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Longitudinal trajectories and determinants of plasma per- and polyfluoroalkyl substance (PFAS) levels from birth to early childhood and metabolomic associations: A pilot study in the Boston Birth Cohort

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Abstract

Background: Per- and polyfluoroalkyl substances (PFAS) are a major public health concern worldwide due to their ubiquitous exposures, environmental persistence, maternal-to-fetal transfer, and multi-organ toxicity. This pilot study aimed to generate preliminary data to inform future studies to address data gaps in the field, including early life PFAS exposure levels, longitudinal changes, determinants, and associated metabolomic alterations in understudied Black and Hispanic children in the United States (U.S.).

Methods: This study leveraged existing biosamples and data in the Boston Birth Cohort and measured 12 legacy and emerging PFAS, including Me-PFOSA-AcOH, PFDA, PFDoA, PFHxS, PFNA, PFOA, PFOS, PFUnA, GenX, ADONA, 9Cl-PF3ONS, and PFHpS, in paired cord and early childhood plasma samples. Summary statistics and graphic plots were used to depict PFAS

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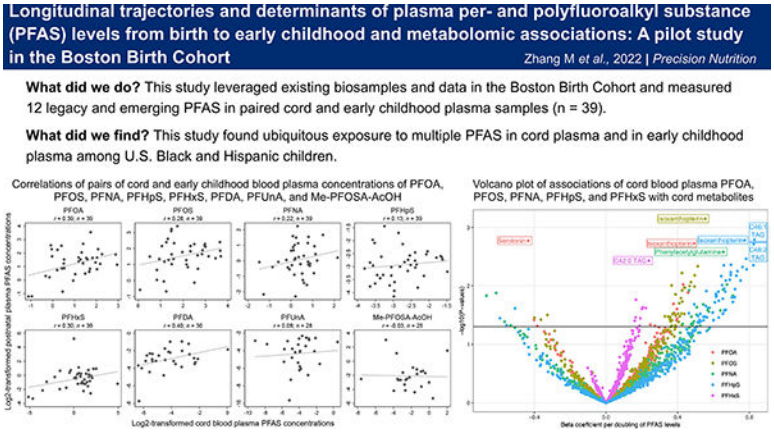
levels at the two time points and their longitudinal changes. Linear regression models were used to identify the early-life factors associated with cord and early childhood PFAS levels. Associations of cord PFAS with cord metabolites were explored using a metabolome-wide association approach and a targeted approach.

Results: This study included 39 children, of whom 25 (64%) were Black, 14 (36%) were Hispanic, and 15 (38%) were female. PFOA, PFOS, PFNA, and PFHpS were detectable in all cord and early childhood plasma samples, while GenX and ADONA were not detectable in any sample. Cord PFAS levels were weakly-to-moderately correlated with early childhood PFAS levels (r = −0.03 to 0.40). Several maternal and child factors, including gestational age, year at blood collection, and race/ethnicity, were associated with cord and early childhood PFAS levels. The metabolome-wide association study and the targeted study identified several cord metabolites that may have been affected by *in utero* PFAS exposure.

Conclusions: This pilot study found ubiquitous exposure to multiple PFAS in cord plasma (reflects *in utero* exposure) and in early childhood plasma (reflects both prenatal and postnatal exposure) among U.S. Black and Hispanic children. Metabolomic analysis suggests that *in utero* PFAS exposures may alter fetal metabolism. Future large-scale studies are needed to replicate the findings and further examine the associations of fetal PFAS exposure with long-term health outcomes and underlying metabolic pathways.

Graphical Abstract:



1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic fluorinated organic compounds that have been manufactured since the 1940s by replacing hydrogen (H) in organic compounds by fluorene (F)[1,2]. Due to their strong binding to carbon and fluorine (C-F) and unique chemical properties, such as water and oil repellency, friction reduction, surfactant, and temperature resistance, PFAS have been extensively used in the making or as a component of commercial and consumer products, including food packaging, cookware, clothing, carpets, personal care products, and aqueous fire-fighting foams[2,3]. PFAS are water-soluble, so they can be easily transported through aqueous environments, but they are highly resistant to biodegradation and thus persistent in the environment[2,4,5]. They have been found in air, dust, soil, water, and foods; human PFAS exposures may occur through

contact with all these sources[2]. Once in the body, long-chain PFAS are slowly excreted with half-lives ranging from a few years (e.g., for perfluorooctanoic acid [PFOA] and perfluorooctane sulfonic acid [PFOS]) to up to a decade (e.g., for perfluorohexane sulfonic acid [PFHxS]). They bioaccumulate, if exposure is continuing, and bind tightly to proteins and are predominantly present in human serum or plasma[6–8]. Although certain PFAS (e.g., PFOA and PFOS) have been voluntarily phased out in the United States (U.S.) since the 2000s, newer, short-chain PFAS are extensively used to replace the legacy ones[9,10]. With these phase-outs, levels of legacy PFAS in the U.S. population have decreased in the past decade, but they remain widespread due to their long half-lives[11].

From a life-course perspective, the fetal period corresponds to the most rapid cell division, differentiation, and organ and system formation and functional development, representing the most sensitive period to environmental perturbations[12]. PFAS exposures start *in utero*, as studies have demonstrated the trans-placental passage of several PFAS from mothers to fetuses[8,13–17]. Perinatal and early childhood PFAS exposure routes include breastfeeding, maternal and infant dietary intake, drinking or using PFAS-contaminated water for baby formula, house dust, and contact with consumer products[13,18]. PFAS are endocrine disruptors[19], and some PFAS have been associated with adverse reproductive and developmental outcomes[2], such as preeclampsia, miscarriage, and preterm birth[20]. Studies have identified associations of early life PFAS exposures with risk of neurodevelopment and cardiometabolic outcomes in childhood[19,21]. To date, however, data on early life PFAS exposure levels are sparse, particularly among Black and Hispanic children in the U.S.[1] Important gaps also include early life determinants and metabolomic signatures of PFAS exposures and longitudinal changes in PFAS levels from birth to early childhood. Also, few studies have examined the exposure levels of emerging PFAS, such as 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS), 4,8-dioxa-3H-perfluorononanoic acid (ADONA), and perfluoro-2-proproxypropanoic acid (GenX).

To address the gaps above, the objectives of this pilot study were to *a)* demonstrate the feasibility of quantifying PFAS levels in cord and early childhood blood plasma samples, *b)* depict interindividual levels, variabilities, and longitudinal changes of PFAS levels from birth to early childhood; *c)* determine early life factors associated with PFAS levels at the two time points, and *d)* explore cord blood metabolomic signatures associated with cord PFAS levels using an untargeted metabolome-wide association approach and a targeted approach. We measured 12 PFAS, including legacy long-chain (i.e., ≥ C8 for perfluorocarboxylic acids [PFCAs], ≥ C6 for perfluorosulfonic acids [PFSAs]) and emerging PFAS, in cord blood and early childhood blood plasma in 39 children in the Boston Birth Cohort (BBC). The BBC is a well-established, prospective birth cohort that comprises a sample of the patient population at the Boston Medical Center, the largest safety-net hospital in the New England area, serving predominantly urban, low-income, Black, and Hispanic populations from Boston, MA. With its longitudinal design and rich databases, the BBC is well-suited to investigate early life PFAS exposure on a broad range of child health outcomes and metabolomic underpinnings.

2. Methods

2.1 Study population

The BBC has recruited participants from the Boston Medical Center in Boston, MA, since 1998, using rolling enrollment. Cohort descriptions and recruitment strategies have been provided in previous publications[22,23]. The BBC has been registered on ClinicalTrials.gov (Identifier: NCT03228875). Mothers who delivered a singleton live birth were eligible for participation in the study. Pregnancies resulting from *in vitro* fertilization or with multiple gestations and newborns with fetal chromosomal abnormalities or major birth defects were excluded. Trained research staff contacted eligible mothers 24 to 72 hours after delivery. After written informed consent was obtained by research staff at the maternal bedside, a structured questionnaire was administered that included medical history, dietary intake, home environment, sociodemographic information, substance use, pregnancy history, and health status. This analysis included a random sample of 39 children in the BBC who had necessary data and archived paired cord blood and early childhood blood samples. Initially, 40 children were selected, but one child was excluded from pair-wise analyses due to missing postnatal blood samples.

The BBC is under the oversight of institutional review boards at the Boston Medical Center and the Johns Hopkins Bloomberg School of Public Health and has received initial and continuation approval since its inception. We obtained written informed consent from each child's biological mother.

2.2 PFAS measurement

Labor and delivery nursing staff collected cord blood samples, and pediatric phlebotomists obtained early childhood blood samples at children's first postnatal follow-up study visit. Research staff separated plasma and red blood cells by centrifugation and stored the samples at −80°C. Plasma samples were transported on dry ice to the Environmental and Chemical Laboratory Services (ECLS), Public Health and Environmental Laboratories at the New Jersey Department of Health. As part of the U.S. Centers for Disease Control and Prevention's (CDC) State Biomonitoring Programs, the ECLS lab currently monitors ~130 chemicals in the State of New Jersey, including12 PFAS measured in serum/plasma: 2-(N-methyl-PFOSA) acetic acid (Me-PFOSA-AcOH), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), PFHxS, perfluorononanoic acid (PFNA), PFOA, PFOS, perfluoroundecanoic acid (PFUnA), GenX, ADONA, 9Cl-PF3ONS, and perfluoroheptanesulfonic acid (PFHpS)[24]. For all 39 children included in this study, we measured the 12 PFAS in paired cord and early childhood plasma samples.

The lab used an online solid-phase extraction unit coupled to a high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS), described in detail elsewhere[25]. The lab strictly followed the CDC protocols and Clinical Laboratory Improvement Amendments (CLIA) guidance for quality control steps. To accurately measure each PFAS, a known mass of internal standards (mass labeled PFAS purchased from Wellington Labs) was spiked onto each plasma sample and calibration standard to monitor and normalize the responses in the analytical system. Among the 12 target PFAS analytes, internal standards

for ADONA, PFHpS, and 9Cl-PF3ONS were unavailable. Therefore, internal standards for GenX, PFHxS, and PFOS were respectively utilized to quantify the above three PFAS compounds. Rigorous method validation studies and CLIA approval process confirmed the use and report of the above three PFAS concentrations with neighboring internal standards. Low- and high-concentration quality control materials, prepared from a calf serum pool, were included to ensure the overall analysis steps are in control and reported concentrations are accurate and reliable. The lab participates in an external proficiency testing program for PFAS in human samples (three times per year) and has passed all tests since 2020. The detailed lab methods and method performance were published previously[26–28]. For samples below the limit of detection (LOD), the lab provided machine-read values and reported values as "<0" if the concentrations were negative. Negative concentrations occur when samples with a very low PFAS level were plugged into a calibration curve with a positive intercept that represents the background portion; these values were obtained mathematically but do not have biological or analytical meanings and are usually treated as non-detectable or zero in exposure science literature.

2.3 Cord metabolome profiling

We performed metabolome profiling using stored cord blood plasma samples collected at birth. Metabolome profiling was conducted at the Broad Institute of MIT and Harvard with two LC-MS/MS techniques: 1) hydrophilic interaction liquid chromatography in the positive ionization mode (HILIC-pos) for water-soluble metabolites, and 2) C8 chromatography with positive ion mode (C8-pos) for polar and non-polar plasma lipids. The Broad Institute has published their protocols for analyzing metabolites[29], and we strictly followed these protocols. Detailed quality control and assurance steps for metabolome profiling have been provided in our previous publication[30]. Briefly, all the samples were analyzed in a blinded manner. We created a pooled reference sample composed of all study samples and randomly inserted the pooled samples across study samples (per 20–30 samples). We calculated the coefficient of variation (CV) of each metabolite using the reference samples. We monitored the internal standard peak to ensure system performance. Using an untargeted approach, we identified 397 metabolites that represent both endogenous (e.g., lipids, amino acids, nucleotides) and exogenous (e.g., xenobiotics) compounds. This analysis included 378 cord metabolites with CV < 20%. We imputed the non-detectable values as one-half of the minimal value of each metabolite, and we used inverse normal transformation to render an approximately normal distribution of the metabolites.

2.4 Covariates

We extracted data on maternal age at delivery and child sex, gestational age, birthweight, and birth date from the electronic medical records. We collected information on maternal race and ethnicity, educational level, marital status, pre-pregnancy weight and height, cigarette smoking history during pregnancy, and parity from the standardized Maternal Postpartum Questionnaire, and child breastfeeding status from a postnatal follow-up questionnaire. We calculated maternal pre-pregnancy body mass index (BMI) as pre-pregnancy weight (kilograms) divided by height (meters) squared and defined overweight or obese (OWO) as BMI ≥ 25 kg/m^2. We defined preterm birth as gestational age at birth < 37 weeks and low birthweight as birthweight < 2,500 grams. We calculated standardized

birthweight-for-gestational-age (BW-GA) using an internal population consisting of >15,000 deliveries from pregnant women admitted to the Boston Medical Center labor and delivery service, and we defined small for gestational age as BW-GA < 10^{th} percentile and large for gestational age as BW-GA > 90^{th} percentile of the BW-GA distributions in the reference population[31].

2.5 Statistical analysis

2.5.1 Descriptive analysis—We described the characteristics of the 39 children in this analysis using n (%) for categorical variables and mean [standard deviation (SD)] or median [interquartile range (IQR)] for continuous variables. We quantified the distributions of the 12 PFAS. We calculated the detection frequency for each PFAS as the number of samples with concentrations above the LOD divided by the total number of samples × 100. Samples with concentrations below the LOD or negative concentrations were classified as "<LOD."

We used scatterplots to show the correlations of pairs of cord and early childhood plasma PFAS concentrations (log2-transformed). We calculated the Pearson correlations of each pair of cord and early childhood PFAS samples. We used machine-read values for samples below the LOD unless the concentrations were negative. Pairs with at least one negative concentration were excluded (n = 9 for PFDA, 11 for PFUnA, and 14 for Me-PFOSA-AcOH). PFDoA, 9Cl-PF3ONS, GenX, and ADONA were excluded due to their extremely low detection frequencies. We used fractional polynomial prediction plots to show the average cord and early childhood blood PFAS levels by child's birth year.

2.5.2 Reliability analysis of duplicate samples—We included 9 duplicate samples (4 cord samples and 5 early childhood samples) whose identities were blinded to the lab. To assess the reliability of the duplicate measures, we calculated the CV and the relative percent difference (RPD) for each duplicate pair as described in detail by Kannan et al[32]. Briefly, the CV for each duplicate pair was calculated as:

$$CV\% = \frac{SD}{X} * 100\%$$

Where SD is the standard deviation and X is the mean of each duplicate pair. RPD for each duplicate pair was calculated as:

$$RPD\% = \frac{|sample\ result\ -\ repeat\ result|}{(sample\ result\ +\ repeat\ result)/2} * 100\%$$

We also used a Bland-Altman plot to assess the agreement of the duplicate measurements[33].

2.5.3 Association analysis—The subsequent analyses included 5 PFAS (PFOA, PFOS, PFNA, PFHpS, and PFHxS). PFOA, PFOS, PFNA, and PFHpS were detected in all cord and early childhood plasma samples, and PFHxS was detected in all but one cord plasma sample (the machine-read value was used for this sample) and all early childhood plasma samples.

To identify early-life factors associated with cord and early childhood PFAS levels, we used univariable linear regression to assess the associations of maternal factors: maternal race/ethnicity [Hispanic vs. Black], educational level [some college or above vs. high school graduate or below], parity [multiparous vs. nulliparous], maternal pre-pregnancy OWO [yes vs. no]) and child factors (birthweight [per 500-gram increment], gestational age at birth [per week increment], sex [male vs. female], year at blood sample collection [cord blood collected ≥ 2005 vs. before; early childhood blood collected ≥ 2008 vs. before; defined by the respective median year at sample collection]) with cord and early childhood PFAS concentrations (log2-transformed). We also included cord PFAS levels (a doubling of concentration), breastfeeding status (yes vs. no), and child age at postnatal blood sample collection (per year increment) and examined their associations with early childhood PFAS levels.

For factors suggested to be associated with (defined as $p < 0.20$ for the factor in the univariable linear regression model) at least one cord and early childhood PFAS, we included them in multivariable linear regression models for each PFAS to assess their independent associations with cord and early childhood PFAS levels. Because breastfeeding is a known route for early life PFAS exposure[34–36], we used kernel density plots and histograms to examine the distributions of early childhood PFAS levels by breastfeeding status.

2.5.4 Metabolomics analysis—We examined the associations of cord PFAS with cord plasma metabolites using an untargeted metabolome-wide association approach and a targeted approach. Three children were excluded from these analyses due to the lack of metabolome data. For the untargeted approach, we used univariable linear regression models to examine associations of each cord PFAS (log2-transformed) with each metabolite. Given the limited power and the exploratory nature of the analyses, we did not adjust for multiple comparisons. For the targeted approach, *a priori,* we focused on metabolites that included thyroxine (a biomarker for thyroid function that has been associated with PFAS exposures[37,38]) and 25 other metabolites recently identified by our group to be associated with *in utero* metal exposures. We focused on these metabolites because they may represent common exposure pathways (e.g., consumer products, dietary intake) or biological mechanisms of *in utero* exposures on child health[39]. We estimated the Pearson correlations of cord PFAS (log2-transformed) with these 26 metabolites.

We used Stata 15.1 (Stata Corp) and R 4.1.1 (The R Foundation for Statistical Computing) to conduct the analyses.

4. Results

Table 1 shows the characteristics of the 39 children in this study. Of their mothers, 25 (64%) were Black, 14 (36%) were Hispanic, 21 (54%) had pre-pregnancy OWO, and 26 (67%) were multiparous. Of children, 15 (38%) were female and most were full term (n = 35; 90%) and had normal birthweight (n = 36; 92%). The calendar year of delivery ranged from 2001 to 2010, and the median (IQR) age at postnatal blood sample collection was 2.1 (1.3 to 3.8) years.

Table 2 shows the distributions of the cord and early childhood plasma PFAS levels of the 39 children. PFOA, PFOS, PFNA, and PFHpS were 100% detectable in all cord and early childhood samples, while PFHxS was detectable in 97.4% of the cord and 100% of the early childhood samples. The detection frequencies were lower for PFDA (43.6% in cord and 84.6% in early childhood samples), PFUnA (53.8% in both cord and early childhood samples), and Me-PFOSA-AcOH (71.8% in cord and 59.0% in early childhood samples). Only 3 (7.7%) and 1 (2.6%) cord samples had detectable levels of PFDoA and 9Cl-PF3ONS, but neither PFAS was detectable in early childhood samples. GenX and ADONA were not detectable in any cord or early childhood samples.

The intra-assay CV and RPD (Table S1) and the Bland-Altman plot (Figure S1) of the 9 pairs of blinded duplicate samples showed a relatively good test-retest reliability. The median CV ranged from 11.5% (PFHpS) to 31.8% (Me-PFOSA-AcOH) and the median RPD ranged from 16.3% (PFHpS) to 45.0% (Me-PFOSA-AcOH) (Table S1). In the Bland-Altman plot, all but 3 data points fell between the 95% limits of agreement (i.e., the mean difference ± 1.96*SD) (Figure S1).

Figure 1 shows the correlations of cord and early childhood plasma PFAS levels, respectively; PFAS levels in cord plasma were weakly to moderately correlated with PFAS levels in early childhood plasma. Pearson correlations of cord–early childhood sample pairs ranged from −0.03 (Me-PFOSA-AcOH) to 0.40 (PFDA).

Factors suggested to be associated (i.e., $p < 0.20$) with cord and early childhood PFOA, PFOS, PFNA, PFHpS, or PFHxS in univariable linear regression models included gestational age at birth, year at blood sample collection, maternal race/ethnicity, maternal education, and pre-pregnancy OWO. Birthweight and child sex were additionally associated with cord PFAS (Table S2). Cord PFAS, child age at postnatal blood sample collection, and parity were additionally associated with early childhood PFAS (Table S3). Table 3 and Table 4 provide the estimates when we included these factors in a multivariable regression model for each PFAS. For cord PFAS, larger gestational age at birth was associated with higher PFOS and PFNA, samples collected ≥ year 2005 (vs. before) had lower PFOA and PFOS, higher maternal educational level was associated with lower PFOA, and maternal pre-pregnancy OWO was associated with higher PFHpS; the overall R-squared values ranged from 0.20 (PFHxS) to 0.44 (PFOS) (Table 3). For early childhood PFAS, higher cord PFOA levels were associated with higher early childhood PFOA levels, samples collected ≥ year 2008 (vs. before) had lower levels of PFHxS, and maternal pre-pregnancy OWO was associated with lower PFOA and PFOS levels; the overall R-squared values ranged from 0.23 (PFHpS) to 0.38 (PFOA) (Table 4).

Figure S2 shows the change in mean cord and early childhood plasma PFAS levels by child's birth year. In cord blood, PFOA, PFOS, and Me-PFOSA-AcOH levels decreased monotonically by year, PFNA, PFHxS, PFDA, and PFUnA levels increased and then decreased, and the other PFAS appeared to be stable across years. Most PFAS levels appeared to be stable in early childhood blood except that PFOS and PFHxS levels decreased and PFDA levels increased and then decreased. Figure S3 shows the kernel density plots and histograms of early childhood plasma PFAS levels by breastfeeding status.

Compared to exclusively bottle-fed children, those who experienced any breastfeeding had a wider range of PFAS concentrations.

The metabolome-wide association analysis identified 13, 35, 25, 58, and 9 cord metabolites associated with (i.e., $p < 0.05$) cord PFOA, PFOS, PFNA, PFHpS, and PFHxS, respectively (Figure 2). Metabolites that were most significantly associated with cord PFAS included serotonin (negatively associated with PFOA; $p = 0.002$), isoxanthopterin (positively associated with PFOA, PFOS, and PFHpS; $p < 0.002$), phenylacetylglutamine (positively associated with PFNA; $p = 0.003$), C46:1 and C48:2 TAG (positively associated with PFHpS; $p < 0.002$), and C42:0 TAG (positively associated with PFHxS; $p = 0.004$). Figure 3 shows the Pearson correlation matrix for cord PFOA, PFOS, PFNA, PFHpS, and PFHxS with select cord metabolite biomarkers in the targeted analyses. All 5 PFAS were positively correlated with thyroxine. There were negative correlations (blue color) of: *a)* piperine with PFNA, *b)* C22:5 CE with PFNA and PFHpS, *c)* C56:8 TAG with PFOA, *d)* C56:11 TAG with PFOA and PFOS, *e)* C60:12 TAG with PFOA, PFOS, and PFNA, *f)* guanidinoacetic acid (GAA) with PFOS, and *g)* C18:0 LPE B with PFHxS, and there were positive correlations (red color) of: *a)* adenosine with PFNA, *b)* hypoxanthine with PFOA, PFOS, and PFHpS, *c)* inosine with PFOA and PFOS, *d)* xanthosine with PFOS, *e)* cadaverine with PFOA, PFOS, PFHpS, and PFHxS, *f)* 8-hydroxy-deoxyguanosine (8-OHdG) with PFOS and PFHpS, and *g)* C36:4 hydroxy-PC with PFOA, PFOS, PFNA, and PFHxS. None of the 5 PFAS were significantly associated with birthweight or gestational age at birth ($p > 0.05$).

5. Discussion

This pilot study contributes to the field in the following aspects. It demonstrated the feasibility of using cord blood collected at delivery to assess BBC newborn's PFAS exposure levels and found ubiquitous exposure to multiple PFAS among Black and Hispanic children. Using PFAS data from two time points, we demonstrated weak-to-moderate PFAS correlations in cord and early childhood plasma and identified several factors associated with PFAS levels at both time points. We showed the temporal trend of PFAS levels that coincided with PFAS regulations in the U.S. and observed associations of cord PFAS with multiple cord metabolites in both the metabolome-wide association analysis and the targeted analysis.

We identified factors associated with early childhood PFAS levels, including higher cord PFAS levels (indicating prenatal source) and multiple maternal and infant characteristics. The weak-to-moderate correlations ($r = -0.03$ to 0.40) of cord and early childhood PFAS levels showed that both prenatal and postnatal factors might be important determinants of PFAS in children[18]. The low R-squared values (≤ 0.38 for all 5 models) showed that known predictors of postnatal PFAS levels (e.g., cord PFAS levels, parity, and breastfeeding) did not account for much of the variability of early childhood PFAS levels even with the additional factors included. In this population, early childhood PFAS exposures appear to be coming from other routes not accounted for in this analysis, which warrants future investigations. PFAS share multiple common exposure pathways. In a scenario-based risk assessment study, Trudel et al. estimated that hand-to-mouth contact with consumer products (e.g., contaminated carpets) and ingestion of house dust were the main routes of PFOA

and PFOS uptake in infants and toddlers in the U.S.[40] Dietary sources such as food and water are also routes of PFOS and PFOA uptake, and they show stronger contribution as the child grows into adolescence and adulthood[40,41]. Breastfeeding is another major source of early life PFAS exposures[34–36]; however, we did not observe differences in early childhood PFAS levels by breastfeeding status, which could be due to several possibilities. First, we did not measure maternal PFAS. The association between breastfeeding and child PFAS would greatly depend on lactating mother's PFAS level. This possibility is supported by the observation that the distribution of PFAS is much wider among breastfed than non-breastfed infants. Second, the age of postnatal blood collection varied (median = 2.1 years, IQR = 1.3 to 3.8 years), which may dilute the strength of association between breastfeeding and child PFAS levels. Lastly, this could also be due to the lack of detailed data on the duration and exclusivity of breastfeeding. With the normal PFAS excretion processes, as the child grows older, the main contributor of their plasma PFAS levels shift from maternal inputs (e.g., placental transfer, breastfeeding) to child exposures (e.g., consumer products, dietary sources). Future studies with child plasma PFAS data from multiple postnatal time points should examine this dynamic and explore key contributors at each time point. One interesting finding is that breastfed children had wider ranges of PFAS levels, but one should note the small sample size for the exclusively bottle-fed group (n = 9); these distributions could be driven by the sparse data at upper and/or lower concentration ranges. Another possibility is that whether breastfeeding is a source of early childhood PFAS depends on maternal PFAS levels. To clarify this question, maternal PFAS levels need to be measured as well.

The temporal trends of PFAS levels by calendar year of birth in our study were similar to what was observed in the U.S. population (i.e., National Health and Nutrition Examination Survey [NHANES])[42] and in U.S. blood donors[43]. Due to the Environmental Protection Agency (EPA)'s Stewardship Program, some legacy PFAS such as PFOA and PFOS have been voluntarily phased out since the 2000s[9]. Since then, blood levels of most PFAS have been declining in NHANES for all age groups[44,45]. Our data showed a similar trend as children born in the late-2000s had lower levels of multiple cord PFAS compared to those born in the early-2000s. Emerging PFAS such as GenX, ADONA, and 9Cl-PF3ONS were measured in serum samples from NHANES 2017–2018 participants. Early-release data showed that serum levels of GenX and ADONA were below the LOD for almost all participants, which is consistent with our data in this study; 9Cl-PF3ONS was detectable in some NHANES populations, especially in Asians[11]. These data are also consistent with a prior CDC study that found extremely low detection frequency of GenX and 9Cl-PF3ONS in urine from NHANES 2013–2014 participants[46]. In October 2021, the U.S. EPA released its final human health toxicity assessment for GenX chemicals, reducing their chronic reference doses to 3×10^{-6} mg/kg, lower than that of PFOA and PFOS by an order of mangnitude[47]; this demonstrated the strong toxicity of GenX chemicals. GenX and ADONA were not detectable in any plasma sample in our study, which is expected since these two PFAS were used to replace legacy PFAS (e.g., PFOA) since the late-2000s, and the chance of detecting them in human biofluids in the 2001 to 2010 timeframe is small. With the emergence of these new, short-chain PFAS, there is a need to monitor the prevalence of exposure to these PFAS in pregnant women and newborns and examine the health effects

of these PFAS. Newborn blood, however, is difficult to collect, and cord blood may be a promising alternative matrix for screening neonatal PFAS levels. In the U.S., blood samples are collected from newborns 24–48 hours after birth via heel sticks for newborn screening, but this test only provides <30 μL of whole blood, from which serum/plasma needs to be further separated because they are preferred matrices (vs. whole blood) for PFAS testing[48]; this results in inadequate sample volume for PFAS testing (at least 50 μL of serum or plasma sample is needed). Our study supports the feasibility of using cord blood as an alternative to capillary blood for PFAS testing and screening in newborns.

We observed associations of cord PFAS with several cord metabolite biomarkers. In the metabolome-wide association analysis, cord metabolites such as serotonin, isoxanthopterin, phenylacetylglutamine, and certain TAGs were most strongly associated with cord PFAS. We also observed correlations of cord PFAS with several cord metabolites in the targeted analyses. To highlight a few, PFOA was positively correlated with cotinine, a biomarker of tobacco smoke exposure[49]; this is consistent with a prior study that found higher PFAS levels among smokers (vs. non-smokers), which might reflect sociodemographic factors[50]. PFOA, PFOS, and PFHpS were positively correlated with inosine and hypoxanthine, both in the purine catabolism pathway that leads to the production of uric acid[51], an independent risk factor for cardiometabolic diseases in human[52]. PFOS and PFHpS were also positively correlated with 8-OHdG, an established biomarker for oxidative stress[53] associated with cardiovascular diseases and cancer risk[54–56]. Additionally, PFOA, PFOS, and PFHxS were positively correlated with C36:4 hydroxy-PC, an oxidized lipid metabolite associated with a higher risk of coronary heart disease[57]. These associations are consistent with studies that found adverse effects of prenatal PFAS exposures on child cardiometabolic health[58–61]. The metabolite positively correlated with all 5 PFAS was thyroxine, a biomarker for thyroid function associated with PFAS exposures in previous studies[37,38]. Although we did not observe differences in birth outcomes (i.e., birthweight and gestational age at birth) by cord PFAS levels, likely due to a lack of statistical power, these associations mentioned above of PFAS with cord metabolite biomarkers may be relevant to future child health outcomes.

6. Limitations and future perspectives

Limitations of this study include the small sample size and the possible non-representativeness of the participants of the larger cohort. Given the pilot and hypothesis-generating nature of this work and that we were unable to adjust for covariates or control for multiple comparisons, results from the association analyses and the metabolomics analyses should be interpreted with caution. Nonetheless, our results provided important preliminary data to inform future large-scale studies. One strength of this study is the high quality of the PFAS testing performed by the Public Health and Environmental Laboratories at the New Jersey Department of Health. Our study confirmed the excellent performance of the PFAS testing method, providing confidence in the data for newborn PFAS exposure assessment. It is known that exposure routes and distributions of PFAS differ by geographic region[18]; to our knowledge, ours is the first prospective cohort in the Boston area to measure cord and early childhood PFAS levels in a predominantly Black and Hispanic population.

Foods, food packaging, and cookware are important sources of PFAS exposure. The BBC provides an ideal study design to examine early life exposure to PFAS. In addition, the BBC has collected comprehensive child health outcomes including birth (e.g., birthweight, gestational age), cardiometabolic (e.g., BMI, blood pressure), neuro-developmental (e.g., autism spectrum disorder [ASD], attention deficit hyperactivity disorder [ADHD]), and allergy and asthma outcomes. With the repository of existing multi-omics (e.g., metabolome, whole genome, DNA methylation) and *in utero* environmental exposure data (e.g., maternal particulate matter, toxic metals such as lead, mercury, and cadmium, trace elements such as manganese and selenium), adding PFAS data in the BBC—as demonstrated feasible in this pilot study—will afford a unique opportunity to assess early life PFAS exposure and their implications in long-term child health. This aligns with the EPA's priority to understand "how PFAS contribute to the cumulative burden of pollution in communities with environmental justice concerns," as outlined in its recent 2021–2024 Strategic Roadmap and Commitment to Action[62]. The BBC also offers an excellent opportunity to study co-exposure to environmental chemicals and the role of maternal micronutrients as protective factors, as we already demonstrated the protective role of maternal folate in the presence of low levels of lead and mercury exposure[63,64]. This line of research advances the emphasis on healthy dietary patterns during pregnancy and lactation in the latest U.S. Dietary Guideline for Americans (2020–2025)[65]. Recent evidence suggests that diets during pregnancy may be sources of exposure to metals and PFAS[66,67], calling for the need to better understand the interplay between nutrients and environmental chemicals[68,69]. With data on pregnancy dietary intake[70] and environmental exposures, we have opportunities to assess interindividual variability in response to foods and dietary exposures, a key goal in the 2020–2030 Strategic Plan for National Institutes of Health (NIH) Nutrition research[71].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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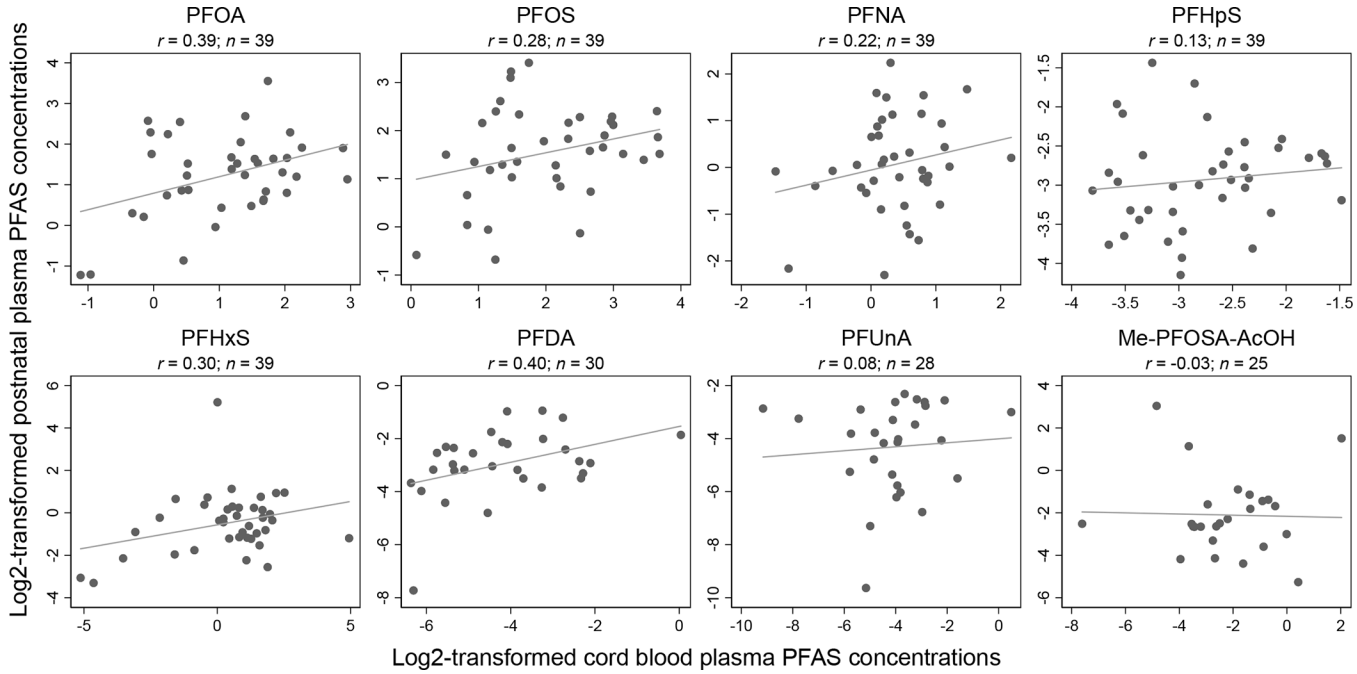

Figure 1.

Correlations of pairs of cord and early childhood blood plasma concentrations of PFOA, PFOS, PFNA, PFHpS, PFHxS, PFDA, PFUnA, and Me-PFOSA-AcOH. All PFAS concentrations were log2-transformed. Pearson correlation coefficients for each pair of cord and early childhood blood plasma PFAS concentrations (log2-transformed) and sample sizes (*n*) are provided. For samples below the limit of detection (LOD), machine-read values were used unless the concentration was negative. Pairs with at least one negative concentration were excluded (n = 9 for PFDA, n = 11 for PFUnA, n = 14 for Me-PFOSA-AcOH). Abbreviations: PFAS indicates per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFDA, perfluorodecanoic acid; PFUnA, perfluoroundecanoic acid; Me-PFOSA-AcOH, 2-(N-methyl-PFOSA) acetic acid.

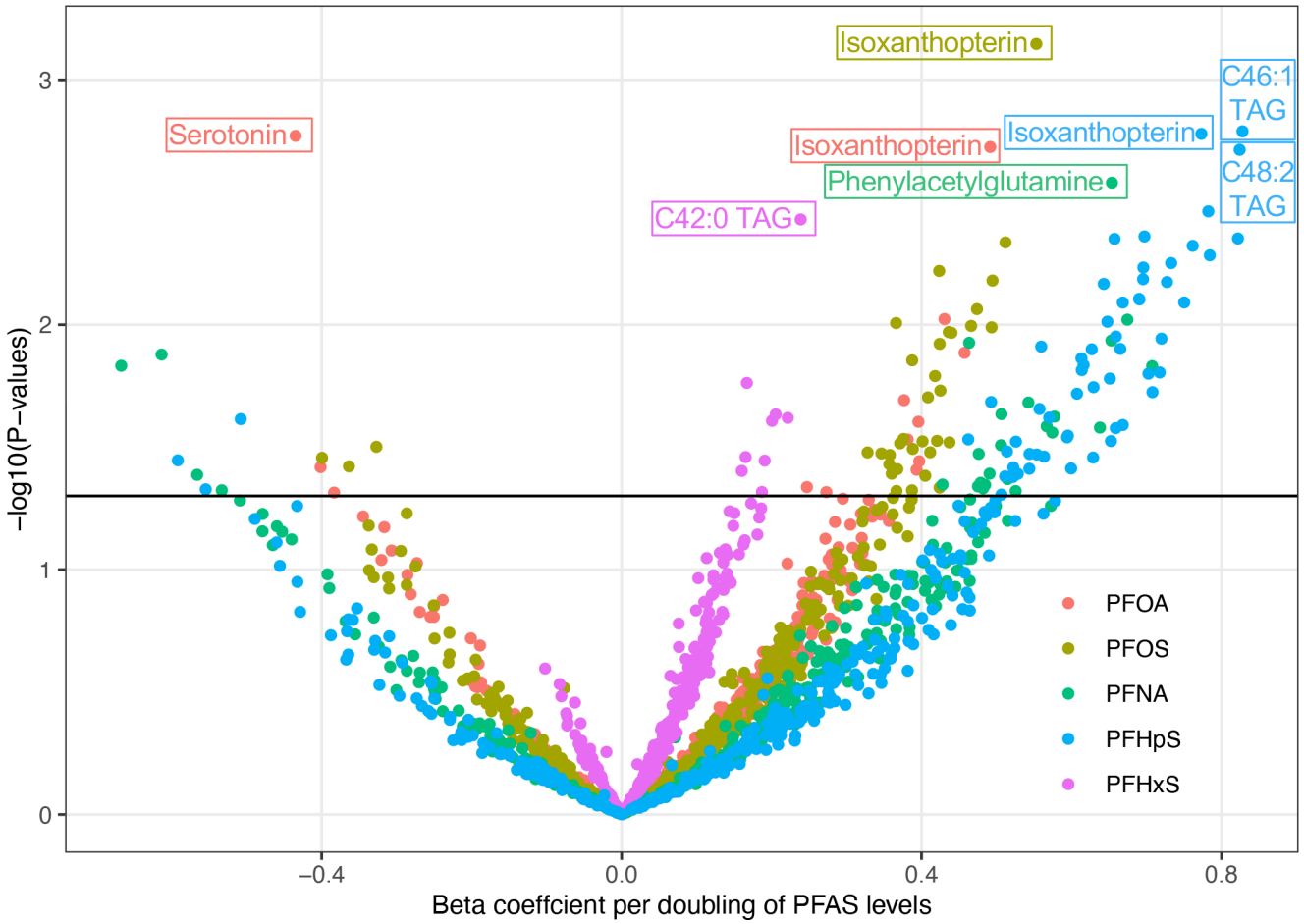

Figure 2.

Volcano plot of associations of cord blood plasma PFOA, PFOS, PFNA, PFHpS, and PFHxS with cord metabolites. The black horizontal line represents $p = 0.05$. Abbreviations: PFOA indicates perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxS, perfluorohexane sulfonic acid; TAG, triacylglycerol.

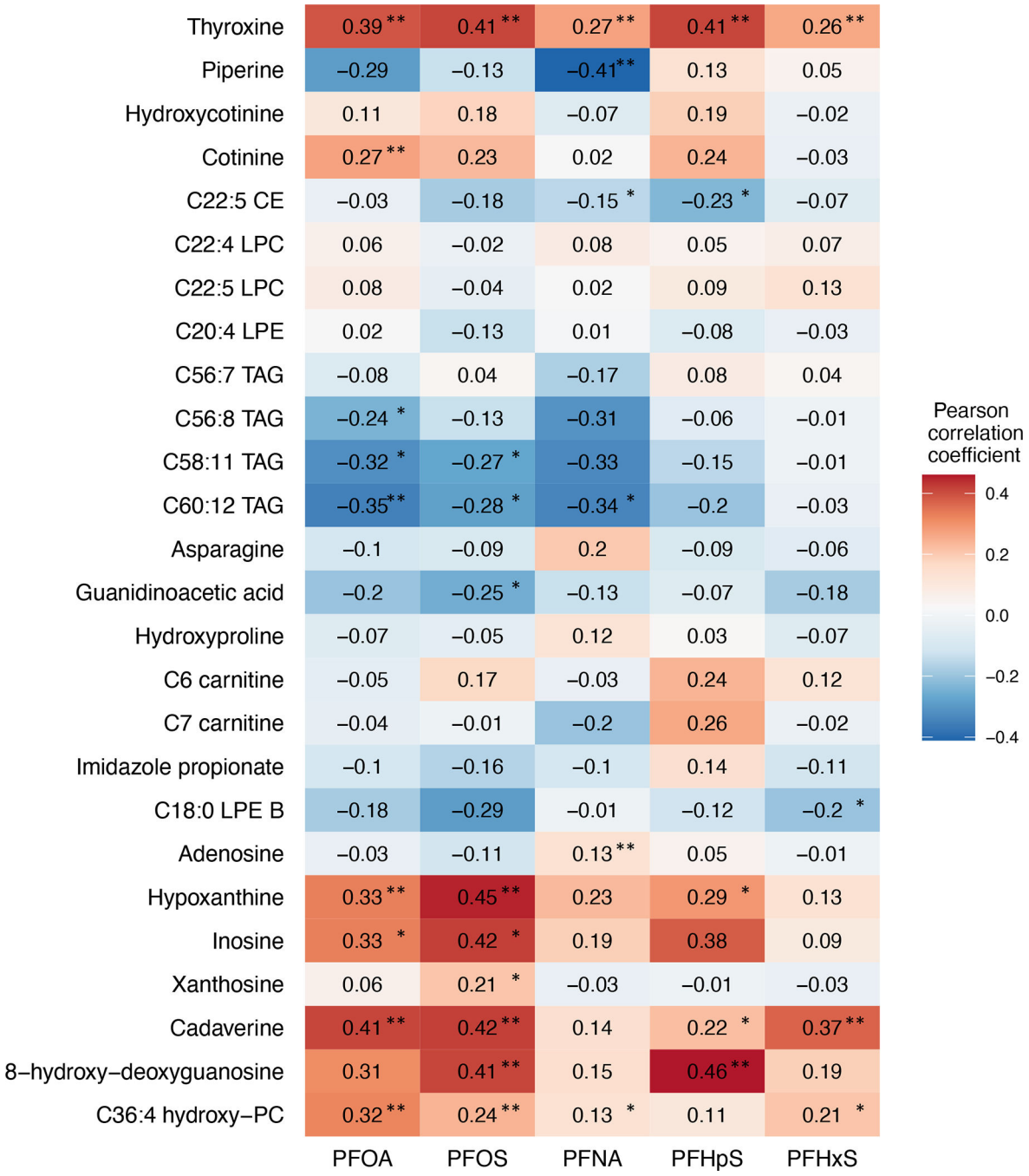

Figure 3.

Heatmap that shows the Pearson correlations of cord blood plasma PFOA, PFOS, PFNA, PFHpS, and PFHxS concentrations with select cord metabolites. Asterisks indicate the significance levels of the correlation coefficients ("**" indicates $p < 0.10$ and "*" indicates $p < 0.05$). Abbreviations: PFOA indicates perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptanesulfonic acid;

PFHxS, perfluorohexane sulfonic acid; CE, cholesterol ester; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; TAG, triacylglycerol; PC, phosphatidylcholine.

Table 1.

Characteristics of the 39 children in this analysis.

Characteristics	n (%), unless otherwise indicated
Maternal Characteristics	
Age at delivery, year, mean (SD)	27.4 (7.2)
Race/ethnicity	
Black	25 (64%)
Hispanic	14 (36%)
Educational level	
High school graduate or below	29 (74%)
Some college or above	10 (26%)
Marital status	
Married	13 (33%)
Single	26 (67%)
Pre-pregnancy overweight or obese	
No	18 (46%)
Yes	21 (54%)
Maternal smoking during pregnancy	
Never smoked	32 (82%)
Quit smoking before pregnancy	2 (5%)
Smoked during pregnancy	3 (8%)
Missing	2 (5%)
Multiparous	
No	13 (33%)
Yes	26 (67%)
Child Characteristics	
Child sex	
Female	15 (38%)
Male	24 (62%)
Gestational age, week, mean (SD)	38.6 (1.8)
Preterm birth	
No	35 (90%)
Yes	4 (10%)
Birthweight, gram, mean (SD)	3193.7 (570.4)
Low birthweight	
No	36 (92%)
Yes	3 (8%)
Intrauterine growth restriction	
Average for gestational age	31 (79%)
Large for gestational age	5 (13%)

Characteristics	n (%), unless otherwise indicated
Small for gestational age	3 (8%)
Calendar year of delivery	
2001	1 (3%)
2002	2 (5%)
2003	8 (21%)
2004	8 (21%)
2005	2 (5%)
2006	6 (15%)
2007	8 (21%)
2008	1 (3%)
2009	2 (5%)
2010	1 (3%)
Child age at postnatal blood sample collection, year, median (IQR)	2.1 (1.3 to 3.8)
Breast vs. bottle-feeding	
Bottle-fed	9 (23%)
Exclusive breastfed	3 (8%)
Both	27 (69%)

Abbreviations: SD indicates standard deviation; PFAS, per- and polyfluoroalkyl substances; IQR, interquartile range.

Table 2.

Distributions of cord and early childhood blood plasma PFAS concentrations of the 39 children in this analysis.

PFAS (ng/ml)	LOD	Cord blood plasma						Early childhood blood plasma					
		Min	Percentile			Max	Detection frequency† (%)	Min	Percentile			Max	Detection frequency† (%)
			25th	50th	75th				25th	50th	75th		
PFOA	0.046	0.46	1.32	2.42	3.34	7.74	100	0.43	1.55	2.47	3.74	11.73	100
PFOS	0.046	1.06	2.39	3.92	7.19	12.86	100	0.62	2.04	2.99	4.56	10.62	100
PFNA	0.049	0.36	1.06	1.26	1.75	4.48	100	0.20	0.74	1.01	1.83	4.72	100
PFHpS	0.015	0.07	0.10	0.14	0.20	0.36	100	0.06	0.10	0.13	0.17	0.37	100
PFHxS	0.038	< LOD	0.78	1.76	3.11	30.81	97.4	0.10	0.43	0.78	1.18	37.04	100
PFDA	0.049	< LOD	< LOD	< LOD	0.11	1.03	43.6	< LOD	0.08	0.12	0.20	0.52	84.6
PFUnA	0.047	< LOD	< LOD	0.06	0.11	1.40	53.8	< LOD	< LOD	0.06	0.11	0.20	53.8
Me-PFOSA-AcOH	0.061	< LOD	< LOD	0.15	0.53	4.09	71.8	< LOD	< LOD	0.16	0.31	8.22	59.0
PFDoA	0.040	< LOD	< LOD	< LOD	< LOD	0.28	7.7	< LOD	< LOD	< LOD	< LOD	0.74	0.0
9Cl-PF3ONS	0.032	< LOD	< LOD	< LOD	< LOD	0.17	2.6	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
GenX	0.049	< LOD	< LOD	< LOD	< LOD	< LOD	0.0	< LOD	< LOD	< LOD	< LOD	< LOD	0.0
ADONA	0.041	< LOD	< LOD	< LOD	< LOD	< LOD	0.0	< LOD	< LOD	< LOD	< LOD	< LOD	0.0

Abbreviations: PFAS indicates per- and polyfluoroalkyl substances; LOD, limit of detection; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFDA, perfluorodecanoic acid; PFUnA, perfluoroundecanoic acid; Me-PFOSA-AcOH, 2-(N-methyl-PFOSA) acetic acid; PFDoA, perfluorododecanoic acid; 9Cl-PF3ONS, 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid; GenX, perfluoro-2-proproxypropanoic acid; ADONA, 4,8-dioxa-3H-perfluorononanoic acid.

†Detection frequency is calculated as the number of samples with concentrations above the LOD divided by the total number of samples × 100. Samples with concentrations below the LOD or negative concentrations were classified as "<LOD."

Table 3.

Mean differences (95% confidence interval) in log2-transformed cord blood plasma PFOA, PFOS, PFNA, PFHpS, and PFHxS concentrations by levels of child and maternal factors.

Factors	PFOA	PFOS	PFNA	PFHpS	PFHxS
Child factors					
Birthweight (Scale: per 500-gram increment)	0.09 (−0.24, 0.42)	0.04 (−0.25, 0.33)	0.17 (−0.08, 0.43)	0.01 (−0.19, 0.22)	0.26 (−0.47, 1.00)
Gestational age at birth (Scale: per week increment)	0.07 (−0.16, 0.30)	0.25 (0.05, 0.46)	−0.02 (−0.20, 0.16)	0.14 (−0.00, 0.29)	0.22 (−0.29, 0.74)
Male sex (Ref: female sex)	0.02 (−0.60, 0.63)	−0.24 (−0.78, 0.31)	−0.04 (−0.52, 0.44)	−0.15 (−0.54, 0.24)	−0.85 (−2.25, 0.55)
Cord blood collected ≥ 2005 (Ref: collected prior to year 2005)	−0.85 (−1.50, −0.19)	−1.05 (−1.63, −0.47)	−0.12 (−0.63, 0.38)	−0.38 (−0.79, 0.03)	−1.27 (−2.74, 0.21)
Maternal factors					
Hispanic (Ref: Black)	0.41 (−0.27, 1.09)	−0.03 (−0.64, 0.57)	0.34 (−0.19, 0.87)	−0.01 (−0.44, 0.41)	−0.72 (−2.26, 0.81)
Some college or above (Ref: high school graduate or below)	−0.78 (−1.47, −0.09)	−0.61 (−1.22, 0.004)	−0.33 (−0.86, 0.21)	−0.30 (−0.74, 0.13)	−0.51 (−2.07, 1.05)
Pre−pregnancy overweight or obese (Ref: not overweight or obese)	−0.26 (−0.87, 0.36)	0.19 (−0.36, 0.74)	−0.10 (−0.58, 0.38)	0.48 (0.09, 0.87)	0.20 (−1.20, 1.60)
R−squared	0.34	0.44	0.22	0.41	0.20

Abbreviations: PFAS indicates per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxS, perfluorohexane sulfonic acid.

Table 4.

Mean differences (95% confidence interval) in log2-transformed early childhood blood plasma PFOA, PFOS, PFNA, PFHpS, and PFHxS concentrations by levels of child and maternal factors.

Factors	PFOA	PFOS	PFNA	PFHpS	PFHxS
Child factors					
Cord PFAS (Scale: a doubling of concentration)	0.58 (0.17, 0.99)	0.30 (−0.13, 0.73)	0.21 (−0.32, 0.74)	0.15 (−0.26, 0.56)	0.14 (−0.11, 0.38)
Gestational age at birth (Scale: per week increment)	0.17 (−0.03, 0.38)	0.18 (−0.04, 0.39)	0.02 (−0.21, 0.25)	0.13 (−0.02, 0.27)	0.19 (−0.10, 0.49)
Age at postnatal blood collection (Scale: per year increment)	0.001 (−0.18, 0.18)	0.08 (−0.11, 0.26)	0.13 (−0.06, 0.32)	0.02 (−0.10, 0.14)	0.12 (−0.12, 0.36)
Postnatal blood collected ≥ 2008 (Ref: collected prior to year 2008)	0.001 (−0.73, 0.73)	−0.40 (−1.13, 0.33)	0.31 (−0.44, 1.07)	−0.24 (−0.71, 0.22)	−1.32 (−2.36, −0.29)
Maternal factors					
Hispanic (Ref: Black)	−0.12 (−0.84, 0.60)	−0.21 (−0.88, 0.47)	0.57 (−0.24, 1.38)	−0.15 (−0.61, 0.31)	−0.77 (−1.80, 0.27)
Some college or above (Ref: high school graduate or below)	0.81 (0.01, 1.61)	0.54 (−0.20, 1.28)	0.29 (−0.56, 1.13)	0.39 (−0.11, 0.88)	0.83 (−0.23, 1.89)
Multiparous (Ref: nulliparous)	0.51 (−0.22, 1.25)	0.33 (−0.36, 1.01)	−0.12 (−0.92, 0.68)	0.15 (−0.32, 0.62)	0.26 (−0.78, 1.31)
Pre−pregnancy overweight or obese (Ref: not overweight or obese)	−0.67 (−1.35, 0.00)	−0.94 (−1.59, −0.28)	−0.17 (−0.92, 0.58)	−0.39 (−0.88, 0.10)	−0.76 (−1.73, 0.21)
R-squared	0.38	0.35	0.24	0.23	0.36

Abbreviations: PFAS indicates per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxS, perfluorohexane sulfonic acid.