Research

Prenatal Exposure to Per- and Polyfluoroalkyl Substances, Umbilical Cord Blood DNA Methylation, and Cardio-Metabolic Indicators in Newborns: The Healthy Start Study

Anne P. Starling,[1,2] Cuining Liu,[3] Guannan Shen,[3] Ivana V. Yang,[1,4,5] Katerina Kechris,[3,4] Sarah J. Borengasser,[6] Kristen E. Boyle,[6] Weiming Zhang,[3] Harry A. Smith,[2,3] Antonia M. Calafat,[7] Richard F. Hamman,[1,2] John L. Adgate,[8] and Dana Dabelea[1,2,6]

[1]Department of Epidemiology, Colorado School of Public Health, Aurora, Colorado, USA
[2]Lifecourse Epidemiology of Adiposity and Diabetes (LEAD) Center, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
[3]Department of Biostatistics and Informatics, Colorado School of Public Health, Aurora, Colorado, USA
[4]Division of Biomedical Informatics and Personalized Medicine, Department of Medicine, University of Colorado School of Medicine, Aurora, Colorado, USA
[5]Center for Genes, Environment and Health, National Jewish Health, Denver, Colorado, USA
[6]Department of Pediatrics, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA
[7]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
[8]Department of Environmental and Occupational Health, Colorado School of Public Health, Aurora, Colorado, USA

BACKGROUND: Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent chemicals widely detected in women of reproductive age. Prenatal PFAS exposure is associated with adverse health outcomes in children. We hypothesized that DNA methylation changes may result from prenatal PFAS exposure and may be linked to offspring cardio-metabolic phenotype.

OBJECTIVES: We estimated associations of prenatal PFAS with DNA methylation in umbilical cord blood. We evaluated associations of methylation at selected sites with neonatal cardio-metabolic indicators.

METHODS: Among 583 mother–infant pairs in a prospective cohort, five PFAS were quantified in maternal serum (median 27 wk of gestation). Umbilical cord blood DNA methylation was evaluated using the Illumina HumanMethylation450 array. Differentially methylated positions (DMPs) were evaluated at a false discovery rate (FDR) < 0.05 and differentially methylated regions (DMRs) were identified using comb-p (Šidák-adjusted $p < 0.05$). We estimated associations between methylation at candidate DMPs and DMR sites and the following outcomes: newborn weight, adiposity, and cord blood glucose, insulin, lipids, and leptin.

RESULTS: Maternal serum PFAS concentrations were below the median for females in the U.S. general population. Moderate to high pairwise correlations were observed between PFAS concentrations ($\rho = 0.28 - 0.76$). Methylation at one DMP (cg18587484), annotated to the gene *TJAP1*, was associated with perfluorooctanoate (PFOA) at FDR < 0.05. Comb-p detected between 4 and 15 DMRs for each PFAS. Associated genes, some common across multiple PFAS, were implicated in growth (*RPTOR*), lipid homeostasis (*PON1*, *PON3*, *CIDEB*, *NR1H2*), inflammation and immune activity (*RASL11B*, *RNF39*), among other functions. There was suggestive evidence that two PFAS-associated loci (cg09093485, cg09637273) were associated with cord blood triglycerides and birth weight, respectively (FDR < 0.1).

DISCUSSION: DNA methylation in umbilical cord blood was associated with maternal serum PFAS concentrations during pregnancy, suggesting potential associations with offspring growth, metabolism, and immune function. Future research should explore whether DNA methylation changes mediate associations between prenatal PFAS exposures and child health outcomes. https://doi.org/10.1289/EHP6888

Introduction

Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent chemicals that have been widely detected in blood among general populations worldwide (Centers for Disease Control and Prevention 2019; Vestergren and Cousins 2009), including pregnant women (Bjerregaard-Olesen et al. 2017; Kato et al. 2014; Yang et al. 2019). PFAS have unique surfactant qualities and a wide variety of consumer and industrial applications that led to widespread global use of this class of chemicals for several decades (Buck et al. 2011; Wang et al. 2017). Some

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehponline@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

PFAS have relatively long elimination half-lives in the human body, ranging from 2 to 8 y (Li et al. 2018; Olsen et al. 2007). Unlike many persistent organic pollutants that are lipophilic, PFAS circulate in blood bound to carrier proteins, primarily albumin (Beesoon and Martin 2015; Forsthuber et al. 2020).

The PFAS most frequently studied to date have been perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). In animal studies, developmental toxicity results from PFAS exposure during gestation, resulting in impaired growth, metabolic disruption, and neonatal mortality (Abbott et al. 2012; Lau et al. 2007). PFAS have been shown to cross the placenta in humans, with transfer efficiency varying by chemical structure and chain length (Gao et al. 2019; Gützkow et al. 2012; Midasch et al. 2007). In human epidemiological studies, concentrations of PFAS, and particularly PFOA, in the blood of pregnant women have been associated with delivering infants with lower birth weight (Bach et al. 2015; Johnson et al. 2014) and lower adiposity at birth (Starling et al. 2017). Moreover, exposures to PFOS and PFOA *in utero* have been associated with a variety of adverse health effects in children, including reduced antibody response to vaccinations (Grandjean et al. 2012), increased adiposity (Braun et al. 2016; Høyer et al. 2015; Lauritzen et al. 2018), and altered lipid profile (Mora et al. 2018). Prenatal exposure to PFOA has also been associated with greater risk of being overweight in early adulthood (Halldorsson et al. 2012).

One mechanism by which prenatal exposure to PFAS may affect later health outcomes may be through modifications to the fetal

epigenome, particularly changes to DNA methylation (Martin and Fry 2018) that persist through cell division and influence gene expression, consequently affecting cardio-metabolic phenotype and disease risk. This theory is supported by findings that umbilical cord blood concentrations of PFOA, suggesting prenatal exposure, were associated with global hypomethylation of cord blood DNA (Guerrero-Preston et al. 2010). More recently, greater maternal PFOA concentrations during pregnancy were associated with hypomethylation of *IGF2* in cord blood, which may mediate associations of maternal PFOA with lower offspring birth weight and adiposity at birth (Kobayashi et al. 2017).

However, few studies have examined associations between prenatal exposure to PFAS and cord blood DNA methylation at specific sites in the genome (Kingsley et al. 2017; Kobayashi et al. 2017; Leung et al. 2018; Miura et al. 2018), and none have examined associations with commonly measured PFAS other than PFOA or PFOS. In particular, perfluorohexane sulfonate (PFHxS) is a substance of increasing global concern because it has been detected in communities exposed to drinking water contaminated with aqueous film-forming foams from firefighting and training activities (Barton et al. 2020; Daly et al. 2018; Gyllenhammar et al. 2015). Additionally, previous studies have not included multiethnic participants and therefore may not be generalizable to the diverse U.S. population.

We conducted an epigenome-wide analysis to examine the associations between concentrations of five PFAS in maternal serum collected during pregnancy and DNA methylation in umbilical cord blood cells among mother–infant pairs in Healthy Start, a Colorado longitudinal prebirth cohort study with racial and ethnic diversity reflecting the population of the Denver metropolitan area. We additionally evaluated associations between differentially methylated CpGs and neonatal markers of adiposity and metabolic status.

Methods

Participants and Study Design

The Healthy Start prospective cohort study recruited 1,410 pregnant women from outpatient obstetrics clinics at the University of Colorado Hospital during the period 2009–2014. Eligible women were 16 years of age or older, pregnant with a single fetus, having completed fewer than 24 wk of gestation at enrollment, with no history of extremely preterm birth or stillbirth, and no self-reported diabetes, asthma, cancer, or psychiatric illness. Participants completed questionnaires and provided blood samples during pregnancy and authorized review of their medical records. The study procedures were approved by the Colorado Multiple Institutional Review Board. All participants provided written informed consent. The analysis of blinded specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

Of the 1,410 enrolled participants, 867 participants had umbilical cord blood collected when delivery conditions allowed. Of these, 600 mother–infant pairs were selected for DNA methylation analysis based on the availability of both maternal midpregnancy serum samples and cord blood DNA samples, and 589 had PFAS measured in maternal serum (Figure S1).

Exposure Assessment

A panel of 11 PFAS were quantified in maternal midpregnancy serum samples collected at a median of 27 wk of gestation (range 20–34 wk) and promptly separated and frozen at $-80°C$. Analyses were conducted at the CDC's National Center for Environmental Health, Division of Laboratory Sciences, using a previously published method (Kato et al. 2011). The 11 PFAS measured were

perfluorooctane sulfonamide (FOSA; also known as PFOSA), 2-(*N*-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA; also known as Et-PFOSA-AcOH), 2-(*N*-methyl perfluorooctane sulfonamido) acetate (MeFOSAA; also known as Me-PFOSA-AcOH), PFHxS, linear PFOA (n-PFOA), sum of branched isomers of PFOA (Sb-PFOA), perfluorodecanoate (PFDA; also known as PFDeA), linear PFOS (n-PFOS), sum of perfluoromethylheptane sulfonate isomers (Sm-PFOS), sum of perfluorodimethylhexane sulfonate isomers (Sm2-PFOS), and perfluorononanoate (PFNA). PFOA and PFOS were calculated as the sum of the concentrations (nanogram per milliliter) of linear and branched isomers of PFOA and PFOS, respectively. The limit of detection (LOD) for all PFAS was 0.1 ng/mL. This analysis is restricted to those PFAS detectable in >60% of participants in this sample: PFOA, PFOS, PFNA, PFDA, and PFHxS. Concentrations of all PFAS measured are reported in Table S1. For PFAS concentrations below the LOD, instrument values were used when available, and concentrations reported as zero were replaced with the LOD/2 for this analysis.

Analysis of DNA Methylation in Cord Blood

Briefly, DNA was extracted from stored buffy coats using the QIAamp DNA Blood Mini Kit (Qiagen). DNA purity was assessed using the Nanodrop 2000 spectrophotometer (ThermoFisher), DNA quality was assessed using the Bioanalyzer 2100 (Agilent), and DNA quantity was determined on a Qubit fluorometer (ThermoFisher). Samples with $260/280 > 1.8$, DNA Integrity Score (DNA) > 7, and ≥500 ng DNA were used for DNA methylation analyses. Five hundred nanograms of DNA were bisulfite converted using the Zymo EZ DNA Methylation kit (Zymo Research). Each conversion assay included a commercially available positive and negative control sample. Genome-wide DNA methylation in cord blood was assessed using the Illumina Infinium HumanMethylation450 BeadChip array using previously published methods (Yang et al. 2017).

The relative proportions of seven cell types in umbilical cord blood (B cells, CD4 T cells, CD8 T cells, granulocytes, monocytes, NK cells, and nucleated red blood cells) were estimated using estimateCellCounts2 function in the R package FlowSorted (R version 3.6.2, R Foundation for Statistical Computing). CordBloodCombined.450k, using a combined cord blood reference data set (Gervin et al. 2019). In our quality control procedures, we excluded 587 probes with high detection *p*-value (>0.05) in at least 10% of samples, and 664 probes with a beadcount <3 in at least 5% of samples. Cross-reactive probes ($n = 29,101$) (Chen et al. 2013) and polymorphic probes with minor allele frequency ≥1% ($n = 23,941$) were excluded from the analysis of differentially methylated positions. Probes on the X and Y chromosomes were also excluded ($n = 11,648$). Raw methylation (beta) values were converted to *M*-values to better approximate a normal distribution, where $M = \log[\text{beta}/(1 - \text{beta})]$. Stratified quantile normalization was performed using the preprocessQuantile function in minfi (Touleimat and Tost 2012). Batch effects were removed using ComBat (Johnson et al. 2007). Reported sex of the infant was compared with predicted sex and participants were excluded if the reported and predicted sex did not match ($n = 6$). Extreme methylation outliers were removed by trimming *M*-values for each probe more than three times the interquartile range below the 25th percentile or above the 75th percentile (Hoaglin et al. 1986; Merid et al. 2020).

Assessment of Maternal and Neonatal Characteristics

Infant birth weight was measured by clinical personnel at delivery. Infant body composition (fat mass and fat-free mass) within 3 d of birth was measured via air displacement plethysmography using the PEAPOD (COSMED), which uses a two-compartment model

to estimate whole-body fat mass and fat-free mass (Urlando et al. 2003). Adiposity was calculated as fat mass divided by total (fat + fat-free) body mass × 100%. Maternal age, education, gravidity, and race/ethnicity were self-reported at the first research visit. Current maternal smoking was self-reported at multiple visits during pregnancy. Infant sex was reported by the mother shortly after delivery or abstracted from the medical record.

Umbilical cord blood was collected at delivery and concentrations of cardio-metabolic biomarkers were measured at the University of Colorado Clinical and Translational Sciences Institute Core Laboratory. Glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, free fatty acids, and triglycerides were measured using enzymatic kits on an AU400e Chemistry Analyzer (Olympus). Leptin was quantified via ELISA (Alpco) and insulin via radioimmunoassay (EMD Millipore Corporation).

Statistical Analysis

Following descriptive summary statistics and standard checks for normality and outliers, separate linear regression models were fitted to estimate associations between each continuous, natural log-transformed PFAS concentration during pregnancy and umbilical cord blood cell DNA methylation (M-values) at each of 423,151 CpG sites remaining after filtering. Models were adjusted for potential confounders and precision variables, which were identified by the construction of a directed acyclic graph (Figure S2). All models were adjusted for the following common set of covariates: infant sex, gestational age at blood sample collection (days), maternal age (years), education completed (high school or less vs. more than high school), smoking during pregnancy (any vs. none), race/ethnicity (non-Hispanic White vs. all others), body mass index (BMI) prior to pregnancy (kg/m²), previous pregnancies (any vs. none), and imputed proportions of seven cell types.

Raw p-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995) separately for each PFAS and a false discovery rate (FDR) of 0.05 was used as a cutoff for significance of differentially methylated positions (DMPs). For a more stringent cutoff, we also set the Bonferroni threshold for correction of multiple comparisons to $\alpha = (0.05/423,151) = 1.2 \times 10^{-7}$. Genomic inflation was evaluated by constructing Q-Q plots and calculating lambda for each of the five epigenome-wide analyses. Scatter plots of the association between natural log-transformed PFAS concentration and methylation (M-values) were constructed for significant DMPs to evaluate whether the linear association was influenced by outliers.

Pathways (Ren et al. 2019) with false discovery rate ≤0.2 are reported. We additionally compared our results with the findings of the three previous studies (Kingsley et al. 2017; Leung et al. 2018; Miura et al. 2018) that reported associations between prenatal PFAS concentrations and epigenome-wide methylation in umbilical cord blood, and one study that reported associations in adult men between serum PFAS and peripheral blood methylation (van den Dungen et al. 2017). For each of the top CpGs reported in these studies, we examined whether the association between the relevant maternal PFAS concentration and cord blood DNA methylation at that CpG was nominally significant ($p < 0.05$) in our results.

Differentially methylated regions (DMRs) were identified using the program comb-p to group neighboring CpG sites with small p-values (Pedersen et al. 2012). CpGs with raw p-values < 0.1 from the DMP analysis were selected as seeds to detect potential DMRs. Peaks within 750 bps were merged into a single DMR. DMRs containing only one assayed CpG were excluded. We defined significant DMRs based on Šidák-adjusted p-values < 0.05 to adjust for multiple testing. We evaluated whether each DMR was consistently

hyper- or hypomethylated by reporting the proportion of CpGs within the DMR with a consistent direction (positive association with PFAS concentrations). We identified genes overlapping and near (± 5 kB) each DMR using the annotatr R package, version 3.6.2 (Cavalcante and Sartor 2017). Genes associated with each significant DMR were individually searched using PubMed and NCBI's Gene database to identify function, with a particular emphasis on previously published associations with lipid metabolism and adiposity/obesity as hypothesized outcomes.

For each significant DMP and for the top CpG (lowest p-value) from each significant DMR, we separately estimated each association between methylation (M-value) at the selected CpG and the following neonatal cardio-metabolic indicators: birth weight, adiposity (percent fat mass), and cord blood concentrations of glucose, insulin, leptin, total cholesterol, HDL cholesterol, free fatty acids, and triglycerides. Neonatal cardio-metabolic outcome variables were $\log_{10}$ transformed to better approximate a normal distribution and reduce the influence of outliers. M-values were regressed on seven cell types and then cell type-adjusted residuals were entered as predictors in separate linear regression models for each neonatal cardio-metabolic variable, adjusted for infant sex, maternal age, education, smoking, race/ethnicity, prepregnancy BMI, and previous pregnancies. p-Values were adjusted for multiple comparisons across all models by controlling the false discovery rate (FDR) with the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995). FDR control <0.2 was considered suggestive evidence of association.

In sensitivity analyses, we examined the potential for effect modification by infant sex and, separately, by maternal race/ethnicity by including interaction terms in linear regression models for each CpG. We considered an interaction significant if the FDR for the p-value for interaction was <0.05. Results are reported in supplemental Excel tables for all CpGs with raw p-values <0.05 in the population as a whole, and stratified results are reported for all CpGs with raw interaction p-values <0.05 in models including PFAS-by-infant sex or PFAS-by-race/ethnicity interaction terms. Complete results for all CpGs may be obtained from the authors on request.

Results

From the original 589 participants with maternal PFAS data and cord blood DNA methylation data, we excluded 6 participants for whom the predicted sex did not match the reported sex, resulting in a sample size of 583 for the epigenome-wide analysis (Figure S1). Characteristics of mother–infant pairs are presented in Table 1. The characteristics of this sample do not differ notably from the characteristics of all potentially eligible participants in the Healthy Start cohort (Table S2). Serum concentrations of PFAS were somewhat lower than the median concentrations among females in the U.S. general population during the same time period (Centers for Disease Control and Prevention 2019) and displayed moderate to high pairwise Spearman correlations (Table 2; Table S1). Distributions of PFAS concentrations before and after natural-log transformation are shown in Figures S3–S7.

Epigenome-Wide Analysis of Differentially Methylated Positions

We conducted a separate epigenome-wide analysis for each of the five PFAS. Associations between PFAS concentration and methylation for the top CpGs with raw p-values <0.05 are presented in Excel Tables S1–S5, along with the mean and standard deviation of methylation at the CpG for context. There was no evidence of genomic inflation with all lambda values <1 (Table S3). Manhattan, volcano plots, and Q-Q plots for each PFAS are

Table 1. Characteristics of 583 mother–infant pairs in the Healthy Start study who were eligible for this analysis.

Maternal and infant characteristics	Mean ± SD or n (%)
Maternal age (y)	27.6 ± 6.2
Race/ethnicity	
Non-Hispanic White	314 (54)
Hispanic	142 (24)
Non-Hispanic African American	90 (15)
All others	37 (6)
Prepregnancy BMI (kg/m^2)	25.9 ± 6.6
Highest education level completed	
Less than 12th grade	92 (16)
High school degree or equivalent	102 (18)
Some college or Associate's degree	125 (21)
Four-year college degree	128 (22)
Graduate degree	136 (23)
Household income in the past year	
$20,000 or less	86 (15)
$20,001–$40,000	78 (13)
$40,001–$70,000	108 (19)
$70,001 or more	198 (34)
Don't know	113 (19)
Any previous pregnancies	365 (63)
Any smoking during pregnancy	53 (9)
Gestational weight gain (kg)	14.0 ± 6.4
Gestational age at blood sample collection (d)	191 ± 17
Infant gestational age at birth (d)	276 ± 9
Birth weight (g)	3273 ± 435
Adiposity at birth (%)[a]	9.0 ± 3.8
Cord blood glucose (mg/dL)[a]	83.0 ± 20.3
Cord blood insulin (μU/mL)[a]	9.3 ± 8.1
Cord blood leptin (ng/mL)[a]	16.5 ± 16.6
Cord blood total cholesterol (mg/dL)[a]	58.6 ± 19.2
Cord blood HDL cholesterol (mg/dL)[a]	25.8 ± 7.8
Cord blood triglycerides (mg/dL)[a]	46.6 ± 35.1
Cord blood free fatty acids (μmol/L)[a]	280.9 ± 143.9

Note: BMI, body mass index.
[a]Sample size reduced due to missing data: $n = 563$ for adiposity measured within 3 days of birth; $n = 558$ for glucose; $n = 537$ for insulin; $n = 478$ for leptin; $n = 544$ for total cholesterol; $n = 503$ for HDL cholesterol; $n = 536$ for triglycerides; $n = 502$ for free fatty acids.

shown in Figures S8–S22. One DMP (cg18587484) had significantly lower methylation in association with higher maternal PFOA concentrations ($p = 2.5 \times 10^{-8}$, FDR < 0.05) (Figure S23). No other probes reached epigenome-wide significance. None of the PFAS-by-sex interaction terms or PFAS-by-race/ethnicity interaction terms were significant at FDR < 0.05. For CpGs with interaction p-values < 0.05, results stratified by infant sex are reported in Excel Tables S6–S10, and results stratified by category of self-reported race/ethnicity are reported in Excel Tables S11–S15.

Pathway Analysis on Differentially Methylated Positions

DMP results were entered into methylGSA for examination of potentially enriched GO and KEGG pathways, defined by FDR ≤ 0.2. Pathways meeting these criteria (Table 3) included

Table 2. Distribution and spearman correlations[a] of serum concentrations of perfluoroalkyl substances (ng/mL) among 583 pregnant women.

	Median (IQR)	Range	PFOA	PFOS	PFHxS	PFNA	PFDA
PFOA	1.1 (0.9)	0.1–15.4	1	0.67	0.62	0.76	0.55
PFOS	2.4 (2.3)	<LOD–15.6		1	0.67	0.62	0.48
PFHxS	0.7 (0.7)	<LOD–10.9			1	0.45	0.28
PFNA	0.4 (0.3)	<LOD–4.3				1	0.64
PFDA	0.1 (0.1)	<LOD–3.5					1

Note: IQR, interquartile range; LOD, limit of detection; PFDA, perfluorodecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.
[a]All pairwise correlations $p < 0.001$.

antigen processing and presentation (PFOA), response to endoplasmic reticulum stress (PFDA) and protein processing in the endoplasmic reticulum (PFDA), and female sex differentiation (PFHxS).

Look-up Analysis of Results from Previous Studies

Among the top CpGs reported in each of the previous epigenome-wide studies of PFAS concentrations and epigenome-wide methylation (Kingsley et al. 2017; Leung et al. 2018; Miura et al. 2018; van den Dungen et al. 2017), several achieved nominal significance ($p < 0.05$) in our study. Among the top 20 CpGs reported by Miura et al. for PFOA, two had $p < 0.05$ and the same direction of association for PFOA in our study (annotated to genes *DUX2* and *GPR126*; Excel Table S16). One of these was also associated with PFOS in our study, and another with PFDA. Among the top 20 CpGs associated with PFOS in Miura et al., one was nominally associated with PFOS (annotated to *GREB1*) and with the same direction of association (Excel Table S17); this CpG was also nominally associated with PFOA, PFHxS, and PFDA in our study. Among the 20 top CpGs reported by Kingsley et al. to be associated with PFOA, two were nominally associated with PFOA in our study with the same direction of association, both annotated to *OPRD1* (Excel Table S18). None of these previously reported associations reached epigenome-wide significance in our study. Among the top 21 CpGs associated with PFOS in adults in Van den Dungen et al. and also analyzed in our study, none were nominally significant (Excel Table S19). Leung et al. reported 10,598 CpGs that were significantly associated with cord blood n-PFOS in males. Of these, 327 (3%) were nominally associated with PFOS in our study (Excel Table S20).

Identification of Differentially Methylated Regions Using Comb-p

Between 4 and 15 DMRs were identified for each PFAS (Excel Tables S21–S25). All regions identified with FDR < 0.05 also showed consistent hyper- or hypomethylation (≥95%) of CpGs within the region. Certain DMR-annotated genes were associated with multiple PFAS, including *PON1, PON3, TM9SF2, RNF39, RASL11B, OR2L13*, and *PPP1R11*. Other genes were only associated with a single PFAS, such as *RPTOR* and *NR1H2* with PFOA, and *CIDEB* and *LTB4R* with PFDA.

Associations between Differentially Methylated CpGs and Neonatal Cardio-Metabolic Outcomes

We estimated associations between methylation (*M*-values) at 43 CpGs and infant birth weight, adiposity at birth, and the following cardio-metabolic indicators measured in umbilical cord blood: total cholesterol, free fatty acids, glucose, HDL cholesterol, insulin, triglycerides, and leptin. Methylation was suggestively associated with neonatal cardiometabolic outcomes (FDR < 0.1) for 2 CpGs (Excel Table S26). Methylation at one of these probes (cg09093485) was inversely associated with cord blood triglycerides; this probe was annotated to the gene *RNF39*. Methylation at the other probe (cg09637273) was positively associated with birth weight; there was no gene annotated to this probe.

Discussion

In this study, we identified one DMP and several DMRs in umbilical cord blood DNA associated with prenatal exposure to one or more of five commonly detected PFAS. One CpG associated with maternal PFOA concentration met both FDR and Bonferroni cutoffs for epigenome-wide significance and was annotated to the gene *TJAP1*, tight junction associated protein 1, expressed in

Table 3. Pathways enriched (false discovery rate <0.20) in gene sets associated with maternal PFAS concentrations.

Pathway name	Pathway ID	PFAS	*p*-Value	False discovery rate	Top 5 genes in pathway
Antigen processing and presentation	KEGG:04612	PFOA	0.000917	0.112	KIR2DL1, HLA-DRB5, TAP2, HLA-DQB1, CD8A
Malaria	KEGG:05144	PFHxS	0.0000588	0.00718	THBS4, GYPC, TGFB3, CD40, CR1
Protein processing in endoplasmic reticulum	KEGG:04141	PFDA	0.000685	0.0835	CAPN1, RAD23B, NPLOC4, MAN1A2, CANX
Response to endoplasmic reticulum stress	GO:0034976	PFDA	0.0000354	0.122	MANF, ASNS, NPLOC4, DAB2IP, CANX
Female sex differentiation	GO:0046660	PFHxS	0.0000152	0.0522	TBX3, ADCYAP1R1, ESR1, LHFPL2, SOD1
Neural nucleus development	GO:0048857	PFHxS	0.0000959	0.114	DYNLL1, GNB4, MBP, FOXP2, ZNF148
Regulation of osteoblast differentiation	GO:0045667	PFHxS	0.0000993	0.114	DDR2, REST, NOTCH1, RUNX2, HDAC4
Integral component of endoplasmic reticulum membrane	GO:0030176	PFHxS	0.000180	0.126	PIGS, RTN2, HLA-E, HLA-DRA, HLA-DQA1
Intrinsic component of endoplasmic reticulum membrane	GO:0031227	PFHxS	0.0001842	0.126	PIGS, RTN2, HLA-E, HLA-DRA, ESYT2
Development of primary female sexual characteristics	GO:0046545	PFHxS	0.000268	0.154	ADCYAP1R1, ESR1, LHFPL2, SOD1, NUP107

Note: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PFDA, perfluorodecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

epithelia throughout the body including the blood–brain barrier (Burek et al. 2019) and the epididymis (Dubé et al. 2010). Notably, a recent study of PFOA exposure in mice reported changes in the expression of tight junction genes in the small intestine, with possible adverse impacts on gut barrier functions (Rashid et al. 2020). Genes associated with the significant DMRs were involved in lipid metabolism, growth, and cardiovascular disease risk, as well as functions of the immune and nervous systems.

Notably, PFOA, PFNA, and PFDA had DMRs annotated to the *PON1* gene, previously linked to lipid oxidation (Luo et al. 2018), HDL functionality (Mahrooz et al. 2019) and cardiovascular disease (Moreno-Godínez et al. 2018), and the related *PON3* gene, previously linked with atherosclerotic disease (Rull et al. 2012). DMRs associated with PFOA were also annotated to *NR1H2*, involved in lipid homeostasis and inflammation; and *RPTOR*, part of the mTOR complex that regulates cell growth in response to nutrient availability. Other genes that were associated with multiple PFAS include *RNF39*, a gene within the major histocompatibility complex class 1 region on chromosome 6 associated with early synaptic plasticity and previously linked with multiple sclerosis (Maltby et al. 2017); *RASL11B*, a widely expressed gene with proposed roles in inflammation and arteriosclerosis (Stolle et al. 2007); and *TM9SF2*, encoding a transmembrane protein and reported to be a colorectal cancer oncogene (Clark et al. 2018).

Our findings that prenatal exposures to PFAS are associated with differences in methylation at lipid homeostasis genes are consistent with previous literature. Numerous epidemiological studies have reported cross-sectional or prospective associations between PFAS serum or plasma concentrations and lipid concentrations (Fisher et al. 2013; Fu et al. 2014; Maisonet et al. 2015; Mora et al. 2018; Steenland et al. 2009), and more limited evidence of associations with cardiovascular disease (Huang et al. 2018; Lind et al. 2017). Additionally, a cross-sectional study among adults with higher than background exposure to PFOA through contaminated drinking water reported sex-specific and chemical-specific associations with the expression of certain cholesterol transport and mobilization genes (Fletcher et al. 2013).

Genes linked to immune system activity and inflammation were also prominent: PFOA and PFNA were associated with methylation at multiple genes in the HLA-DRB group, with core immune system functionality; PFHxS was associated with *PPP1R11*, a gene in the major histocompatibility complex class 1 shown to affect lung inflammation in mice (McKelvey et al. 2016); PFDA was associated with *PF4* (also called *CXCL4*), a chemokine with a role in platelet aggregation and antimicrobial activity (Palankar et al. 2018). The epigenome-wide results for

PFOA were enriched in the KEGG pathway "antigen processing and presentation." Some of these pathways and gene functions are consistent with previously reported effects of PFAS in animal and human studies, including lipid abnormalities and immunotoxicity (ATSDR 2018).

We further identified two exposure-associated CpGs for which there was suggestive evidence of association (FDR < 0.10) with neonatal cardio-metabolic indicators. Methylation at a locus (cg09093485) annotated to *RNF39* was lower among those more highly exposed to PFHxS *in utero*, and methylation at this locus was also inversely associated with cord blood triglycerides. Although the long-term implications of altered lipid profiles in cord blood are not well established, it may be suggestive of early lipid dysregulation. Methylation at cg09637273, inversely associated with both PFOA and PFOS, was associated with greater birth weight. Maternal concentrations of PFAS during pregnancy have been associated with reduced offspring birth weight in this cohort and others (Bach et al. 2015; Johnson et al. 2014; Starling et al. 2017). Because the neonatal cardiometabolic outcomes in this study are measured concurrently with cord blood DNA methylation at birth, we cannot establish a temporal order and therefore do not make claims to causal mediation. However, we do plan to examine in future studies whether PFAS-associated methylation in cord blood predicts later child cardio-metabolic outcomes in this cohort. Prenatal exposure to some PFAS have been associated with greater risk of being overweight or obese in childhood (Braun et al. 2016; Karlsen et al. 2017; Lauritzen et al. 2018) and young adulthood (Halldorsson et al. 2012), and DNA methylation may be one mechanism linking intrauterine PFAS exposure to later disease risks.

Observed associations between PFAS exposure and methylation of genes involved in immune response and inflammation are consistent with the results of some previous epidemiological studies reporting altered immune system-related outcomes in adults and children exposed to PFAS (Chang et al. 2016; Rappazzo et al. 2017). Previous studies have generally focused on one or more of the following outcomes: allergy and atopic disease (Goudarzi et al. 2016; Stein et al. 2016b; Timmermann et al. 2017), reduced antibody response to vaccination (Grandjean et al. 2017; Looker et al. 2014; Stein et al. 2016a), frequency of infectious disease in children (Dalsager et al. 2016; Goudarzi et al. 2017; Impinen et al. 2018), and inflammatory or autoimmune disease (Steenland et al. 2013, 2018; Webster et al. 2014, 2016). Evidence from animal studies also indicates that PFOA is immunotoxic, and dietary exposure has resulted in a variety of immune system effects, including reduced weight of lymphoid organs and

impaired antibody responses (DeWitt et al. 2009). Specifically, PFOA exposure in mice has been shown to suppress T-cell dependent antibody responses (TDAR) (DeWitt et al. 2016). It is unclear at this time whether methylation at any of the specific CpGs identified in this study could influence clinically relevant immunological outcomes, such as antibody response to vaccination or risk of autoimmune disorders.

Three previous studies have examined the association between prenatal exposure to certain PFAS and epigenome-wide DNA methylation in umbilical cord blood. A pilot study ($n = 22$ high PFOA and 22 low PFOA mother–infant pairs) was conducted in the Health Outcomes and Measures of the Environment (HOME) study in Ohio (Kingsley et al. 2017). A relatively small study ($n = 72$, of which 51 were analyzed) of cord blood concentrations of several persistent chemicals was conducted within a Faroese birth cohort recruited in 1986–1987 (Leung et al. 2018). In addition, a recent epigenome-wide association study was conducted within the Hokkaido cohort in Japan ($n = 190$), and some of the findings were replicated in the Taiwan Maternal and Infant Cohort study ($n = 37$) (Miura et al. 2018). We examined whether the top CpGs from each of these studies, as well as an additional study of adult exposures ($n = 34$ men) and epigenome-wide peripheral blood methylation (van den Dungen et al. 2017), were associated with prenatal PFAS in our study.

The previous studies differed substantially from each other and from the present study in numerous characteristics, including PFAS concentrations, nationality of participants, and covariates adjusted (Table S4). However, we found nominal significance ($p < 0.05$) and the same direction of association for several CpGs from the three cord blood methylation studies, suggesting some reproducibility of results, but no CpGs from the adult blood methylation study. Genes annotated to the consistently PFAS-associated CpGs included *OPRD1*, opioid receptor delta 1; *GPR126* (also called *ADGRG6*), associated with adult height (Gudbjartsson et al. 2008; Soranzo et al. 2009); and *DUX2* (*DUX4L8*), which encodes a homeobox protein, and *GREB1*, involved in the proliferation of hormone-sensitive cancers (Hodgkinson and Vanderhyden 2014). The Leung et al. study reported numerous epigenome-wide significant associations among males only. That study had 72 participants, of which 31 were male. Additionally, 21 total samples were removed in quality control procedures, resulting in an analytic sample size of 19 for males. There was no statistical test for interaction reported. There were no epigenome-wide significant results for females or for other PFAS. No formal comparison was made between male and female results. In contrast, our study found no significant PFAS-by-sex interactions using a criterion of FDR < 0.05 for the interaction term in linear regression models.

The mechanism by which PFAS exposure may cause changes in DNA methylation is not well established. However, experimental studies have clearly demonstrated epigenetic changes following PFAS treatment. In one example, 3T3-L1 preadipocytes treated with PFOA showed global hypomethylation and increased expression of DNA methyltransferase genes, as well as increased expression of peroxisome proliferator activated receptor (PPAR) gamma and other proteins leading to adipogenic differentiation (Ma et al. 2018). Additionally, an animal study demonstrated global DNA hypomethylation in the liver of rats exposed to peroxisome proliferator WY-14,643 (Pogribny et al. 2008). The role of numerous PFAS in activating PPARs has been previously documented (Takacs and Abbott 2007). It is worth noting that the activation of PPARs varies by chemical structure of PFAS, including chain length and functional groups (carboxylates vs. sulfonates) (Wolf et al. 2008). Various PFAS also have activity on other receptors, including estrogen receptor alpha and the constitutive activated receptor (Rosen et al. 2017), and it is largely because of these qualitative differences between PFAS that summation of effects across chemicals is not recommended (Peters and Gonzalez 2011).

Limitations of this study include the lack of gene expression data in cord blood; therefore, we are unable to determine whether observed differences in methylation are correlated with differences in protein abundance and cellular activity. Additionally, genotype data were not available, so we were unable to adjust for ancestry or the role of underlying genetics on DNA methylation changes. However, we included the maternal self-reported race/ethnicity category as a covariate in regression models to reduce the potential for confounding and removed known single-nucleotide polymorphism-associated probes. We additionally examined the potential for effect modification by maternal self-reported race/ethnicity category; however, we found no epigenome-wide significance for PFAS-by-race/ethnicity interaction terms. We examined DNA methylation in cord blood, which is a mixture of cell types and may not accurately reflect methylation differences in target tissues of interest. Results should be interpreted as potential biomarkers rather than causal mechanisms of developmental effects of prenatal PFAS exposure. Finally, we cannot exclude the possibility of residual confounding by factors that may influence both maternal PFAS concentration and cord blood DNA methylation. For example, impaired maternal kidney function could theoretically reduce the excretion of PFAS (Dhingra et al. 2017; Watkins et al. 2013); however, a recent study showed the decline in measured concentration of PFAS across pregnancy to be unrelated to kidney function (Nielsen et al. 2020). The Healthy Start study excluded women with diabetes prior to pregnancy, and kidney function is expected to be normal for the majority of women in this study.

Strengths of this study include the relatively large sample size and the examination of multiple PFAS at concentrations commonly found in the general U.S. population, including emerging drinking water contaminant PFHxS.

Conclusions

This epigenome-wide association study found associations between maternal serum concentrations of five PFAS measured during pregnancy and regions of DNA methylation in umbilical cord blood. Differences in DNA methylation occurred at genes associated with lipid metabolism and growth as well as immune system function, suggesting that DNA methylation may be one mechanism by which prenatal PFAS exposures affect health outcomes later in life. Although the use of certain PFAS has been restricted or phased out of production in the United States and elsewhere, it is important to note that many other PFAS are still in use (Wang et al. 2017), and exposure to the general population is ongoing due to their persistence. Future studies will provide useful data to evaluate the potential role of multiple PFAS in producing epigenetic changes *in utero* that may increase chronic disease risk in the offspring.

References

Abbott BD, Wood CR, Watkins AM, Tatum-Gibbs K, Das KP, Lau C. 2012. Effects of perfluorooctanoic acid (PFOA) on expression of peroxisome proliferator-activated receptors (PPAR) and nuclear receptor-regulated genes in fetal and postnatal CD-1 mouse tissues. Reprod Toxicol 33(4):491–505, PMID: 22154759, https://doi.org/10.1016/j.reprotox.2011.11.005.

ATSDR (Agency for Toxic Substances and Disease Registry). 2018. *Toxicological Profile for Perfluoroalkyls*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Bach CC, Bech BH, Brix N, Nohr EA, Bonde JP, Henriksen TB. 2015. Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review. Crit Rev Toxicol 45(1):53–67, PMID: 25372700, https://doi.org/10.3109/10408444.2014.952400.

Barton KE, Starling AP, Higgins CP, McDonough CA, Calafat AM, Adgate JL. 2020. Sociodemographic and behavioral determinants of serum concentrations of per- and polyfluoroalkyl substances in a community highly exposed to aqueous film-forming foam contaminants in drinking water. Int J Hyg Environ Health 223(1):256–266, PMID: 31444118, https://doi.org/10.1016/j.ijheh.2019.07.012.

Beesoon S, Martin JW. 2015. Isomer-specific binding affinity of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) to serum proteins. Environ Sci Technol 49(9):5722–5731, PMID: 25826685, https://doi.org/10.1021/es505399w.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 57(1):289–300, https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bjerregaard-Olesen C, Bossi R, Liew Z, Long M, Bech BH, Olsen J, et al. 2017. Maternal serum concentrations of perfluoroalkyl acids in five international birth cohorts. Int J Hyg Environ Health 220(2 pt A):86–93, PMID: 28063899, https://doi.org/10.1016/j.ijheh.2016.12.005.

Braun JM, Chen A, Romano ME, Calafat AM, Webster GM, Yolton K, et al. 2016. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: the HOME study. Obesity (Silver Spring) 24(1):231–237, PMID: 26554535, https://doi.org/10.1002/oby.21258.

Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, et al. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr Environ Assess Manag 7(4):513–541, PMID: 21793199, https://doi.org/10.1002/ieam.258.

Burek M, König A, Lang M, Fiedler J, Oerter S, Roewer N, et al. 2019. Hypoxia-induced microRNA-212/132 alter blood-brain barrier integrity through inhibition of tight junction-associated proteins in human and mouse brain microvascular endothelial cells. Transl Stroke Res 10(6):672–683, PMID: 30617994, https://doi.org/10.1007/s12975-018-0683-2.

Cavalcante RG, Sartor MA. 2017. Annotatr: genomic regions in context. Bioinformatics 33(15):2381–2383, PMID: 28369316, https://doi.org/10.1093/bioinformatics/btx183.

Centers for Disease Control and Prevention. 2019. Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2019. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention.

Chang ET, Adami HO, Boffetta P, Wedner HJ, Mandel JS. 2016. A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans. Crit Rev Toxicol 46(4):279–331, PMID: 26761418, https://doi.org/10.3109/10408444.2015.1122573.

Chen YA, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, Zanke BW, et al. 2013. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. Epigenetics 8(2):203–209, PMID: 23314698, https://doi.org/10.4161/epi.23470.

Clark CR, Maile M, Blaney P, Hellweg SR, Strauss A, Durose W, et al. 2018. Transposon mutagenesis screen in mice identifies TM9SF2 as a novel colorectal cancer oncogene. Sci Rep 8(1):15327, PMID: 30333512, https://doi.org/10.1038/s41598-018-33527-3.

Dalsager L, Christensen N, Husby S, Kyhl H, Nielsen F, Høst A, et al. 2016. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4 years among 359 children in the Odense Child Cohort. Environ Int 96:58–64, PMID: 27608427, https://doi.org/10.1016/j.envint.2016.08.026.

Daly ER, Chan BP, Talbot EA, Nassif J, Bean C, Cavallo SJ, et al. 2018. Per- and polyfluoroalkyl substance (PFAS) exposure assessment in a community exposed to contaminated drinking water, New Hampshire, 2015. Int J Hyg Environ Health 221(3):569–577, PMID: 29514764, https://doi.org/10.1016/j.ijheh.2018.02.007.

DeWitt JC, Shnyra A, Badr MZ, Loveless SE, Hoban D, Frame SR, et al. 2009. Immunotoxicity of perfluorooctanoic acid and perfluorooctane sulfonate and the role of peroxisome proliferator-activated receptor alpha. Crit Rev Toxicol 39(1):76–94, PMID: 18802816, https://doi.org/10.1080/10408440802209804.

DeWitt JC, Williams WC, Creech NJ, Luebke RW. 2016. Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: role of PPARα and T- and B-cell targeting. J Immunotoxicol 13(1):38–45, PMID: 25594567, https://doi.org/10.3109/1547691X.2014.996682.

Dhingra R, Winquist A, Darrow LA, Klein M, Steenland K. 2017. A study of reverse causation: examining the associations of perfluorooctanoic acid serum levels with two outcomes. Environ Health Perspect 125(3):416–421, https://doi.org/10.1289/EHP273.

Dubé E, Dufresne J, Chan PT, Hermo L, Cyr DG. 2010. Assessing the role of claudins in maintaining the integrity of epididymal tight junctions using novel human epididymal cell lines. Biol Reprod 82(6):1119–1128, PMID: 20164436, https://doi.org/10.1095/biolreprod.109.083196.

Fisher M, Arbuckle TE, Wade M, Haines DA. 2013. Do perfluoroalkyl substances affect metabolic function and plasma lipids?–analysis of the 2007–2009, Canadian Health Measures Survey (CHMS) Cycle 1. Environ Res 121:95–103, PMID: 23266098, https://doi.org/10.1016/j.envres.2012.11.006.

Fletcher T, Galloway TS, Melzer D, Holcroft P, Cipelli R, Pilling LC, et al. 2013. Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. Environ Int 57–58:2–10, PMID: 23624243, https://doi.org/10.1016/j.envint.2013.03.008.

Forsthuber M, Kaiser AM, Granitzer S, Hassl I, Hengstschläger M, Stangl H, et al. 2020. Albumin is the major carrier protein for PFOS, PFOA, PFHxS, PFNA and PFDA in human plasma. Environ Int 137:105324, PMID: 32109724, https://doi.org/10.1016/j.envint.2019.105324.

Fu Y, Wang T, Fu Q, Wang P, Lu Y. 2014. Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. Ecotoxicol Environ Saf 106:246–252, PMID: 24863755, https://doi.org/10.1016/j.ecoenv.2014.04.039.

Gao K, Zhuang T, Liu X, Fu J, Zhang J, Fu J, et al. 2019. Prenatal exposure to per- and polyfluoroalkyl substances (PFASs) and association between the placental transfer efficiencies and dissociation constant of serum proteins-PFAS complexes. Environ Sci Technol 53(11):6529–6538, https://doi.org/10.1021/acs.est.9b00715.

Gervin K, Salas LA, Bakulski KM, van Zelm MC, Koestler DC, Wiencke JK, et al. 2019. Systematic evaluation and validation of reference and library selection methods for deconvolution of cord blood DNA methylation data. Clin Epigenetics 11(1):125, PMID: 31455416, https://doi.org/10.1186/s13148-019-0717-y.

Goudarzi H, Miyashita C, Okada E, Kashino I, Chen CJ, Ito S, et al. 2017. Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age. Environ Int 104:132–138, PMID: 28392064, https://doi.org/10.1016/j.envint.2017.01.024.

Goudarzi H, Miyashita C, Okada E, Kashino I, Kobayashi S, Chen CJ, et al. 2016. Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children. Environ Int 94:124–132, PMID: 27236407, https://doi.org/10.1016/j.envint.2016.05.020.

Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, et al. 2012. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA 307(4):391–397, PMID: 22274686, https://doi.org/10.1001/jama.2011.2034.

Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Budtz-Jørgensen E. 2017. Serum vaccine antibody concentrations in adolescents exposed to perfluorinated compounds. Environ Health Perspect 125(7):077018, PMID: 28749778, https://doi.org/10.1289/EHP275.

Gudbjartsson DF, Walters GB, Thorleifsson G, Stefansson H, Halldorsson BV, Zusmanovich P, et al. 2008. Many sequence variants affecting diversity of adult human height. Nat Genet 40(5):609–615, PMID: 18391951, https://doi.org/10.1038/ng.122.

Guerrero-Preston R, Goldman LR, Brebi-Mieville P, Ili-Gangas C, Lebron C, Witter FR, et al. 2010. Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. Epigenetics 5(6):539–546, https://doi.org/10.4161/epi.5.6.12378.

Gützkow KB, Haug LS, Thomsen C, Sabaredzovic A, Becher G, Brunborg G. 2012. Placental transfer of perfluorinated compounds is selective–a Norwegian Mother and Child sub-cohort study. Int J Hyg Environ Health 215(2):216–219, PMID: 21937271, https://doi.org/10.1016/j.ijheh.2011.08.011.

Gyllenhammar I, Berger U, Sundström M, McCleaf P, Eurén K, Eriksson S, et al. 2015. Influence of contaminated drinking water on perfluoroalkyl acid levels in human serum–a case study from Uppsala, Sweden. Environ Res 140:673–683, PMID: 26079316, https://doi.org/10.1016/j.envres.2015.05.019.

Halldorsson TI, Rytter D, Haug LS, Bech BH, Danielsen I, Becher G, et al. 2012. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ Health Perspect 120(5):668–673, PMID: 22306490, https://doi.org/10.1289/ehp.1104034.

Hoaglin DC, Iglewicz B, Tukey JW. 1986. Performance of some resistant rules for outlier labeling. J Am Stat Assoc 81(396):991–999, https://doi.org/10.1080/01621459.1986.10478363.

Hodgkinson KM, Vanderhyden BC. 2014. Consideration of GREB1 as a potential therapeutic target for hormone-responsive or endocrine-resistant cancers. Expert Opin Ther Targets 18(9):1065–1076, PMID: 24998469, https://doi.org/10.1517/14728222.2014.936382.

Høyer BB, Ramlau-Hansen CH, Vrijheid M, Valvi D, Pedersen HS, Zviezdai V, et al. 2015. Anthropometry in 5- to 9-year-old Greenlandic and Ukrainian children in relation to prenatal exposure to perfluorinated alkyl substances. Environ Health Perspect 123(8):841–846, PMID: 25809098, https://doi.org/10.1289/ehp.1408881.

Huang M, Jiao J, Zhuang P, Chen X, Wang J, Zhang Y. 2018. Serum polyfluoroalkyl chemicals are associated with risk of cardiovascular diseases in national US population. Environ Int 119:37–46, PMID: 29933236, https://doi.org/10.1016/j.envint.2018.05.051.

Impinen A, Nygaard UC, Lodrup Carlsen KC, Mowinckel P, Carlsen KH, Haug LS, et al. 2018. Prenatal exposure to perfluoroalkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ Res 160:518–523, PMID: 29106950, https://doi.org/10.1016/j.envres.2017.10.012.

Johnson WE, Li C, Rabinovic A. 2007. Adjusting batch effects in microarray expression data using empirical bayes methods. Biostatistics 8(1):118–127, PMID: 16632515, https://doi.org/10.1093/biostatistics/kxj037.

Johnson PI, Sutton P, Atchley DS, Koustas E, Lam J, Sen S, et al. 2014. The navigation guide - evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. Environ Health Perspect 122(10):1028–1039, PMID: 24968388, https://doi.org/10.1289/ehp.1307893.

Karlsen M, Grandjean P, Weihe P, Steuerwald U, Oulhote Y, Valvi D. 2017. Early-life exposures to persistent organic pollutants in relation to overweight in preschool children. Reprod Toxicol 68:145–153, PMID: 27496715, https://doi.org/10.1016/j.reprotox.2016.08.002.

Kato K, Basden BJ, Needham LL, Calafat AM. 2011. Improved selectivity for the analysis of maternal serum and cord serum for polyfluoroalkyl chemicals. J Chromatogr A 1218(15):2133–2137, PMID: 21084089, https://doi.org/10.1016/j.chroma.2010.10.051.

Kato K, Wong LY, Chen A, Dunbar C, Webster GM, Lanphear BP, et al. 2014. Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003–2006. Environ Sci Technol 48(16):9600–9608, https://doi.org/10.1021/es501811k.

Kingsley SL, Kelsey KT, Butler R, Chen A, Eliot MN, Romano ME, et al. 2017. Maternal serum PFOA concentration and DNA methylation in cord blood: a pilot study. Environ Res 158:174–178, PMID: 28645023, https://doi.org/10.1016/j.envres.2017.06.013.

Kobayashi S, Azumi K, Goudarzi H, Araki A, Miyashita C, Kobayashi S, et al. 2017. Effects of prenatal perfluoroalkyl acid exposure on cord blood IGF2/H19 methylation and ponderal index: the Hokkaido study. J Expo Sci Environ Epidemiol 27(3):251–259, PMID: 27553991, https://doi.org/10.1038/jes.2016.50.

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci 99(2):366–394, PMID: 17519394, https://doi.org/10.1093/toxsci/kfm128.

Lauritzen HB, Larose TL, Oien T, Sandanger TM, Odland JO, van de Bor M, et al. 2018. Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study. Environ Health 17(1):9, PMID: 29347948, https://doi.org/10.1186/s12940-017-0338-x.

Leung YK, Ouyang B, Niu L, Xie C, Ying J, Medvedovic M, et al. 2018. Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a Faroese birth cohort. Epigenetics 13(3):290–300, PMID: 29560787, https://doi.org/10.1080/15592294.2018.1445901.

Li Y, Fletcher T, Mucs D, Scott K, Lindh CH, Tallving P, et al. 2018. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occup Environ Med 75(1):46–51, PMID: 29133598, https://doi.org/10.1136/oemed-2017-104651.

Lind PM, Salihovic S, van Bavel B, Lind L. 2017. Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. Environ Res 152:157–164, PMID: 27771570, https://doi.org/10.1016/j.envres.2016.10.002.

Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, et al. 2014. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci 138(1):76–88, PMID: 24284791, https://doi.org/10.1093/toxsci/kft269.

Luo Z, Pu L, Muhammad I, Chen Y, Sun X. 2018. Associations of the PON1 rs662 polymorphism with circulating oxidized low-density lipoprotein and lipid levels: a systematic review and meta-analysis. Lipids Health Dis 17(1):281, https://doi.org/10.1186/s12944-018-0937-8.

Ma Y, Yang J, Wan Y, Peng Y, Ding S, Li Y, et al. 2018. Low-level perfluorooctanoic acid enhances 3 T3-L1 preadipocyte differentiation via altering peroxisome proliferator activated receptor gamma expression and its promoter DNA methylation. J Appl Toxicol 38(3):398–407, PMID: 29094436, https://doi.org/10.1002/jat.3549.

Mahrooz A, Mackness M, Bagheri A, Ghaffari-Cherati M, Masoumi P. 2019. The epigenetic regulation of paraoxonase 1 (PON1) as an important enzyme in HDL function: the missing link between environmental and genetic regulation. Clin Biochem 73:1–10, PMID: 31351988, https://doi.org/10.1016/j.clinbiochem.2019.07.010.

Maisonet M, Näyhä S, Lawlor DA, Marcus M. 2015. Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females. Environ Int 82:49–60, PMID: 26010307, https://doi.org/10.1016/j.envint.2015.05.001.

Maltby VE, Lea RA, Sanders KA, White N, Benton MC, Scott RJ, et al. 2017. Differential methylation at MHC in CD4+ T cells is associated with multiple sclerosis independently of HLA-DRB1. Clin Epigenetics 9:71, PMID: 28729889, https://doi.org/10.1186/s13148-017-0371-1.

Martin EM, Fry RC. 2018. Environmental influences on the epigenome: exposure-associated DNA methylation in human populations. Annu Rev Public Health 39:309–333, PMID: 29328878, https://doi.org/10.1146/annurev-publhealth-040617-014629.

McKelvey AC, Lear TB, Dunn SR, Evankovich J, Londino JD, Bednash JS, et al. 2016. RING finger E3 ligase PPP1R11 regulates TLR2 signaling and innate immunity. Elife 5:e1849, PMID: 27805901, https://doi.org/10.7554/eLife.18496.

Merid SK, Novoloaca A, Sharp GC, Kupers LK, Kho AT, Roy R, et al. 2020. Epigenome-wide meta-analysis of blood DNA methylation in newborns and children identifies numerous loci related to gestational age. Genome Med 12(1):17, https://doi.org/10.1186/s13073-020-0716-9.

Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J. 2007. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int Arch Occup Environ Health 80(7):643–648, PMID: 17219182, https://doi.org/10.1007/s00420-006-0165-9.

Miura R, Araki A, Miyashita C, Kobayashi S, Kobayashi S, Wang SL, et al. 2018. An epigenome-wide study of cord blood DNA methylations in relation to prenatal perfluoroalkyl substance exposure: the Hokkaido study. Environ Int 115:21–28, PMID: 29544137, https://doi.org/10.1016/j.envint.2018.03.004.

Mora AM, Fleisch AF, Rifas-Shiman SL, Woo Baidal JA, Pardo L, Webster TF, et al. 2018. Early life exposure to per- and polyfluoroalkyl substances and mid-childhood lipid and alanine aminotransferase levels. Environ Int 111:1–13, PMID: 29156323, https://doi.org/10.1016/j.envint.2017.11.008.

Moreno-Godínez ME, Galarce-Sosa C, Cahua-Pablo JA, Rojas-García AE, Huerta-Beristain G, Alarcón-Romero LDC, et al. 2018. Genotypes of common polymorphisms in the PON1 gene associated with paraoxonase activity as cardiovascular risk factor. Arch Med Res 49(7):486–496, PMID: 30853125, https://doi.org/10.1016/j.arcmed.2019.02.002.

Nielsen C, Andersson Hall U, Lindh C, Ekström U, Xu Y, Li Y, et al. 2020. Pregnancy-induced changes in serum concentrations of perfluoroalkyl substances and the influence of kidney function. Environ Health 19(1):80, PMID: 32641055, https://doi.org/10.1186/s12940-020-00626-6.

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect 115(9):1298–1305, PMID: 17805419, https://doi.org/10.1289/ehp.10009.

Palankar R, Kohler TP, Krauel K, Wesche J, Hammerschmidt S, Greinacher A. 2018. Platelets kill bacteria by bridging innate and adaptive immunity via platelet factor 4 and FcγRIIA. J Thromb Haemost 16(6):1187–1197, PMID: 29350833, https://doi.org/10.1111/jth.13955.

Pedersen BS, Schwartz DA, Yang IV, Kechris KJ. 2012. Comb-p: software for combining, analyzing, grouping and correcting spatially correlated p-values. Bioinformatics 28(22):2986–2988, PMID: 22954632, https://doi.org/10.1093/bioinformatics/bts545.

Peters JM, Gonzalez FJ. 2011. Why toxic equivalency factors are not suitable for perfluoroalkyl chemicals. Chem Res Toxicol 24(10):1601–1609, PMID: 21913657, https://doi.org/10.1021/tx200316x.

Pogribny IP, Tryndyak VP, Boureiko A, Melnyk S, Bagnyukova TV, Montgomery B, et al. 2008. Mechanisms of peroxisome proliferator-induced DNA hypomethylation in rat liver. Mutat Res 644(1–2):17–23, PMID: 18639561, https://doi.org/10.1016/j.mrfmmm.2008.06.009.

Rappazzo KM, Coffman E, Hines EP. 2017. Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. Int J Environ Res Public Health 14(7):691, PMID: 28654008, https://doi.org/10.3390/ijerph14070691.

Rashid F, Ahmad S, Irudayaraj JMK. 2020. Effect of perfluorooctanoic acid on the epigenetic and tight junction genes of the mouse intestine. Toxics 8(3):64, https://doi.org/10.3390/toxics8030064.

Ren X, Kuan PF. 2019. MethylGSA: a Bioconductor package and Shiny app for DNA methylation data length bias adjustment in gene set testing. Bioinformatics 35(11):1958–1959, PMID: 30346483, https://doi.org/10.1093/bioinformatics/bty892.

Rosen MB, Das KP, Rooney J, Abbott B, Lau C, Corton JC. 2017. PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387:95–107, PMID: 28558994, https://doi.org/10.1016/j.tox.2017.05.013.

Rull A, García R, Fernandez-Sender L, García-Heredia A, Aragonès G, Beltrán-Debón R, et al. 2012. Serum paraoxonase-3 concentration is associated with insulin sensitivity in peripheral artery disease and with inflammation in coronary artery disease. Atherosclerosis 220(2):545–551, PMID: 22153698, https://doi.org/10.1016/j.atherosclerosis.2011.11.021.

Soranzo N, Rivadeneira F, Chinappen-Horsley U, Malkina I, Richards JB, Hammond N, et al. 2009. Meta-analysis of genome-wide scans for human adult stature identifies novel loci and associations with measures of skeletal frame size. PLoS Genet 5(4):e1000445, PMID: 19343178, https://doi.org/10.1371/journal.pgen.1000445.

Starling AP, Adgate JL, Hamman RF, Kechris K, Calafat AM, Ye X, et al. 2017. Perfluoroalkyl substances during pregnancy and offspring weight and adiposity at birth: examining mediation by maternal fasting glucose in the Healthy Start study. Environ Health Perspect 125(6):067016, PMID: 28669937, https://doi.org/10.1289/EHP641.

Steenland K, Kugathasan S, Barr DB. 2018. PFOA and ulcerative colitis. Environ Res 165:317–321, PMID: 29777922, https://doi.org/10.1016/j.envres.2018.05.007.

Steenland K, Tinker S, Frisbee S, Ducatman A, Vaccarino V. 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am J Epidemiol 170(10):1268–1278, PMID: 19846564, https://doi.org/10.1093/aje/kwp279.

Steenland K, Zhao L, Winquist A, Parks C. 2013. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. Environ Health Perspect 121(8):900–905, https://doi.org/10.1289/ehp.1206449.

Stein CR, Ge Y, Wolff MS, Ye X, Calafat AM, Kraus T, et al. 2016a. Perfluoroalkyl substance serum concentrations and immune response to FluMist vaccination among healthy adults. Environ Res 149:171–178, PMID: 27208468, https://doi.org/10.1016/j.envres.2016.05.020.

Stein CR, McGovern KJ, Pajak AM, Maglione PJ, Wolff MS. 2016b. Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. Pediatr Res 79(2):348–357, PMID: 26492286, https://doi.org/10.1038/pr.2015.213.

Stolle K, Schnoor M, Fuellen G, Spitzer M, Cullen P, Lorkowski S. 2007. Cloning, genomic organization, and tissue-specific expression of the RASL11B gene. Biochim Biophys Acta 1769(7–8):514–524, PMID: 17628721, https://doi.org/10.1016/j.bbaexp.2007.05.005.

Takacs ML, Abbott BD. 2007. Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate. Toxicol Sci 95(1):108–117, PMID: 17047030, https://doi.org/10.1093/toxsci/kfl135.

Timmermann CA, Budtz-Jorgensen E, Jensen TK, Osuna CE, Petersen MS, Steuerwald U, et al. 2017. Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination. J Immunotoxicol 14(1):39–49, PMID: 28091126, https://doi.org/10.1080/1547691X.2016.1254306.

Touleimat N, Tost J. 2012. Complete pipeline for Infinium® Human Methylation 450k Beadchip data processing using subset quantile normalization for accurate DNA methylation estimation. Epigenomics 4(3):325–341, PMID: 22690668, https://doi.org/10.2217/epi.12.21.

Urlando A, Dempster P, Aitkens S. 2003. A new air displacement plethysmograph for the measurement of body composition in infants. Pediatr Res 53(3):486–492, PMID: 12595599, https://doi.org/10.1203/01.PDR.0000049669.74793.E3.

van den Dungen MW, Murk AJ, Kampman E, Steegenga WT, Kok DE. 2017. Association between DNA methylation profiles in leukocytes and serum levels of persistent organic pollutants in Dutch men. Environ Epigenet 3(1):dvx001, PMID: 29492303, https://doi.org/10.1093/eep/dvx001.

Vestergren R, Cousins IT. 2009. Tracking the pathways of human exposure to perfluorocarboxylates. Environ Sci Technol 43(15):5565–5575, PMID: 19731646, https://doi.org/10.1021/es900228k.

Wang Z, DeWitt JC, Higgins CP, Cousins IT. 2017. A never-ending story of per- and polyfluoroalkyl substances (PFASs)? Environ Sci Technol 51(5):2508–2518, PMID: 28224793, https://doi.org/10.1021/acs.est.6b04806.

Watkins DJ, Josson J, Elston B, Bartell SM, Shin HM, Vieira VM, et al. 2013. Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environ Health Perspect 121(5):625–630, https://doi.org/10.1289/ehp.1205838.

Webster GM, Rauch SA, Marie NS, Mattman A, Lanphear BP, Venners SA. 2016. Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in U.S. adults: variation according to TPOAb and iodine status (NHANES 2007–2008). Environ Health Perspect 124(7):935–942, PMID: 26517287, https://doi.org/10.1289/ehp.1409589.

Webster GM, Venners SA, Mattman A, Martin JW. 2014. Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environ Res 133:338–347, PMID: 25019470, https://doi.org/10.1016/j.envres.2014.06.012.

Wolf CJ, Takacs ML, Schmid JE, Lau C, Abbott BD. 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol Sci 106(1):162–171, PMID: 18713766, https://doi.org/10.1093/toxsci/kfn166.

Yang IV, Pedersen BS, Liu AH, O'Connor GT, Pillai D, Kattan M, et al. 2017. The nasal methylome and childhood atopic asthma. J Allergy Clin Immunol 139(5):1478–1488, https://doi.org/10.1016/j.jaci.2016.07.036.

Yang J, Wang H, Du H, Xu L, Liu S, Yi J, et al. 2019. Factors associated with exposure of pregnant women to perfluoroalkyl acids in North China and health risk assessment. Sci Total Environ 655:356–362, PMID: 30471604, https://doi.org/10.1016/j.scitotenv.2018.11.042.