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Maternal serum PFOA concentration and DNA methylation in cord blood: A pilot study

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ABSTRACT

Perfluorooctanoic acid (PFOA), a perfluoroalkyl substance, is commonly detected in the serum of pregnant women and may impact fetal development via epigenetic re-programming. In a pilot study, we explored associations between serum PFOA concentrations during pregnancy and offspring peripheral leukocyte DNA methylation at delivery in women with high (n = 22, range: 12–26 ng/mL) and low (n = 22, range: 1.1–3.1 ng/mL) PFOA concentrations. After adjusting for cell type, child sex, and income, we did not find differences in CpG methylation in the two exposure groups that reached epigenome-wide significance. Among the 20 CpGs with the lowest p-values we found that seven CpG sites in three genes differed by exposure status. In a confirmatory cluster analysis, these 20 CpGs clustered into two groups that perfectly identified exposure status. Future studies with larger sample sizes should confirm these findings and determine if PFOA-associated changes in DNA methylation underlie potential health effects of PFOA.

1. Introduction

Perfluoroalkyl substances (PFAS) are man-made fluorinated chemicals used in some stain/water resistant textile coatings, non-stick cookware, food container coatings, floor polish, fire-fighting foam, and industrial surfactants (EFSA, 2008). There is concern over the potential adverse health effects of PFAS, including the PFAS perfluorooctanoic acid (PFOA), due to their widespread use, biological persistence, detection in the blood of pregnant women and neonates, and association with adverse human health outcomes (Anon, 2008; Braun, 2016; Kato et al., 2014).

The developing fetus and infant may be especially sensitive to PFOA exposure because they have less developed biologically protective mechanisms and enhanced sensitivity to environmental toxicants. Cellular growth and large-scale epigenetic programming of lineage development that occur early in development could be affected by PFOA exposure and have long lasting impacts on various health outcomes (Jirtle and Skinner, 2007). Thus, changes in DNA methylation may be early markers of PFAS-induced alterations in fetal programming.

Several studies have reported that PFOA or perfluorooctane sulfonate (PFOS) exposures are associated with lower global DNA cytosine methylation in neonates, higher Long Interspersed Nuclear Element-1 (LINE-1) methylation in adults, changes in the expression of cholesterol metabolism genes in adults, and decreased insulin-like growth factor-2 methylation in neonates (Guerrero-Preston et al., 2010; Watkins et al., 2014; Fletcher et al., 2013; Kobayashi et al., 2016). However, we are not aware of any studies that have examined epigenome-wide DNA methylation to identify PFOA-affected biological pathways. The purpose of this prospective pilot study was to determine if maternal serum PFOA concentrations during pregnancy were associated with differences in newborn peripheral leukocyte DNA methylation.

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Table 1
Participant characteristics by prenatal PFOA group, n (%).

	Total (n = 44)	High PFOA exposure (n = 22)	Low PFOA exposure (n = 22)
Maternal characteristics			
Age (years), mean ± SD	31.3 ± 5.0	31.2 ± 5.7	31.5 ± 4.4
Serum cotinine (ng/mL), GM ± SD	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.5
Prenatal vitamin use, Yes	41 (93)	20 (91)	21 (95)
Annual household income (thousands), mean ± SD	73.6 ± 47.0	74.5 ± 42.2	72.7 ± 52.4
Education			
< Bachelor's degree	16 (36)	9 (41)	7 (32)
≥ Bachelor's degree	28 (64)	13 (59)	15 (68)
Newborn characteristics			
Gestational age (weeks), mean ± SD	39.4 ± 1.3	39.5 ± 1.4	39.2 ± 1.3
Infant sex, Male	23 (52)	11 (50)	12 (55)
PFOA (ng/mL), median (IQR)	7.5 (2.4, 15)	15 (13, 17)*	2.4 (2.0, 2.6)*

* Statistically significant difference between exposure groups ($p < 0.05$).

2. Materials and methods

We conducted a pilot study using data from 44 mother-infant pairs in the Health Outcomes and Measures of the Environment (HOME) Study, a prospective pregnancy and birth cohort that recruited pregnant women in the greater Cincinnati, OH metropolitan area between March 2003 and January 2006. See the Supplemental Methods for additional details about the presented study and Braun et al. for additional details regarding participant eligibility, recruitment, and follow-up (Braun et al., 2016). The institutional review boards of Cincinnati Children's Hospital Medical Center and the cooperating delivery hospitals approved this study, and all women provided informed consent for themselves and their infants.

We collected blood samples from women at ~16 weeks of pregnancy during their prenatal clinic appointments and measured serum PFOA concentrations using online solid phase extraction coupled to high performance liquid chromatography-isotope dilution tandem mass spectrometry (limit of detection: 0.1 ng/mL) (Kato et al., 2011). We examined 22 children born to women with the highest PFOA concentrations in the study (median: 15 ng/mL; range: 12–26) and 22 children born to women with the lowest concentrations (median: 2.5 ng/mL; range: 1.1–3.1) to estimate a potential range of effect sizes. For all analyses we dichotomized PFOA into high and low exposure groups.

We collected venous cord blood samples immediately after delivery by venipuncture, extracted DNA from frozen cord blood, and used the Illumina Infinium Human Methylation 450 BeadChip Kit to measure DNA methylation at 485,512 loci. We analyzed samples in four batches, and each row of each chip contained DNA from one high and one low PFOA group infant, with the columns counterbalanced. We followed recommended best practices to process, convert, and normalize raw DNA methylation data (Breton et al., 2017). After removing sex chromosomes, SNP-affected probes, cross-hybridizing probes, and probes having detection p-values > 0.05, the final data set included 358,933 autosomal probes.

Trained research assistants collected information about maternal age, education, household income, insurance, prenatal vitamin use, gestational age, and infant sex from questionnaires and medical records. We measured serum cotinine as a biomarker of tobacco smoke exposure. Due to the small sample size of this pilot study we considered only child sex and household income as potential confounders in the analysis. In addition, we estimated and adjusted for the proportion of B cells, CD4, CD8, granulocytes, monocytes, natural killer cells, and nucleated red blood cells using reference data from cord blood samples and an unsupervised optimal differentially methylated region subset finder to predict the proportions of each cell type for each subject in our data (Bakulski et al., 2016; Koestler et al., 2016).

We examined bivariate associations between covariates and PFOA exposure group. Then, after adjusting the methylation beta values for cell type, we used the Limma package (R version 3.2.1 version 3.2.1) to estimate the difference in the mean methylation beta values for those in the high exposure group compared to those in the low exposure group, adjusting for infant sex and income. We adjusted for multiple comparisons using the Benjamini-Hochberg method to calculate q-values to control the false discovery rate (FDR < 0.05) (Benjamini and Hochberg, 1995). This approach can be sensitive to outliers, thus, we performed a sensitivity analysis using robust linear regression.

We used the UCSC Genome Browser (http://genome.ucsc.edu/) to map exonic and intronic regions using the February 2009 human reference sequence (GRCh37) for genes with multiple CpG sites in the top 20 loci from our primary analysis (Kent, 2002). As a confirmatory analysis, we used k-means clustering to cluster individuals based on the top 20 loci. The optimal number of clusters was chosen by the cubic clustering criterion. We then cross-tabulated cluster with PFOA exposure group. Next, we calculated the proportion of significant ($p < 0.001$) and non-significant CpGs located in promoter and enhancer regions. Finally, we examined differences in cell type proportions between the high and low serum PFOA concentration groups. Analyses were performed in SAS 9.4 (SAS Institutes, Inc., Cary, NC) and in R (v3.0.0).

3. Results

On average, women in this pilot study were 31 years old at delivery and had an average annual household income of $73,600 (Table 1). Most women used prenatal vitamins (93%) and had a bachelor's degree or higher (64%).

In adjusted linear regression models, there were no differences in methylation at any CpG sites in the two PFOA exposure groups at a false discovery rate < 0.05 level (Table 2). The distribution of p-values slightly deviates from the null expected distribution for those near statistical significance (Supplemental Figure 1). Results did not change appreciably in sensitivity analyses using robust linear regression. Among the 20 CpGs with the smallest p-values, we found seven CpG sites in three genes that were hypomethylated in women who had higher PFOA exposure compared with women who had lower exposure. We highlight these seven CpGs because they were associated with PFOA more than once in a particular gene. The seven CpGs were found in *RASA3* (n = 3), *UCK1* (n = 2), and *OPRD1* (n = 2) with q-values ranging from 0.133 to 0.339 (p-values ≤ 1.87e-05). In each gene, these CpGs were located in close proximity to each other (< 200 base pairs); most were in intronic regions (Supplemental Figure 2, Supplemental Table 1). In addition, the loci associated with PFOA exposure ($p < 0.001$) were more likely to be located in promoter regions (23.8 vs 16.3%; Fig. 1, Supplemental Table 2) compared to loci not associated with PFOA.