



Associations between serum perfluoroalkyl acids and LINE-1 DNA methylation

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ABSTRACT

Perfluoroalkyl acids (PFAAs) are persistent, synthetic compounds that are used in a number of consumer products. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been associated with cardiovascular risk factors, and changes in gene expression and DNA methylation in animals and cellular systems. However, whether PFAA exposure is associated with LINE-1 DNA methylation, a potential marker of cardiovascular risk, in humans remains unknown. We sought to evaluate the cross-sectional associations between serum PFAAs and LINE-1 DNA methylation in a population highly exposed to PFOA. We measured serum PFAAs twice four to five years apart in 685 adult participants (47% male, mean age $\pm$ SD = 42 ± 11 years). We measured percent LINE-1 DNA methylation in peripheral blood leukocytes at the second time point (follow-up), and estimated absolute differences in LINE-1 methylation associated with an interquartile (IQR) shift in mean PFAA serum levels. IQR increases in mean serum PFOA, PFOS, perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS) were associated with differences of -0.04 ($p = 0.16$), 0.20 ($p = 0.001$), 0.06 ($p = 0.19$), and 0.02 ($p = 0.57$), respectively, in % LINE-1 methylation at follow-up after adjustment for potential confounders. We observed a monotonic increase in LINE-1 DNA methylation across tertiles of PFOS and PFNA ($p_{\text{trend}} = 0.02$ for both associations), but not across tertiles of PFOA or PFHxS ($p_{\text{trend}} = 0.71$ and 0.44 , respectively). In summary, serum PFOS was associated with LINE-1 methylation, while serum PFOA, PFHxS, and PFNA were not. Additional research is needed to more precisely determine whether these compounds are epigenetically active.

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1. Introduction

Perfluoroalkyl acids (PFAAs) are a class of synthetic compounds that have been widely used in commercial and industrial applications. As PFAAs are extremely stable and repel both oil and water, they have been used in non-stick cooking surfaces, oil-resistant coatings for food contact paper, and stain resistant and waterproofing sprays for fabrics, upholstery, and carpets (Lau et al., 2007). PFAAs are now ubiquitous

in the environment (Lindstrom et al., 2011), and over 95% of Americans participating in the 2007–2008 National Health and Nutrition Examination Survey (NHANES) had detectable serum concentrations of four common PFAAs — perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS) (Kato et al., 2011).

Although PFAA exposure has not yet been directly linked to cardiovascular disease (CVD), epidemiological studies do suggest that exposure to PFOA and PFOS may be associated with CVD risk factors such as hyperuricemia (Costa et al., 2009; Shankar et al., 2011; Steenland et al., 2010) and increased total and low density lipoprotein cholesterol (LDL-C) (Costa et al., 2009; Eriksen et al., 2013; Fitz-Simon et al., 2013; Frisbee et al., 2010; Nelson et al., 2010; Olsen et al., 2003; Sakr et al., 2007; Steenland et al., 2009), although studies have not been entirely consistent (e.g. Fisher et al., 2013; Olsen et al., 2012), and have been predominantly cross-sectional, limiting causal inference. Identifying mechanisms by which PFAAs may possibly give rise to adverse health effects, including changes in CVD risk factors, could potentially provide deeper understanding of these relationships. Epigenetic changes have been

Abbreviations: BMI, body mass index; C8, 8 carbon chain, referring to perfluorooctanoic acid; CDC, Centers for Disease Control and Prevention; CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; IQR, interquartile range; IRB, Institutional Review Board; LDL-C, low density lipoprotein cholesterol; LINE-1, long interspersed nuclear element 1; NHANES, National Health and Nutrition Examination Survey; NIOSH, National Institute for Occupational Safety and Health; PFAA, perfluoroalkyl acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PPAR, peroxisome-proliferator activated receptor.

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proposed as one possible mechanism (Tian et al., 2012; Wan et al., 2010), but evidence in humans is largely lacking.

DNA methylation is an epigenetic mechanism that involves the addition of a methyl group to cytosine residues primarily in the context of CpG dinucleotides, a combination which is underrepresented in the human genome but concentrated in the promoter regions of many genes and in DNA repeat regions and known to regulate gene expression (Bird, 2002; Tost, 2010). In general, methylation of CpG regions suppresses expression of related genes, while demethylation of CpG regions is associated with increased gene expression. One type of repeat region is the long interspersed nuclear element 1 (LINE-1), a group of retrotransposon sequences that are highly methylated (i.e. not expressed) in non-diseased states (Nelson et al., 2011). Methylation of LINE-1 elements may impact expression of surrounding genes depending on the location of the repeat element as well as the specific genes involved. LINE-1 elements make up approximately 17% of the human genome (International Human Genome Sequencing Consortium, 2001), and the methylation extent of these regions may provide an indication of whether compounds are epigenetically active (Nelson et al., 2011). Hypomethylation of LINE-1 elements has been associated with genomic instability (Belancio et al., 2009), risk of cancer (Belancio et al., 2010), ischemic heart disease, stroke, and hypertension (Baccarelli et al., 2010), increased LDL-C, and decreased high density lipoprotein cholesterol (HDL-C) (Cash et al., 2011), as well as age, sex, race, ethnicity, and environmental exposures (Nelson et al., 2011).

To our knowledge, there has been only one prior epidemiological study evaluating associations between PFAAs and DNA methylation. In a study of newborns, Guerrero-Preston et al. (2010) reported a negative association between PFOA concentrations in cord blood and total methylated cytosine in DNA isolated from cord serum (Guerrero-Preston et al., 2010), but saw no such association with PFOS. Toxicological studies also suggest that exposure to PFOA or PFOS may affect DNA methylation (Tian et al., 2012; Wan et al., 2010) and alter gene expression (Guruge et al., 2006; Rosen et al., 2007; Rosen et al., 2010; Yeung et al., 2007), but information on other PFAAs is limited. In addition, a

recent study of a subset of the population studied here found that serum PFOA and PFOS concentrations were associated with the expression of genes related to cholesterol transport and mobilization (Fletcher et al., 2013), although the involvement of epigenetic mechanisms in this relationship has not been established.

Accordingly, we sought to evaluate the epigenetic activity of PFAAs by examining cross-sectional associations between serum PFAA concentrations and LINE-1 DNA methylation in peripheral leukocytes in a population exposed to high levels of PFOA in the environment, but background levels of PFOS, PFNA, and PFHxS.

2. Materials and methods

2.1. Study population

Participants included in this analysis are a subset of adults enrolled in the C8 Health Project between August 1, 2005 and August 31, 2006 (Fig. 1). The C8 Health Project was a cross-sectional survey of residents in the mid-Ohio River Valley who were exposed to high levels of PFOA via contaminated drinking water, as previously described (Frisbee et al., 2009). A subset of participants in the C8 Health Project who consented to future contact was subsequently invited in 2010 to participate in the Short-Term Follow-Up Study, which was conducted to evaluate changes in several clinical markers including lipids, in relation to changes in serum PFOA and PFOS concentrations (Fitz-Simon et al., 2013). Participation in the follow-up study was restricted to residents in the region affected by PFOA contamination who were between 20 and 60 years of age at the time of the C8 Health Project. Participants were ineligible if they had a history of cancer, were taking anti-inflammatory medications, or had an active or recent infection. The study population for the present analysis is comprised of these participants who provided a blood sample in both the C8 Health Project and the Short-Term Follow-Up Study. For purposes of this analysis, “at enrollment” refers to measurements made upon enrollment in the C8 Health Project in 2005–2006, and “at follow-up” refers to measurements

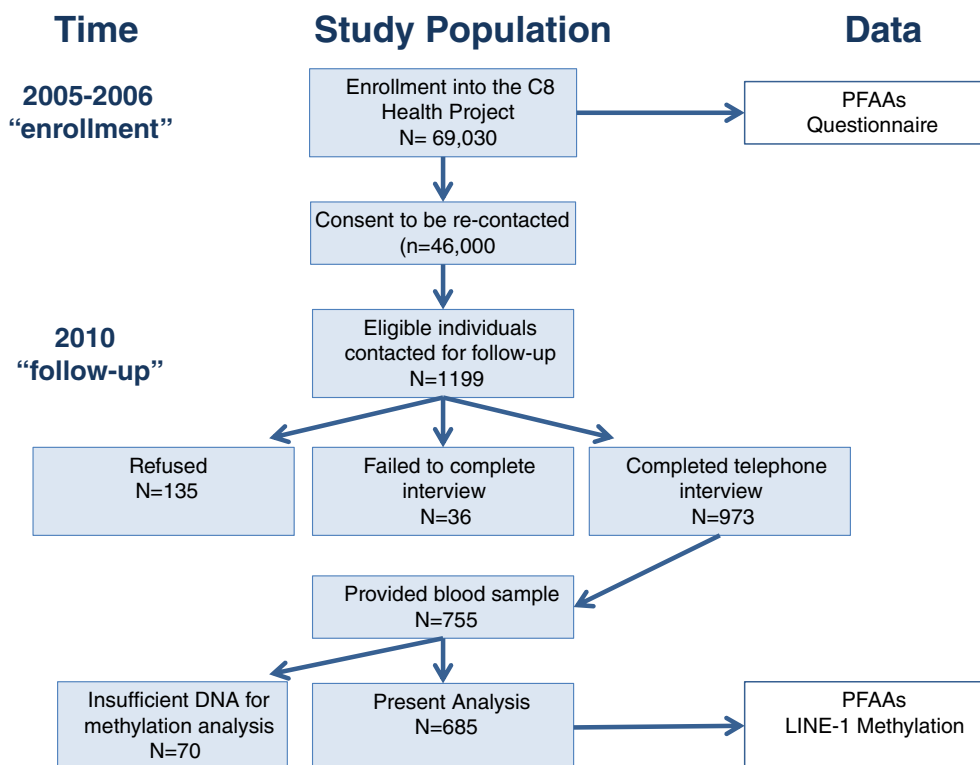


Fig. 1. Flow diagram describes our study population and data included in the present analysis. C8 Health Project participants were eligible if they lived in a region affected by PFOA contamination, were between 20 and 60 years of age at the time of the C8 Health, did not have a history of cancer, an active or recent infection, and were not taking anti-inflammatory medications.

made in 2010 (Fig. 1). All participants provided written informed consent prior to enrollment in both the C8 Health Project and the Short-Term Follow-Up Study, and the Brown University IRB approved this analysis.

2.2. Questionnaires

Upon enrollment in the C8 Health Project, participants completed self-administered questionnaires on demographics, personal health history, and lifestyle habits (Frisbee et al., 2009) and provided a venous blood sample. Information collected included age, gender, race (non-Hispanic white vs. other), household income ($\leq \$30,000$ vs. $> \$30,000$), education (some college or college graduate, yes vs. no), current drinker (yes vs. no), smoking status (ever vs. never) and regular exercise (yes vs. no). At follow-up we administered a questionnaire to obtain current participant information, including health and smoking status.

2.3. Blood sample handling

Participants were not required to fast prior to sample collection. Blood samples collected upon enrollment in the C8 Health Project (2005–2006) were centrifuged, divided into aliquots, and refrigerated until shipment to the laboratory for analysis. DNA samples from the time of enrollment into the C8 Health Project are not available. Blood samples for the Short-Term Follow-Up Study, collected between March and July of 2010, were clotted at room temperature for at least 30 min, and centrifuged for at least 15 min at 2400 rpm. Serum was transferred to polypropylene Nalgene vials and stored at $-30\text{ }^{\circ}\text{C}$ for up to 1 week before being shipped on dry ice to the Centers for Disease Control and Prevention (CDC). Blood clots (including buffy coats) were kept in the red-top serum separator tubes in which they were drawn, stored at $-30\text{ }^{\circ}\text{C}$ until shipment on dry ice to the National Institute for Occupational Safety and Health (NIOSH), and later shipped on dry ice to Brown University for DNA methylation analysis.

2.4. PFAA and lipid analysis

Laboratory analysis of PFAAs in blood samples collected at enrollment (Exxygen Research Inc., State College, Pennsylvania) has been previously described (Frisbee et al., 2009). In follow-up samples, PFOA and PFOS were measured using online solid phase extraction coupled with reversed-phase high-performance liquid chromatography separation and detection by isotope-dilution tandem mass spectrometry at the CDC (Kato et al., 2011). At both enrollment and follow-up, total cholesterol, HDL cholesterol, and triglycerides were measured enzymatically at a commercial accredited laboratory. LDL-C was calculated by the Friedewald equation when triglycerides were less than 400 mg/dl.

2.5. DNA methylation analysis

We used quantitative bisulfite PCR pyrosequencing to measure LINE-1 DNA methylation (Cash et al., 2011; Yang et al., 2004) in blood samples collected at follow-up. Genomic DNA was isolated from peripheral blood leukocytes using Puregene Blood Core Kit C with Clotspin Baskets to extract blood clots, and then sodium bisulfite treated using the EZ-96 DNA Methylation Kit, converting non-methylated cytosine residues into uracil (Zymo Research, Orange, CA). Bisulfite converted DNA was PCR amplified using HotStatTaq Polymerase (Qiagen Inc., Valencia, CA) and the product was pyrosequenced in triplicate. Each replicate provided a measure of methyl cytosine relative to the total cytosine and thymine (%) at 4 CpG sites in the LINE-1 region.

2.6. Statistical analysis

We measured percent LINE-1 DNA methylation in samples from 685 participants. Of these, complete covariate information was available on

671 participants. Average LINE-1 methylation across the 4 CpG sites for each replicate was used to calculate an average of the replicates for each sample. We averaged serum PFAA concentrations measured in samples collected at enrollment and at follow-up as a summary measure of PFAA body burden over the four to five years prior to methylation analysis (2005–2010).

We used linear regression to evaluate the association between mean serum concentrations of PFOA, PFOS, PFNA, and PFHxS and percent LINE-1 methylation. In initial analyses, we modeled serum PFAA concentrations as linear continuous variables and report the results for an interquartile range (IQR) shift of each PFAA. In sensitivity analyses, we repeated these same models considering instead the natural log of serum PFAA levels or tertiles of each PFAA as exposure measures. All models were adjusted for age, gender, body mass index (BMI), smoking status (ever/never), and current drinker (yes/no). In additional sensitivity analyses we stratified all models by gender, since men and women have been found to have differences in percent LINE-1 methylation (Zhu et al., 2012). We also evaluated models that included serum PFAAs measured at either enrollment or follow-up, as opposed to the average measure, as predictors of LINE-1 methylation.

Total cholesterol and LDL-C have been associated with PFAAs (Costa et al., 2009; Frisbee et al., 2010; Nelson et al., 2010; Olsen et al., 2003; Sakr et al., 2007; Steenland et al., 2009) and could also potentially be associated with LINE-1 methylation (Cash et al., 2011). However, the direction of these associations is not clear, such that total cholesterol and LDL-C could be downstream effects of both PFAA and epigenetic changes, in which case should not be included in the model, or they could be potential confounders. Accordingly, we did not adjust for total cholesterol or LDL-C in our primary analyses but then performed sensitivity analyses with additional adjustment for these variables.

In additional sensitivity analyses we excluded participants reporting a history of heart disease, diabetes, or currently taking lipid-lowering or antihypertensive medication. All analyses were conducted using SAS v. 9.3 (Cary, NC) and a 2-sided p -value of <0.05 was considered statistically significant.

3. Results

Characteristics of study participants are presented in Table 1. Serum concentrations of PFOA decreased substantially between enrollment in the C8 Health Project (2005–2006) and follow-up (2010) (Table 2).

Table 1
Participant characteristics by gender.

Characteristic	Men (<i>n</i> = 322)		Women (<i>n</i> = 363)	
At enrollment (2005–2006)				
Age, years - mean (SD)	41.8	(11.3)	41.5	(11.2)
White - <i>n</i> (%)	315	(97.8)	357	(98.4)
BMI, kg/m ² - mean (SD)	28.6	(5.2)	29.1	(7.5)
Ever smoker - <i>n</i> (%)	149	(46.3)	135	(37.2)
Current drinker - <i>n</i> (%)	198	(61.5)	193	(53.5)
Regular exercise - <i>n</i> (%)	102	(31.7)	101	(27.8)
Household income <\$30,000 - <i>n</i> (%)	80	(26.5)	94	(28.6)
At least some college - <i>n</i> (%)	196	(61.3)	246	(68.3)
Total cholesterol, mg/dl - mean (SD)	198	(43.6)	202	(41.0)
HDL-C, mg/dl - mean (SD)	42.8	(10.2)	54.9	(14.6)
LDL-C, mg/dl - mean (SD)	113	(36.6)	115	(35.1)
Heart disease - <i>n</i> (%)	17	(5.3)	9	(2.5)
Diabetes mellitus - <i>n</i> (%)	19	(5.9)	24	(6.6)
Lipid lowering medication - <i>n</i> (%)	53	(16.5)	51	(14.1)
Antihypertensive medication- <i>n</i> (%)	52	(16.2)	72	(19.8)
Neutrophils, % - mean (SD)	61.5	(8.3)	61.8	(8.2)
Lymphocytes, % - mean (SD)	28.8	(7.1)	29.9	(7.3)
At follow-up (2010)				
Total cholesterol, mg/dl - mean (SD)	189	(41.4)	200	(40.5)
HDL-C, mg/dl - mean (SD)	41.4	(11.1)	54.2	(13.9)
LDL-C, mg/dl - mean (SD)	108	(36.5)	113	(33.7)
LINE-1 methylation, % - mean (SD)	84.05	(1.27)	83.61	(1.19)

Table 2

Serum PFAA concentrations at C8 Health Project enrollment (2005–2006) and at follow-up (2010).

	Serum PFAAs at enrollment (2005–2006)		Serum PFAAs at follow-up (2010)		Mean serum PFAAs (2005–2010)	
	GM (GSD) ^a ng/ml		GM (GSD) ng/ml		GM (GSD) ng/ml	
All participants (n = 685)						
PFOA	79.3	(3.2)	32.9	(3.5)	57.9	(3.2)
PFOS	19.0	(2.1)	8.5	(2.3)	14.1	(2.0)
PFNA	1.4	(1.7)	1.3	(1.7)	1.4	(1.6)
PFHxS	3.1	(2.3)	1.9	(2.3)	2.6	(2.1)
Men (n = 322)						
PFOA	93.3	(3.2)	41.9	(3.5)	70.0	(3.2)
PFOS	22.4	(2.2)	10.9	(2.3)	17.1	(2.1)
PFNA	1.5	(1.6)	1.4	(1.7)	1.5	(1.6)
PFHxS	4.0	(2.2)	2.5	(2.2)	3.3	(2.1)
Women (n = 363)						
PFOA	68.7	(3.1)	26.5	(3.3)	48.9	(3.0)
PFOS	16.4	(2.0)	6.8	(2.1)	11.9	(1.9)
PFNA	1.2	(1.7)	1.2	(1.7)	1.3	(1.5)
PFHxS	2.6	(2.2)	1.5	(2.2)	2.1	(2.0)

^a GM = geometric mean, GSD = geometric standard deviation.

Serum concentrations of PFOS also decreased during this time period among participants. Consistent with previous studies, LINE-1 methylation differed by gender, with women having on average 0.45 lower percent LINE-1 methylation compared to men ($p = <0.0001$). LINE-1 methylation was not associated with age, BMI, drinking alcohol, or ever being a smoker (Supplementary Material, Table S1).

LINE-1 methylation was not significantly associated with PFOA in any analyses (Table 3, Fig. 2). For example, an IQR increase in serum PFOA was associated on average with a 0.04 decrease in percent LINE-1 methylation ($p = 0.16$) after adjustment for age, gender, BMI, smoking status, and drinking status.

In contrast, we observed 0.20 higher percent LINE-1 methylation per IQR increase in mean serum PFOS ($p = 0.001$), after adjusting for covariates (Table 3). Results were qualitatively similar when we instead considered natural log-transformed serum PFOS (see Supplementary Material, Table S2). We also observed a monotonic increase in LINE-1 methylation in tertiles of serum PFOS after controlling for covariates (p for trend = 0.02; Fig. 2).

There was inconsistent evidence of PFNA being positively associated with LINE-1 methylation. In the primary analysis modeled as continuous variables (Table 3) the adjusted coefficient was associated with a p -value of 0.19, but after log-transformation (see Supplementary Material, Table S2), the association was slightly stronger and in analyses considering tertiles of serum PFNA we observed a monotonic increase in LINE-1 methylation (p -value for trend = 0.02, Fig. 2). Serum concentrations of PFHxS were not associated with LINE-1 methylation in any analysis.

Table 3

Associations between mean serum PFAA concentrations and percent LINE-1 methylation^a.

	Unadjusted (n = 685)			Adjusted ^b (n = 671)		
	Difference in % LINE-1 methylation (SE)	p -value		Difference in % LINE-1 methylation (SE)	p -value	
PFOA (ng/ml)	−0.014 (0.029)	0.64		−0.041 (0.029)	0.16	
PFOS (ng/ml)	0.265 (0.054)	<0.0001		0.204 (0.058)	0.001	
PFNA (ng/ml)	0.102 (0.048)	0.03		0.064 (0.048)	0.19	
PFHxS (ng/ml)	0.056 (0.035)	0.11		0.020 (0.036)	0.57	

^a Expressed as the difference in % LINE-1 methylation per interquartile range increase (IQR) in mean concentrations of each PFAA. PFOA IQR = 106 ng/ml, PFOS IQR = 12 ng/ml, PFNA IQR = 0.8 ng/ml, PFHxS IQR = 2.6 ng/ml.

^b Adjusted for age, gender, BMI, smoking status (ever/never), and current drinker (yes/no).

Results were similar in sensitivity analyses additionally adjusting for total cholesterol or LDL-C, percent lymphocytes, percent neutrophils, or excluding participants reporting a history of heart disease, or taking lipid lowering medication (data not shown). Gender stratified results (see Supplementary Material, Table S3) and results from models evaluating serum PFAAs measured at either enrollment or follow-up (data not shown), or models evaluating each of the four measured CpG sites individually (data not shown), were not substantially different from those observed in our main analyses.

4. Discussion

We examined cross-sectional associations between PFAAs measured in serum at two different time points and LINE-1 DNA methylation among participants in the C8 Health Project, a large epidemiologic study of a community highly exposed to PFOA. In summary, we observed consistent positive associations between percent LINE-1 methylation and serum PFOS concentrations, but no significant association with PFOA, PFNA, or PFHxS.

The fall in serum PFOA and PFOS by a little over 50% between enrollment and follow-up may have played a role in the observed associations. Local population intake of PFOA fell during the study period, presumably due to efforts to reduce plant emissions of PFOA, provision of bottled drinking water and the installation of filtration for local water supplies beginning in 2007. The decrease in serum PFOS concentrations is similar to that observed in the US population overall (Kato et al., 2011), likely due to the cessation of production of PFOS by US manufacturers during this time. If there were a relationship between either PFOA and PFOS and methylation, and it was reversible, this pattern over time might complicate observed relationships depending on delays in the response to changing serum levels. However, since results were more or less identical using the measures at enrollment or follow up, this suggests that the falling serum levels do not have an impact on LINE-1 methylation.

We are aware of only one prior study to consider the association between PFAAs and DNA methylation in humans. Guerrero-Preston et al. (2010) reported a negative association between PFOA concentrations and a marker of global DNA methylation in cord blood, while we observed some evidence of a negative, non-significant association between LINE-1 methylation and serum PFOA level (Table 3). However, Guerrero-Preston et al. (2010) measured total methylated cytosine while we evaluated LINE-1 DNA methylation, two methods which are not directly comparable (Nelson et al., 2011). In addition, Guerrero-Preston et al. (2010) examined cord blood serum from newborns and selected study participants based on smoking status, while we looked at peripheral blood leukocytes from adults in a community-based study, further complicating comparisons between these studies.

A recent analysis of gene expression among a subset of the population studied here suggests that serum PFOA and PFOS concentrations are associated with gender-specific changes in the expression of genes related to cholesterol transport (Fletcher et al., 2013). However, whether these associations may be mediated by changes in DNA methylation or other epigenetic mechanisms remains unknown.

We observed associations between percent LINE-1 methylation and other covariates in our data similar to those previously reported in other studies. For example, women in our study had approximately 0.45 lower percent LINE-1 methylation compared to men (Table S1), consistent with gender differences reported in prior studies (Cash et al., 2011; Cash et al., 2012; Zhu et al., 2012). Our observed weak, non-significant associations between LINE-1 methylation and age, BMI, smoking status, drinking status, and the proportion of specific leukocyte types present (data not shown) were also consistent with previous reports (Cash et al., 2011; Jintaridh and Mutirangura, 2010; Terry et al., 2011; Zhu et al., 2012).

Interestingly, although our study population was highly exposed to PFOA, LINE-1 methylation was not associated with PFOA concentrations

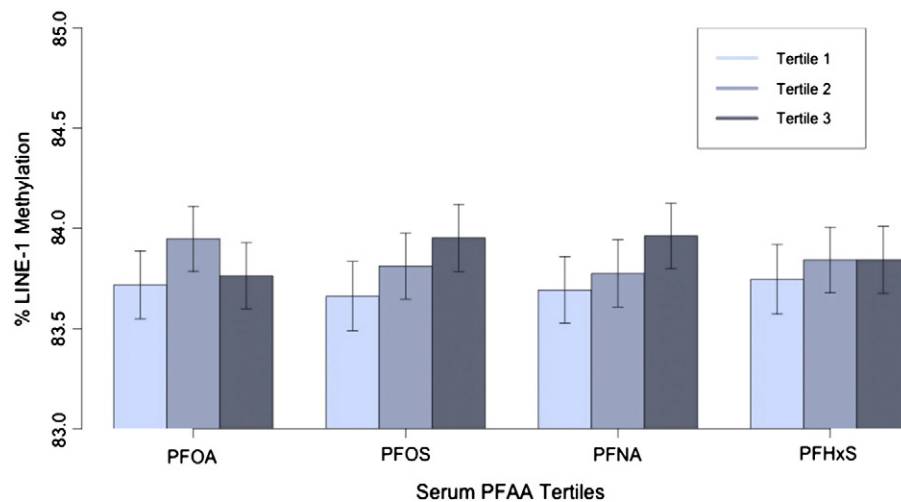


Fig. 2. Mean percent LINE-1 methylation by serum PFAA tertiles adjusted for covariates. Error bars represent 95% confidence intervals. *P*-value for trend: PFOA = 0.71, PFOS = 0.02, PFNA = 0.02, PFHxS = 0.44. Minimum and maximum concentrations in each PFAA tertile (T): PFOA: T1 = 0.8–33.4, T2 = 33.5–91.1, T3 = 91.2–1689; PFOS: T1 = 0.17–11.7, T2 = 11.8–18.7, T3 = 18.8–64.1; PFNA: T1 = 0.15–1.2, T2 = 1.2–1.6, T3 = 1.7–10.0; PFHxS: T1 = 0.15–1.9, T2 = 2.0–3.5, T3 = 3.6–68.3.

in serum, but was positively associated with serum PFOS. Differences in the magnitude of exposure to the different PFAAs among this population could potentially explain these observations. For example, if PFAAs affect DNA methylation only at relatively low serum concentrations, there would be little variability in LINE-1 methylation in relation to PFOA since many of the participants were highly exposed. Differences in the chemical properties of PFOA and PFOS may be a possible explanation, since studies suggest that PFOA and PFOS may differentially bind to plasma lipoproteins (Butenhoff et al., 2012), activate PPARs (Bjork and Wallace, 2009; Bjork et al., 2011), and affect gene expression (Fletcher et al., 2013). It is also interesting that we observed a positive relationship between PFOS and LINE-1 methylation, whereas in many studies, decreased methylation of LINE-1 regions has generally been associated with poorer health outcomes and various environmental exposures. We are unable to provide an explanation for this inconsistency since so little is known regarding potential relationships between PFAAs and DNA methylation.

Our study has other limitations. First, we did not have DNA samples, and thus information on LINE-1 methylation, from participants at the time of enrollment into the C8 Health Project. As a result, we were not able to evaluate temporal changes in LINE-1 methylation, relationships with PFAA exposure over time, or cross-sectional relationships with participant information collected at enrollment. Second, if PFAAs affect DNA methylation primarily through activation of PPARs, DNA methylation in the promoter regions of specific genes activated by PPARs may provide a more precise measure of changes related to PFAA exposure and should be explored in future studies. Third, LINE-1 methylation differs across various types of leukocytes (Zhu et al., 2012). If PFAA exposure is associated with changes in the types of leukocytes present in peripheral blood, then leukocyte type may confound associations between serum PFAAs and LINE-1 methylation. Unfortunately, differential leukocyte information for the DNA samples used in this analysis, which were collected at follow-up, was not available. As an alternative, we controlled for the percent lymphocytes or percent neutrophils measured in samples collected at enrollment and our results were not materially different. Finally, we had limited health information from the time of follow-up, and most covariates were self-reported, potentially leading to some measurement error and residual confounding.

Nonetheless, strengths of this study includes the evaluation of a novel hypothesis in a large, community-based sample of individuals exposed to high levels of PFOA and background levels of other PFAAs.

5. Conclusion

In this community-based sample we found that mean serum levels of PFOS were associated with LINE-1 DNA methylation, suggesting that this compound may be epigenetically active. More research is needed to further elucidate the mechanisms by which epigenetics may be involved in PFAA exposure related disease, such as exploring gene-specific methylation in the affected tissues and studying the effects of PFAA exposure on DNA methylation in different populations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.envint.2013.10.018>.

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