to the manual of outcome classification can be found in Figure S2. Briefly, the PFL was categorized into three groups as follows: A (>mean–1 standard deviation (SD)), B (mean–1 to 2 SD), and C (≤mean–2 SD). According to the Scandinavian PFL growth charts, the average values (SD) at 5 years of age are 24.6 mm (2.1) for girls, and 25.1 mm (2.2) for boys. The philtrum grooved measure was categorized based on visual inspection into three groups: A (grooved; philtrum ranks 1 and 2 in the guide), B (normal; philtrum rank 3 in the guide), and C (smooth; philtrum ranks 4 and 5 in the guide). The upper-lip measure was quantified using circularity, which is defined as perimeter^2/area and also categorized into three groups: A (thick;

lip ranks 1 and 2 in the guide), B (normal; lip rank 3 in the guide), and C (thin; lip ranks 4 and 5 in the guide). These facial feature measures can vary across race/ethnicity groups (Astley 2016). Children included in the DNBC are predominantly (>95%) white and of Scandinavian or European descent, thus outcome misclassification due to racial differences is not expected in this cohort.

We also obtained other neuropsychological test results in the LDPS to estimate the correlations between facial features and brain function measures in this sample (Kesmodel et al. 2019). Child's IQ was measured using the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) (Wechsler 2012), whereas attention and executive function were measured using the Test of Everyday Attention for Children at Five (TEACh-5) (Manly et al. 2001) or the Behavior Rating Inventory of Executive Function (BRIEF) questionnaire (Gioia et al. 2000), respectively. WPPSI-R and TEACh-5 were administered by trained psychologists, and the BRIEF questionnaire was completed by the parents. Lower IQ or TEACh-5 standardized scores are indicative of worse outcomes, whereas higher BRIEF scores suggest increasing difficulties.

Potential Confounders

Potential confounders were selected *a priori* according to directed acyclic graphs (see Figure S3). Information on child's sex, birth year, maternal age at delivery (19–29, 30–34, 35–39 y), and parity (0 or ≥1) was retrieved from the Danish Medical Birth Register, whereas other potential confounders including socio-occupational status (high, medium, low), prepregnancy body mass index (<25 or ≥25 kg/m^2), GW of blood sampling (<8, 8–11, >11 GW), smoking during the first trimester (yes or no), and alcohol intake during the first trimester (no; ≤1 drink/wk; >1 drinks/wk) were collected from maternal interviews conducted during pregnancy. Socio-occupational status was classified according to education and job titles of the parents. One parent with higher education (4 y beyond secondary school education) or in management level jobs was classified as high social status, one parent with middle-range training and skilled workers was classified as middle social status, and unskilled workers and unemployed were classified as low social status. These potential confounders were included and treated as categorical variables in the regression models.

Statistical Analysis

To compare the measurements between anatomical features and neuropsychiatric function, we estimated the mean difference and 95% confidence interval (CI) for the intelligence, attention, and executive function standardized scores according to the three facial features classifications. Next, we used multinomial logistic regression to estimate the odds ratio (OR) and 95% CI for each of the facial features according to PFAS exposure, adjusting for potential confounders. Maternal PFAS concentration (in nanograms per milliliter) was first analyzed as a continuous variable after $\log_2$-transformed to reduce influence of outliers. The effect estimates in the $\log_2$-PFAS model represented a contrast of 2-fold increase of prenatal PFAS concentration. Moreover, we evaluated potential nonlinear relations between prenatal PFAS exposures and outcomes using PFAS tertiles and general additive models (GAMs) with penalized smoothing regression splines with a degree of 3. Potential departure from linearity assessed using a p-value threshold of <0.1 in the GAM was compared with a model that included the PFAS level as a linear term.

For outcome measures, philtrum smoothness was analyzed as a three-level variable comparing grooved (A) or smooth (C) with the normal group (B) as the reference. Upper-lip thickness was also analyzed as a three-level variable, comparing thick (A) or thin (C) with normal (B) as the reference. Only 25 children were classified in group C of PFL measure; therefore, group C and B were combined. We compared a binary classification of a shorter PFL (C and B) to the normal group (A) as reference.

Moreover, we used a weighted quantile sum approach (WQS) to estimate the association between the joint exposure of six PFAS (i.e., "PFAS mixture") and each of the facial feature measures (Carrico et al. 2015). A WQS index assigned each PFAS a weight that reflected the strength of its association with the outcome and the collinearity between that PFAS and other PFAS in the mixture (Carrico et al. 2015). We constrained the sum of the weights to 1.0 to incorporate each mixture component into a single effect estimate. The WQS index was created using the PFAS quartiles, and a 1-unit increase in the WQS index was interpreted as a per-quartile increase in the PFAS mixture exposure. Because WQS performs unidirectional evaluation of mixture effects, we used the results from individual chemical analyses to guide the directionality of WQS analysis. For PFL, we estimated only the positive mixture effect, whereas for smooth philtrum, only the negative mixture effect. There were no clear associations for other end points in individual chemical analysis, thus we estimated both positive and negative mixture associations and reported estimates that were further away from null for these end points.

Because the study population was a subset of the full cohort, all analyses were weighted by the baseline sampling fraction and the probabilities of having outcome available among those invited using inverse-probability selection weight (IPSW) (Kesmodel et al. 2010; Liew et al. 2018b). Sampling of LDPS from all DNBC participants was random within alcohol intake categories, and the sampling probabilities were available for adjustment and have been used in previous studies (Kesmodel et al. 2010). Reweighting by the sampling fractions could enhance the generalizability of findings to the entire DNBC cohort. In addition, our IPSW procedure adjusted for potential selection bias by accounting for the probability of nonparticipation in the LDPS among the invited according to factors measured for all women in the DNBC at baseline. Details of the weighting procedures have been described previously (Inoue et al. 2019; Liew et al. 2018b; Luo et al. 2020). Multiple imputation was used for missing values in covariates in the regression models. Less than 3% of all participants had missing values in at least one covariate. We assumed that they were missing at random (Lubin et al. 2004). Ten simulated complete data sets were generated via imputation, and we employed the analytical procedure PROC MIANALYZE in SAS to combine the results (Yuan 2010).

We also performed sex-stratified analyses to evaluate potential effect modification by child's sex because some sex-specific associations between prenatal PFAS exposure and neurological and pubertal end points have been suggested in previous DNBC studies (Ernst et al. 2019). A prior study suggested that only heavy but not moderate/low alcohol intake during pregnancy was shown to affect these facial feature measures in this cohort (Kesmodel et al. 2019). In sensitivity analyses, we excluded women who reported binge drinking or heavy alcohol use (defined as >4 drinks/wk) intake in the first trimester. Furthermore, we performed a stratified analysis by maternal alcohol intake (yes or no) in the first trimester. Tests of heterogeneity were performed by assessing the p-value of the interaction term on a multiplicative scale for each PFAS and child's sex or maternal alcohol intake in the regression models. All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc.).

Results

Table 1 presents the distributions of selected demographic characteristics of the study participants. The majority (76.3%) of the

Table 1. Distribution of demographic characteristics of the study participants ($n = 656$) from the Lifestyle During Pregnancy Study–Danish National Birth Cohort.

Characteristics	*N*	%
Maternal age (y)		
≤24	47	7.2
25–29	235	35.8
30–34	266	40.5
≥35	108	16.5
Birth year		
1997–2000	360	54.9
2001–2003	296	45.1
Socio-occupational status		
High	255	39.2
Medium	204	31.3
Lower	<197[a]	<30.3
Missing	<5[a]	—
Alcohol drinking during the first trimester (drinks/wk)		
None	318	48.8
≤1	181	27.8
>1	152	23.3
Missing	5	—
Smoking during pregnancy		
No	433	66.0
Yes	223	34.0
Prepregnancy BMI (kg/m^2)		
Underweight (<18.5)	26	4.1
Normal (18.5–25)	444	69.5
Overweight (>25)	169	26.4
Missing	17	—
Gestational week of blood draw		
<8	290	44.2
8–10	204	31.1
≥11	162	24.7
Sex		
Female	338	51.5
Male	318	48.5
Parity		
Nulliparous	312	49.1
Parous	324	50.9
Missing	20	—

Note: —, not applicable; BMI, body mass index.
[a]Due to data regulation, we are not allowed to show cells with a frequency <5.

mothers were 25–34 years of age at the time of delivery, nearly half (49.1%) were nulliparous, 34.0% reported smoking during the first trimester, and 23.3% had >1 drink of alcohol/wk during the first trimester. The distributions of PFAS concentrations in maternal plasma are shown in Table S1. The PFAS exposure distribution in our sample was comparable with a separate study that selected maternal samples at random representing the whole DNBC cohort (Liew et al. 2015), as well as those reported for adults 20–39 years of age of the U.S. National Health and Nutrition Examination Survey 1999–2000 (https://www.cdc.gov/nchs/nhanes/index.htm).

The distributions of the three facial feature measures are presented in Table S2. Children with a shorter PFL had lower verbal and performance IQ scores and poorer attention and executive function at 5 years of age compared with the normal group (Table S3). Specifically, children classified as group C in the PFL had 7.2 (95% CI: 1.8, 12.7) points lower in full-scale IQ and 0.5 (95% CI: 0, 0.9) points lower in attention function compared with children of group A in PFL. For philtrum smoothness and upper-lip thickness, there was no large difference observed for the neuropsychological test scores between the normal and other groups.

Results based on the continuous concentrations of PFAS in maternal plasma found that all six prenatal PFAS [per doubling level (in nanograms per milliliter)] were associated with elevated odds of a shorter PFL at 5 years of age, with the strongest association found for PFDA (OR = 2.02; 95% CI: 1.11, 3.70) (Table 2). There were no associations observed for prenatal PFAS exposure and philtrum measures, except for one compound PFNA that was inversely associated with lower odds for smooth philtrum (OR = 0.52; 95% CI: 0.30, 0.90) (Table 2). No association was found for thin or thick upper-lip measure compared with the normal group for any of the PFAS based on continuous concentrations.

In analysis of exposure to PFAS based on tertiles, higher PFDA was associated with a shorter PFL [OR = 1.80 (95% CI: 0.96, 3.37) for the second tertile; OR = 2.01 (95% CI: 1.07, 3.79) for the third tertile] (Figure 1; effect estimates can be found in Table S4). Some nonlinearity was noted for philtrum measures where the second tertile of PFDA (OR = 1.65; 95% CI: 1.09, 2.52) or PFNA (OR = 1.72; 95% CI: 1.14, 2.60) was associated with grooved philtrum, and the second tertile of PFHpS (OR = 0.36; 95% CI: 0.18, 0.76) was associated with smooth philtrum, whereas no association was found for the third tertiles for any PFAS compounds (Figure 1). There were no apparent associations found for any of the PFAS tertiles and upper-lip measures. The GAM analyses also suggested nonlinearity between PFNA and grooved philtrum ($p_{Nonlinearity} = 0.08$), PFDA and grooved philtrum ($p_{Nonlinearity} = 0.02$), and PFHpS and smooth philtrum ($p_{Nonlinearity} = 0.01$) (Table S5).

In mixture analysis, prenatal exposure to the six-PFAS mixture was associated with a shorter PFL [OR = 1.31 (95% CI: 1.01, 1.72) for a 1-unit increase in WQS index] (Table 2), whereas the associations for the PFAS mixture were close to null for philtrum and upper-lip measures. Exposure to PFHpS and PFDA contributed larger weights (0.30–0.36) in the estimated mixture effect on shorter PFL, followed by PFOS and PFHxS exposure (0.12–0.14), whereas PFOA and PFNA exposure contributed only minimally (0.03–0.05) (Table S6).

In sensitivity analysis where maternal binge or heavy alcohol drinkers were excluded, the results were consistent with our main findings for all three facial features (Table S7). In stratified analysis by maternal alcohol intake during the first trimester, the associations between a shorter PFL and PFDA exposure remained similar, but stronger positive associations were observed for exposure to five other PFAS only among the alcohol users (Table 3). In addition, PFHxS exposure was associated with a thick upper-lip [OR = 2.40 (95% CI: 1.00, 5.79); $p_{Interaction} = 0.08$] among drinkers only, whereas PFOA exposure was associated with a grooved philtrum [OR = 2.02 (95% CI: 1.04, 3.91); $p_{Interaction} = 0.01$] in nondrinkers.

There were no sex differences in the associations between per doubling increase of each of the PFAS and short PFL (Figure 2; effect estimates can be found in Table S8). Some sex-specific differences were observed for philtrum measures where a higher level of PFDA, PFNA, or PFHxS exposure was associated with lower odds for grooved or smooth philtrum in girls only. Increasing PFDA and PFHxS exposure were associated with higher odds for a thinner or thicker lip measure only in boys. However, the $p_{Multiplicative\ interaction}$ was >0.10 in all sex-specific association comparisons (Table S8).

Discussion

Among a subset of children enrolled in the DNBC, prenatal exposure to PFAS, particularly PFDA and a mixture of PFAS, was associated with short PFL in children at 5 years of age. Some nonlinear patterns were observed for philtrum measure in the PFAS tertile analysis, with associations found for the second tertile of PFDA, PFNA, and PFHpS. The associations between exposures to multiple types of PFAS and short PFL could be specific among women who consumed alcohol in pregnancy; the

Table 2. Odds ratio (OR) and 95% confidence interval (CI) for facial features according to per doubling increase in prenatal PFAS level (in ng/mL) in logistic regression models.

	Palpebral fissure length	Philtrum smoothness		Upper lip thickness	
PFAS	Shorter (B,C) vs. normal (A)	Grooved (A) vs. normal (B)	Smooth (C) vs. normal (B)	Thick (A) vs. normal (B)	Thin (C) vs. normal (B)
	$n = 84$ vs. $n = 398$	$n = 256$ vs. $n = 310$	$n = 69$ vs. $n = 310$	$n = 100$ vs. $n = 168$	$n = 184$ vs. $n = 168$
PFOS	1.66 (0.85, 3.22)	1.27 (0.81, 1.98)	1.02 (0.50, 2.06)	1.08 (0.53, 2.21)	0.63 (0.35, 1.14)
PFOA	1.32 (0.73, 2.40)	1.09 (0.72, 1.64)	0.71 (0.38, 1.35)	1.29 (0.68, 2.47)	0.67 (0.39, 1.15)
PFDA	2.02 (1.11, 3.70)	0.84 (0.58, 1.22)	0.85 (0.47, 1.53)	0.97 (0.57, 1.64)	1.21 (0.75, 1.95)
PFNA	1.41 (0.77, 2.58)	0.85 (0.57, 1.26)	0.52 (0.30, 0.90)	0.74 (0.43, 1.27)	0.81 (0.50, 1.31)
PFHxS	1.45 (0.85, 2.46)	0.82 (0.59, 1.14)	0.78 (0.47, 1.29)	1.47 (0.86, 2.51)	1.11 (0.73, 1.67)
PFHpS	1.70 (0.93, 3.10)	1.09 (0.74, 1.60)	0.99 (0.54, 1.80)	1.15 (0.61, 2.15)	0.75 (0.45, 1.25)
WQS (mixture)[a]	1.31 (1.01, 1.72)	0.94 (0.76, 1.16)	0.85 (0.68, 1.08)	1.07 (0.90, 1.28)	0.93 (0.68, 1.28)

Note: Models were adjusted for sex, birth year, maternal age, parity, maternal social-occupational status, maternal prepregnancy BMI, gestational week of blood draw, smoking during the first trimester, and alcohol intake during the first trimester. BMI, body mass index; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; WQS, weighted quantile sum approach.

[a]A WQS index was created using all six types of PFAS to reflect the mixture exposure level. The OR indicates the association between a per quartile increase in the WQS index and each of the facial feature measures, adjusting for the same set of confounders.

modifying role of alcohol exposures on these associations observed for PFL warrants further research.

Examination of prenatal influences on craniofacial development is an understudied area that can add to existing research on assessments of neurological outcomes. Early gestational exposure to environmental factors have been identified to influence both fetal craniofacial and brain development. For instance, alcohol drinking during pregnancy has been established as a risk factor for impaired offspring neurodevelopment and specific facial features as exhibited in FAS (Astley and Clarren 2000). Maternal smoking and exposure to air pollutants during pregnancy have been associated with orofacial cleft and neuropsychological deficits (Kummet et al. 2016; Polanska et al. 2017; Rao et al. 2016; Volk et al. 2021). Children with an orofacial cleft are also more likely to be affected by intellectual disability, language disorders, and psychiatric disorders (Tillman et al. 2018). Multiple environmental risk factors have been suggested to affect autism spectrum disorder (Becerra et al. 2013; Modabbernia et al. 2017), where neurological conditions have also been recognized to be accompanied with distinct facial phenotypes (Aldridge et al. 2011). However, epidemiological studies devoted to investigating how xenobiotics exposures might affect craniofacial development and their associated domains of neurodevelopment are still sparse.

Fetal craniofacial and brain tissues are developed in part from the same cell lineages in early embryo (Couly and Le Douarin 1985, 1987). For instance, neural crest cells develop into different cell types including neuroglia and craniofacial cartilage (Cordero et al. 2011; Simões-Costa and Bronner 2013), and the processes can be sensitive to disruption from exogenous chemicals, including PFAS exposures (Doupe et al. 1985; Shi et al. 2017).

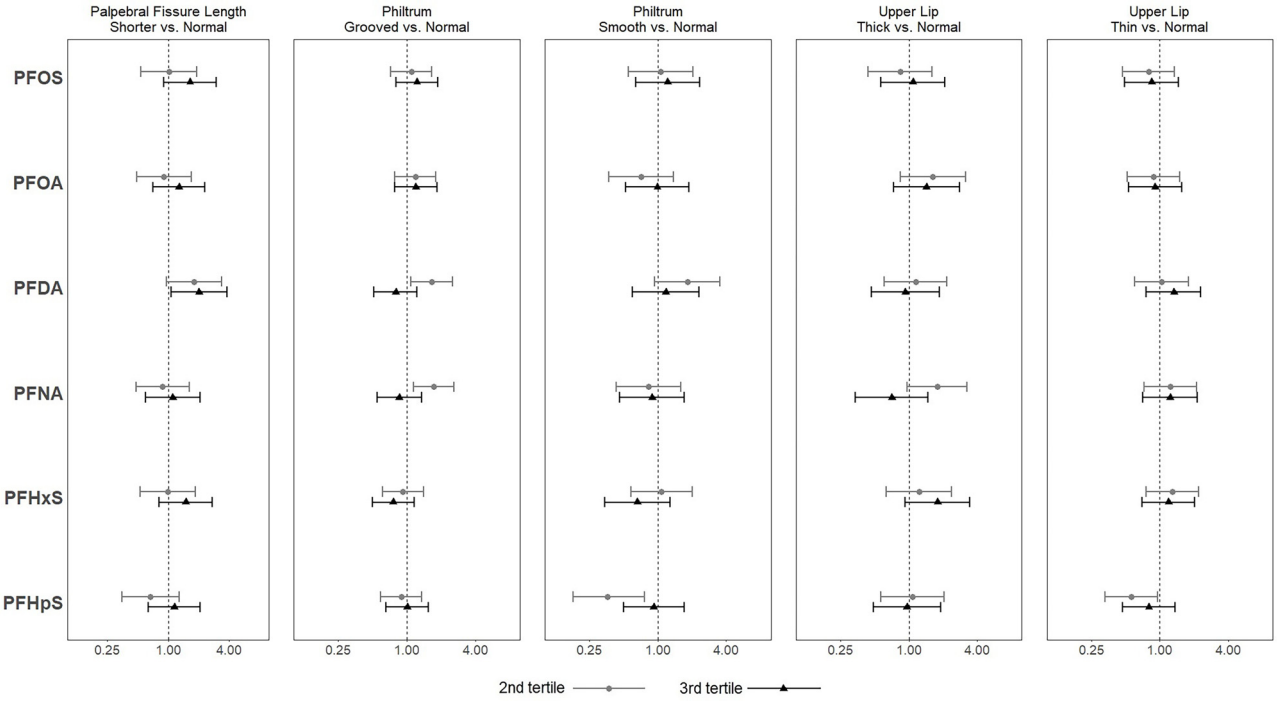

Figure 1. Odds ratio (OR) and 95% confidence interval (CI) for facial features according to prenatal PFAS tertiles in logistic regression models. Models were adjusted for sex, birth year, maternal age, parity, maternal social-occupational status, maternal prepregnancy BMI, gestational week of blood draw, smoking during the first trimester, and alcohol intake during the first trimester. The effect estimates can be found in Table S4. Note: BMI, body mass index; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.

Table 3. Odds ratio (OR) and 95% confidence interval (CI) for facial features according to per doubling increase in prenatal PFAS level (in ng/mL) in logistic regression models, stratified by maternal alcohol intake during the first trimester.

	Palpebral fissure length	Philtrum smoothness		Upper lip thickness	
Maternal alcohol intake/*p*-Value	Shorter (B,C) vs. normal (A)	Grooved (A) vs. normal (B)	Smooth (C) vs. normal (B)	Thick (A) vs. normal (B)	Thin (C) vs. normal (B)
No alcohol drinking during the first trimester	$n=34$ vs. $n=200$	$n=121$ vs. $n=149$	$n=38$ vs. $n=149$	$n=47$ vs. $n=83$	$n=95$ vs. $n=83$
PFOS	0.80 (0.28, 2.33)	1.94 (0.97, 3.91)	0.96 (0.36, 2.56)	0.93 (0.31, 2.77)	0.51 (0.21, 1.22)
PFOA	0.55 (0.22, 1.37)	2.02 (1.04, 3.91)	0.71 (0.29, 1.71)	1.17 (0.43, 3.17)	0.54 (0.24, 1.19)
PFDA	2.22 (0.83, 5.90)	0.90 (0.51, 1.59)	0.50 (0.22, 1.14)	1.01 (0.44, 2.33)	1.34 (0.67, 2.66)
PFNA	0.73 (0.30, 1.76)	1.12 (0.61, 2.06)	0.47 (0.23, 1.00)	0.70 (0.30, 1.67)	0.66 (0.34, 1.29)
PFHxS	0.74 (0.38, 1.44)	0.94 (0.61, 1.44)	1.00 (0.50, 2.02)	0.94 (0.49, 1.80)	0.97 (0.56, 1.66)
PFHpS	1.04 (0.43, 2.51)	1.33 (0.75, 2.38)	1.23 (0.53, 2.83)	1.07 (0.43, 2.69)	0.76 (0.38, 1.54)
Alcohol drinking during the first trimester	$n=50$ vs. $n=198$	$n=132$ vs. $n=161$	$n=31$ vs. $n=161$	$n=53$ vs. $n=85$	$n=89$ vs. $n=85$
PFOS	2.67 (1.08, 6.60)	0.91 (0.49, 1.66)	1.16 (0.42, 3.25)	1.19 (0.44, 3.21)	0.75 (0.33, 1.70)
PFOA	2.68 (1.15, 6.23)	0.68 (0.39, 1.20)	0.85 (0.32, 2.23)	1.46 (0.58, 3.65)	0.85 (0.40, 1.84)
PFDA	2.20 (0.96, 5.03)	0.79 (0.47, 1.31)	2.48 (0.86, 7.14)	0.99 (0.49, 2.00)	1.17 (0.57, 2.39)
PFNA	2.81 (1.19, 6.67)	0.67 (0.38, 1.21)	1.11 (0.38, 3.23)	0.68 (0.30, 1.58)	1.45 (0.62, 3.35)
PFHxS	3.67 (1.60, 8.45)	0.69 (0.40, 1.18)	0.55 (0.23, 1.34)	2.40 (1.00, 5.79)	1.44 (0.71, 2.94)
PFHpS	2.62 (1.13, 6.11)	0.87 (0.50, 1.51)	0.73 (0.29, 1.85)	1.20 (0.48, 3.01)	0.74 (0.35, 1.56)
$p_{Interaction}$					
PFOS	0.18	0.07	0.75	0.96	0.62
PFOA	0.01	0.01	0.70	0.83	0.45
PFDA	0.81	0.93	0.02	0.70	0.87
PFNA	0.05	0.33	0.16	0.77	0.11
PFHxS	<0.01	0.37	0.34	0.08	0.29
PFHpS	0.22	0.17	0.57	0.97	0.92

Note: Models were adjusted for sex, birth year, maternal age, parity, maternal social-occupational status, maternal prepregnancy BMI, gestational week of blood draw, and smoking during the first trimester. BMI, body mass index; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.

Multiple PFAS compounds were detected in maternal placentas and dead embryos/fetuses tissues in a study of elective pregnancy terminations (Mamsen et al. 2019). PFAS exposure can induce oxidative stress in cells, causing cellular dysfunction or cell death (Wielsøe et al. 2015). PFAS have also demonstrated endocrine-disrupting properties through interfering with thyroid hormone transportation and regulating deiodinase (Ghassabian and Trasande 2018). Moreover, subclinical changes in maternal thyroid hormone are important for fetal brain development even after birth, given that thyroid hormones are involved in the migration of neural crest cells (Auso et al. 2004; Bronchain et al. 2017).

In the present study, the association of prenatal exposure to multiple PFAS outcomes with facial features was most consistent for shorter PFL. A shorter PFL could reflect forebrain damages

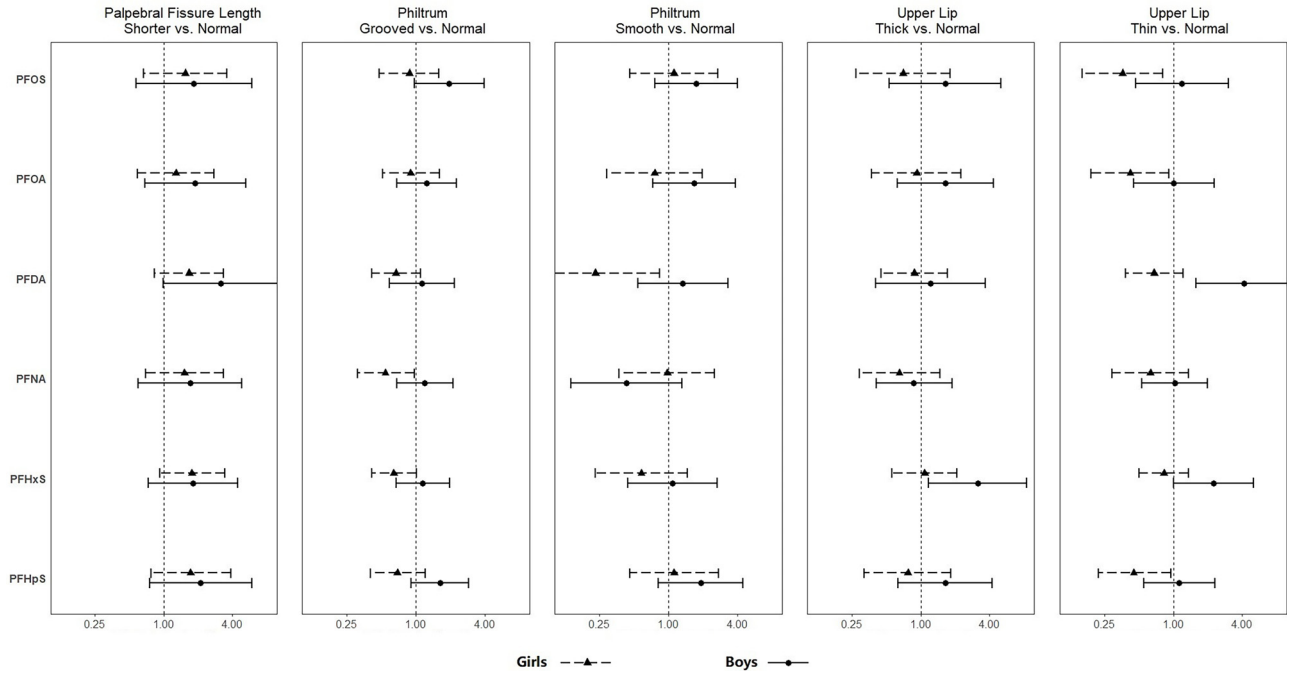

Figure 2. Odds ratio (OR) and 95% confidence interval (CI) for facial features by sex according to per doubling increase in prenatal PFAS level (in ng/mL) in logistic regression models. Models were adjusted for birth year, maternal age, parity, maternal social-occupational status, maternal prepregnancy BMI, gestational week of blood draw, smoking during the first trimester, and alcohol intake during the first trimester. The effect estimates can be found in Table S8. Note: BMI, body mass index; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.

during fetal development (Brennan and Giles 2014). We also found that children with shorter PFL had lower IQ scores and more behavioral difficulties at 5 years of age in this cohort. However, in previous analyses within the same cohort, we did not find consistent associations between prenatal PFAS exposures and child IQ scores (Liew et al. 2018b), and only specific PFAS compounds were related to externalizing or internalizing behaviors in older ages (Luo et al. 2020). One plausible explanation is that physical measures of facial features are sensitive enough to detect even subtle effects of external exposures during early embryonic development, as compared with neuropsychological test scores in young children, which can be influenced by other social/familial factors (Eriksen et al. 2013). Our study sample size precluded analyses that combined both facial features and neuropsychological function as outcomes, which should be addressed in larger studies. In addition, our findings for PFL indicated that an evaluation of prenatal PFAS effects on eye malformations and vision impairments would also be needed.

The LDPS study oversampled mothers who consumed alcohol during pregnancy in the DNBC, thus enabling the opportunity to examine the modifying effects of prenatal alcohol on the associations between PFAS exposure and child facial features at 5 years of age. The overall findings for PFAS exposure remained after excluding pregnant women who reported binge drinking and high alcohol intake, the only alcohol exposure groups that were associated with FAS facial phenotypes in this cohort (Kesmodel et al. 2019). We detected some effect modification of ever alcohol intake, especially for PFL, for which strong positive associations were observed for exposure to multiple PFAS only among women who consumed alcohol during the first trimester. It is biologically plausible that alcohol and PFAS exposure influence embryonic and fetal development by targeting common biological pathways, for example, neurotoxicity induced by oxidative and endocrine effects of these exposures (Wilcoxon et al. 2005; Wu and Cederbaum 2003). However, a lack of statistical precision and chance errors in stratified analyses might be possible because the number of children classified as having a short PFL among nondrinkers was small. Future studies should explore the role of prenatal alcohol exposure in the associations between PFAS and fetal development.

Our study has several strengths. First, to our knowledge, this is the first study to explore facial features as a novel marker to study the potential effect of PFAS exposure on fetal development. Quantification of facial features using digital photographs offers the promise of a more objective outcome assessment and could advance our understanding of the role of intrauterine environmental exposures on early embryonic and fetal central nervous system development. Second, we were able to control for a wide range of potential confounders, and our PFAS mixture analyses suggested robust results for PFAS exposure and shorter PFL. Participants were selected from a well-designed longitudinal cohort, with sampling and nonparticipation accounted for using weighted regression methods that minimized the influence of selection bias on our results. The PFAS levels found in this LDPS sample were comparable to other samples from the DNBC unaffected by dropout or missing the facial feature end points for analyses (Liew et al. 2014; 2015).

Several limitations should be considered. First, the sample size for the facial outcome measures varied because not all features were measurable with high quality using the standard digital photographs collected (Kesmodel et al. 2019). Some specific categories of facial feature classification were small in this study sample, such as group C of PFL, which was combined with group B to avoid sparse data bias in analysis. Second, the facial feature classifications were made based on recommended distribution but were not meant to indicate diagnosis of specific congenital neurological disorders. Despite an increasing interest to explore the use of facial phenotypes to predict neurodevelopmental disorders in childhood (e.g., autism), studies that examined the relations between craniofacial markers and neurological function in general populations are lacking. In our sample, children with a shorter PFL scored lower in cognitive and behavioral tests, but whether philtrum and upper-lip features are associated with any neurological functional impairment was not clear. A larger study that clarifies the predictive link between multiple craniofacial markers and specific domains of neurological deficits would strengthen the implications of findings. Third, although we were able to account for many important confounders, we cannot rule out residual confounding from other unmeasured confounders, such as dietary habits, lifestyles, and household factors (Halldorsson et al. 2008). Residual confounding from imperfect measures of confounders, such as an underreporting of alcohol intake, is also possible. Finally, although genetic factors are also important contributors to craniofacial features, we had no information on genetics in this study sample. Genetics could play a modifying role and studies on gene–environment interaction for craniofacial development are warranted.

In summary, prenatal PFAS exposures were associated with facial features at 5 years of age in the DNBC. The strongest association was found for short PFL being associated with a higher level of multiple PFAS exposure, particularly among women who consumed alcohol during pregnancy. There were also some nonlinear and sex-specific associations observed for specific PFAS exposures and philtrum smoothness or upper-lip thickness. These novel findings require replication in larger populations and further examinations of whether the craniofacial markers reflect specific neurologic functional deficits in offspring exposed to PFAS are needed.

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