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Authors
Huen, K
Yousefi, P
Bradman, A
et al.

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Effects of Age, Sex, and Persistent Organic Pollutants on DNA Methylation in Children

Karen Huen,1* Paul Yousefi,1 Asa Bradman,1 Liying Yan,2 Kim G. Harley,1 Katherine Kogut,1 Brenda Eskenazi,1 and Nina Holland1

1Center for Children’s Environmental Health, School of Public Health, University of California, Berkeley, California
2EpigenDx, Hopkinton, Massachusetts

Epigenetic changes such as DNA methylation may be a molecular mechanism through which environmental exposures affect health. Methylation of Alu and long interspersed nucleotide elements (LINE-1) is a well-established measure of DNA methylation often used in epidemiologic studies. Yet, few studies have examined the effects of host factors on LINE-1 and Alu methylation in children. We characterized the relationship of age, sex, and prenatal exposure to persistent organic pollutants (POPs), dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), and polybrominated diphenyl ethers (PBDEs), with DNA methylation in a birth cohort of Mexican-American children participating in the CHAMACOS study. We measured Alu and LINE-1 methylation by pyrosequencing bisulfite-treated DNA isolated from whole blood samples collected from newborns and nine-year old children (n = 358). POPs were measured in maternal serum during late pregnancy. Levels of DNA methylation were lower in nine-year olds compared to newborns and were higher in boys compared to girls. Higher prenatal DDT/E exposure was associated with lower Alu methylation at birth, particularly after adjusting for cell type composition (P = 0.02 for o,p’-DDT). Associations of POPs with LINE-1 methylation were only identified after examining the co-exposure of DDT/E with PBDEs simultaneously. Our data suggest that repeat element methylation can be an informative marker of epigenetic differences by age and sex and that prenatal exposure to POPs may be linked to hypomethylation in fetal blood. Accounting for co-exposure to different types of chemicals and adjusting for blood cell types may increase sensitivity of epigenetic analyses for epidemiological studies. Environ. Mol. Mutagen. 55:209–222, 2014. ©2013 Wiley Periodicals, Inc.

Key words: Alu; LINE-1; epigenetics; DDT; DDE; PBDE; co-exposure

INTRODUCTION

Epigenetic mechanisms are heritable changes that regulate gene expression without DNA sequence modifications and include DNA methylation, histone modifications, and noncoding RNAs. Their alterations represent likely molecular mechanisms linking environmental exposures with adverse health effects. Determining DNA methylation levels of retrotransposons, Alu, and long interspersed nucleotide elements (LINE-1) has become a popular approach for assessment of epigenetic modifications in epidemiologic studies because this method is informative, has relatively high throughput, and is cost effective.

There are approximately 1.4 million Alu repetitive elements and a half million LINE-1 elements interspersed throughout the human genome and their methylation represents up to 50% of global genomic methylation [Kazazian and Goodier, 2002; Yang et al., 2004]. Measurement of Alu and LINE-1 repeat methylation has previously been referred to as a marker of global DNA methylation [Bollati et al., 2007; Rusiecki et al., 2008]. However, recent studies do not show a correlation between LINE-1 or Alu methylation with global genomic methylation content in non-cancer cells [Wang et al., 2010; Price et al., 2012]. Furthermore, LINE-1 and Alu are not correlated with each other [Hou et al., 2010; Gao et al., 2012] and...
have differential susceptibility to environmental exposures, meaning that the methylation states in response to an exposure may vary between Alu and LINE-1 elements [Baccarelli et al., 2009; Pavanello et al., 2009; Tarantini et al., 2009; Wright et al., 2010]. These recent findings suggest that LINE-1 and Alu methylation each represent distinct measures of methylation in different parts of the methylome [Price et al., 2012; Alexeeff et al., 2013].

Differences in DNA methylation, the most extensively studied type of epigenetic modification, have been associated in adults with health outcomes ranging from asthma to cancer and to a variety of environmental exposures such as benzene, traffic pollution, and persistent organic pollutants (POPs), such as dichlorodiphenyl trichloroethane (DDT) and polychlorinated biphenyls (PCBs) [Bollati et al., 2007; Hsiung et al., 2007; Rusiecki et al., 2008; Baccarelli and Bollati, 2009; Perera et al., 2009; Kim et al., 2010]. Recent evidence also suggests that DNA methylation may be a key mechanism mediating fetal origins of adult disease. Dolinoy et al. [2007] demonstrated that prenatal exposure to Bisphenol A in agouti mice resulted in hypomethylation of a retrotransposon upstream of the agouti gene that shifted coat color of the offspring, providing compelling evidence that in utero exposure can affect phenotype through epigenetic mechanisms including methylation of retrotransposable elements. Additionally, prenatal exposures to tobacco smoke, famine, and lead have been associated with differences in site specific and/or repetitive element methylation in humans [Breton et al., 2009; Wright et al., 2010; Tobi et al., 2012]. Although two small studies in adults found a relationship between concurrent exposure to POPs, DDT, and PCBs, with hypomethylation of Alu repeats [Rusiecki et al., 2008; Kim et al., 2010], no previous study has examined the relationship of prenatal exposure to POPs and DNA methylation.

DDT is lipophilic, bioaccumulates in tissues, and has a long half-life in the human body. Although DDT use was banned in 1972 in the US, DDT use continued in Mexico for malaria control through 2000 and is currently used for malaria control in a number of countries particularly in Africa. Early life exposure to DDT and its breakdown product dichlorodiphenyldichloroethylene (DDE) have been associated with lowered birthweight and shortened gestation in some studies, [Al-Saleh et al., 2012; Kezios et al., 2013], poorer neurodevelopment in children [Eskenazi et al., 2009], and breast cancer in adults [Cohn et al., 2007].

Another class of POPs includes the polybrominated diphenyl ethers (PBDEs). These flame retardants have been used in the manufacture of common household items including furniture, infant products, and electronics. Originally issued in the 1970s, California’s Technical Bulletin (TB 117) required that furniture, baby products, and other items resist open flames. The penta-BDEs (comprised of congeners BDEs -47, -99, -100, and -153), the compounds most commonly used to comply with TB-117 in products containing foam, were phased out in 2004 but continue to leach into the environment from older household items. Children living in California have higher serum PBDE levels compared to children living in other areas of the United States [Windham et al., 2010; Bradman et al., 2012], as well as children from Mexico and Europe [Rose et al., 2010; Eskenazi et al., 2011]. Exposure to penta-PBDE has been related to lowered fertility [Harley et al., 2010], effects on thyroid hormone [Chervier et al., 2010], and poorer neurodevelopment [Chao et al., 2011; Gascon et al., 2012; Eskenazi et al., 2013].

Although studies of environmental exposure often focus on one chemical or one class of compounds, exposures to toxicants do not generally occur in isolation. For instance, pregnant women participating in the National Health and Nutrition Examination Survey (NHANES) had measurable levels of multiple chemicals, many of which were present in almost all women (e.g. phenols, phthalates, organochlorines, and PBDEs) in their blood and urine [Woodruff et al., 2011]. Furthermore, exposure to one chemical may influence how another chemical can produce health effects [Carpenter et al., 2002]. The relationship of co-exposure to DDT/E and PBDEs on repeat element methylation has not yet been examined. However, one in vivo study of steroid secretion found that the effect of PBDEs was dependent on DDT concentration [Gregoraszczuk et al., 2008] suggesting that considering co-exposure to both chemicals may be important.

The purpose of the present study is to examine the relationship of prenatal exposure to DDT, DDE, and penta-BDEs and DNA methylation of Alu and LINE-1 repetitive elements in fetal and child blood from participants of the Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS), a longitudinal birth cohort study. Although differences in levels of DNA methylation with age and sex have not been previously examined in children, a study in adults suggested that methylation of Alu and LINE-1 repeats decreases with age and that males have lower Alu but higher LINE-1 methylation compared to females [Zhu et al., 2012]. Another study of adults also reported higher LINE-1 methylation in males but found no association of repeat element methylation with age [Zhang et al., 2011]. Thus, for this study, we examine the effects of prenatal POP exposure, including co-exposure to both classes of compounds, as well as age and sex on Alu and LINE-1 DNA methylation in CHAMACOS children.

**MATERIALS AND METHODS**

**Study Subjects**

The CHAMACOS study aims to examine the effects of pesticides and other environmental exposures in a population of pregnant women...
DNA Methylation in Children

527 CHAMACOS Newborns
336 CHAMACOS 9 Year Olds

112 DNA Methylation Only at Birth
134 DNA Methylation at Birth and Age 9
219 DNA Methylation at Birth and Prenatal DDT/E
218 DNA Methylation at Birth and Prenatal PBDEs
219 DNA Methylation at Age 9 and Prenatal DDT/E
201 DNA Methylation at Age 9 and Prenatal PBDEs

Fig. 1. Schematic of CHAMACOS children included in this study.

and children living in the agricultural Salinas Valley, California. Women were eligible for enrollment if they were at least 18 years of age, at less than 20 weeks gestation, Spanish or English speaking, eligible for low-income health insurance, receiving prenatal care at one of the local community clinics, and planning to deliver at the public hospital. Six hundred and one pregnant women were enrolled in 1999–2000 and 527 delivered liveborn singleton newborns [Eskenazi et al., 2003]. CHAMACOS women were interviewed by bilingual, bicultural interviewers near the end of the first (~13 weeks gestation) and second (~26 weeks gestation) trimesters of pregnancy. Information was obtained on sociodemographic characteristics, mother’s reproductive and medical history, prenatal lifestyle exposures, diet, occupational and residential history, exposures to pesticides and other environmental chemicals, and housing quality.

Prenatal POP concentrations were measured in blood specimens collected from mothers during pregnancy (approximately ~27 weeks gestation) or at delivery (if insufficient blood quantity was collected during pregnancy; n = 14 and 40 for DDT/E and PBDEs, respectively) [Harley et al., 2008]. Alu and LINE-1 DNA methylation was measured in blood samples collected from children at delivery (umbilical cord blood representing fetal blood) and when they were nine years old (mean = 9.3 years, SD = 0.3). Our study sample included a total of 358 children who had DNA samples available for methylation analysis at birth and/or age 9. Of these children, 134 had samples available at both time points, 112 had samples only at birth, and 112 has sample only at age 9 (Fig. 1). Furthermore among those children with DNA samples available, prenatal exposure measurements for DDT/E and PBDEs were available for 219 and 218 children at birth and 219 and 201 children at age 9, respectively. Children who were born full term were more likely to have samples and measurements of methylation available at birth. Children who were born full term and whose mothers were older during pregnancy were more likely to have samples and measurements of methylation available at age nine compared to other children in the CHAMACOS cohort. For both time points (birth and nine years), children included in the study did not differ from all children in the cohort by demographic and exposure variables (e.g., poverty level, marriage status, maternal BMI, type of work during pregnancy, alcohol and smoking intake during pregnancy, prenatal exposure to DDT/DDE and PBDEs). Study protocols were approved by the University of California, Berkeley Committee for Protection of Human Subjects. Written informed consent was obtained from all mothers and assent was provided by the children at the nine-year assessment.

Blood Collection and Processing

Whole blood was collected in BD vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) containing no anticoagulant, centrifuged, divided into serum and clot, and stored at ~80°C.

PBDE and DDT/E Measurements

PBDE congeners were measured in serum samples using gas chromatography isotope dilution high resolution mass spectrometry (GC-IDHMRMS) [Sjodin et al., 2004] and included BDE-17, -28, -47, -66, -85, -99, -100, -153, -154, and -183. We focus on the four congeners comprising penta-BDEs—BDE-47, -99, -100, and -153 since these were the only congeners to be detected in almost all mothers (>97% detection frequency). As previously reported, BDE-47 had the highest mean concentration of the congeners measured (Table 1). [Castronie et al., 2011; Bradman et al., 2012; Eskenazi et al., 2013]. PBDE concentrations are expressed on a serum lipid basis (nanograms per gram lipids). Total serum lipid concentrations were determined based on the measurement of triglycerides and total cholesterol using standard enzymatic methods (Roche Chemicals, Indianapolis, IN) [Phillips et al., 1989]. We observed a range of LODs rather than one single value based on differences in total serum lipid concentrations per individual. LODs ranged from 0.2 to 0.7 ng/g lipids for all four congeners with the exception of BDE-47, which ranged from 0.3 to 2.6 ng/g lipids. Levels below the LOD with a detectable signal were coded with the instrument concentration obtained. Data below the LOD with no detectable signal were imputed from a log-normal probability distribution [Lubin et al., 2004]. Serum levels of PBDE congeners in women with measurements at both delivery and 26 weeks gestation (n = 21) were strongly correlated (r ≥ 0.98, P < 0.001) [Eskenazi et al., 2013]. QA/QC procedures included the use of blanks and spiked quality control samples in each set of unknowns. Eleven organochlorine compounds including p,p'-DDT, o,p'-DDT, and p,p'-DDE (DDT/E) were measured in serum samples using gas chromatography mass spectrometry [Barr et al., 2003]. As described for PBDEs, serum DDT/E concentrations were also lipid adjusted (nanograms per gram) by dividing o,p'-DDT, p,p'-DDT, and p,p'-DDE on a wholeweight basis by total serum lipid content [Phillips et al., 1989]. The lipid adjusted limits of detection ranges in ng/g lipid were as follows: 0.06–0.76 for o,p'-DDT, 0.06–1.36 for p,p'-DDT, and 0.06–1.36 for p,p'-DDE. Detection frequency for o,p'-DDT was 95.8% and p,p'-DDT and p,p'-DDE were measurable in all samples. Levels below
the limit of detection (LOD) were assigned the value of LOD/2. We did not impute values as we did for PBDEs because so few readings were below the limit of detection. As previously reported, median material serum levels of $p,p'$-DDE, $p,p'$-DDT, and $o,o'$-DDT were 1013.2, 12.1, and 1.2 ng/g lipid, respectively (Table I) [Eskenazi et al., 2006; Fenster et al., 2006; Harley et al., 2008; Weldon et al., 2010; Warner et al., 2013]. Among women with DDT/E measurements at both 26 weeks gestation and at delivery ($n = 20$), concentrations were highly correlated ($r > 0.98$) [Eskenazi et al., 2006]. Serum concentrations of DDT and DDE isomers were strongly correlated with each other ($r = 0.8–0.9$, $P < 0.0005$).

**DNA Methylation Analyses**

DNA was isolated from clots as described previously [Holland et al., 2006] using a Qiamp Blood DNA Maxi kit (Qiagen, Inc., Santa Clarita, CA). Bisulfite conversion of DNA (500 ng) was performed using EpiTect Bisulfite Conversion Kits (Qiagen, Germantown, MD) and eluted into 20 μL. Elution buffer. Bisulfite DNA conversion efficiency (99%) was confirmed by using a non-CpG cytosine residue as an internal control. Alu and LINE-1 methylation status was analyzed by pyrosequencing of PCR-amplified and bisulfite-treated DNA samples using the Pyromark Q96MD System (Qiagen) as previously described [Yang et al., 2004; Royo et al., 2007]. Each of the Alu and LINE-1 assays reports four CpG sites for all Alu and LINE-1 repeats across the genome. The previously published method for Alu as described by Yang et al. [2004] reported only three CpG sites; however, as pyrosequencing read lengths have improved since that time, we were able to report methylation levels at the same three sites and also include one additional site. Repetitive element methylation (%5-mC) was calculated using Pyro Q-CpG Software (Qiagen). All samples were run in triplicate for each time-point/subject.

Stringent quality control criteria were applied for handling of all samples and DNA methylation data. The quality assurance procedures included use of repeats, internal standards, and positive and negative controls to minimize technical variability. To minimize batch variability, all sample plates were run on the same day. Additionally, all plates contained randomized encoded samples from different age groups in order to minimize experimental bias. The coefficient of variation (CV) for Alu and LINE-1 triplicate measures were 5 and 3%, respectively, and CV's for intraplate replicates were essentially the same.

**Differential Cell Count**

To examine the relationship of blood cell composition with repeat element methylation, we performed differential cell counts in a subset of 103 umbilical cord blood samples. Heparinized whole blood smears

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**TABLE I. Demographic and Exposure Characteristics of CHAMACOS participants**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>%</th>
<th>Median (IQR)</th>
<th>Min</th>
<th>Max</th>
</tr>
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<tbody>
<tr>
<td>Child sex</td>
<td></td>
<td></td>
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<tr>
<td>Boy</td>
<td>179</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Girl</td>
<td>179</td>
<td>50</td>
<td></td>
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<tr>
<td>Child gestational age at birth</td>
<td></td>
<td></td>
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<tr>
<td>≥ 37 weeks</td>
<td>338</td>
<td>94</td>
<td></td>
<td></td>
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<tr>
<td>34–36 weeks</td>
<td>20</td>
<td>6</td>
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<tr>
<td>Child birthweight</td>
<td></td>
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<td></td>
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<tr>
<td>Normal birthweight (≥2500 g)</td>
<td>347</td>
<td>97</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Low birthweight (&lt;2500 g)</td>
<td>11</td>
<td>3</td>
<td></td>
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<tr>
<td>Child BMI at 9 years</td>
<td></td>
<td></td>
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<tr>
<td>Normal (&lt;85th percentile)</td>
<td>111</td>
<td>46</td>
<td></td>
<td></td>
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<tr>
<td>Overweight (&gt;85th, &lt;95th percentile)</td>
<td>39</td>
<td>16</td>
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<td>Obese (≥95th percentile)</td>
<td>92</td>
<td>38</td>
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<tr>
<td>Household poverty at pregnancy</td>
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<td>At or below poverty level</td>
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<td>Within 200% poverty level</td>
<td>121</td>
<td>34</td>
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<tr>
<td>Above poverty level</td>
<td>13</td>
<td>4</td>
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<tr>
<td>Maternal age at pregnancy</td>
<td></td>
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<tr>
<td>18–24</td>
<td>171</td>
<td>48</td>
<td></td>
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<tr>
<td>25–29</td>
<td>105</td>
<td>29</td>
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<tr>
<td>30–34</td>
<td>53</td>
<td>15</td>
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<td>35–45</td>
<td>28</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maternal DDT/DDE exposure during pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p,p'$-DDE (ng/g lipid)</td>
<td>332</td>
<td>1013.2 (547.6-2,616.4)</td>
<td>48.8</td>
<td>159,303.3</td>
<td></td>
</tr>
<tr>
<td>$p,p'$-DDT (ng/g lipid)</td>
<td>332</td>
<td>12.1 (6.5–33.6)</td>
<td>1.6</td>
<td>3,3174.0</td>
<td></td>
</tr>
<tr>
<td>$o,o'$-DDT (ng/g lipid)</td>
<td>330</td>
<td>1.2 (0.7-3.1)</td>
<td>0.0</td>
<td>1,878.1</td>
<td></td>
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<tr>
<td>Maternal PBDE exposure during pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDE SUM (ng/g lipid)</td>
<td>310</td>
<td>24.7 (13.4–42.0)</td>
<td>2.6</td>
<td>1293.7</td>
<td></td>
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<tr>
<td>BDE-153 (ng/g lipid)</td>
<td>310</td>
<td>2.1 (1.3-3.8)</td>
<td>0.1</td>
<td>96.9</td>
<td></td>
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<tr>
<td>BDE-100 (ng/g lipid)</td>
<td>310</td>
<td>2.5 (1.5-4.7)</td>
<td>0.2</td>
<td>138.3</td>
<td></td>
</tr>
<tr>
<td>BDE-99 (ng/g lipid)</td>
<td>310</td>
<td>4.0 (2.3–7.1)</td>
<td>0.2</td>
<td>297.6</td>
<td></td>
</tr>
<tr>
<td>BDE-47 (ng/g lipid)</td>
<td>310</td>
<td>15.5 (8.0-26.7)</td>
<td>0.5</td>
<td>761.0</td>
<td></td>
</tr>
</tbody>
</table>

*aTotal number of observation vary due to missing data.

*bOf the 358 children in this study, only 246 were assessed for DNA methylation at age 9, and of those 242 had 9yr anthropometric data available.

OCs – organochlorines
PBDEs – polybrominated diphenyl ethers
were prepared with the “gold standard” Wright-Push blood smearing technique [Houwen, 2001] and stained utilizing a DiffQuik® staining kit. Slides were fixed for 15 minutes at 23°C, stained in both the basophilic dye and eosinophilic dye for five seconds each and washed after each staining period. Slides were scored under light microscopy (Zeiss Axio- plan) with a magnification lens of 1,000 × and oil immersion. At least 100 cells were scored for each slide and a percentage of each cell type was used for data analysis. To ensure consistency and reproducibility of scoring, 100 cells were scored in sets of 3 (3*100 = 300) for a subset of samples (N = 35). The CV for the repeat scoring in this subset was less than 10%.

### Statistical Analyses

We calculated Pearson’s correlation coefficient to examine the correlation between methylation measurements at each of the four positions used for Alu and LINE-1 methylation assays. To determine the correlations between different measures of DDT/E and PBDE measurements, we also calculated Pearson’s correlation coefficients. Exposure measurements were log_{10} transformed to approximate a normal distribution.

We performed mixed effect regression models to determine the relationship of age, sex, and exposure with LINE-1 and Alu repeat methylation. Mixed effects modeling allows us to account for the correlated measures of methylation among the four adjacent CpG positions and for the triplelicate measures at each position per individual yielding a global estimate for the association of variables of interest (age, sex, and exposure) with DNA methylation. Mixed models have been used previously to model the associations of exposures with repeat element methylation [Byun et al., 2013]. The following model was used:

\[ Y_{ijk} = b_0 + b_1 X_1 + \cdots + b_n X_n + z_{ij} + \epsilon_{ijk} \]

where \( Y_{ijk} \) is the methylation level (Alu or LINE-1) for the \( i \)-th subject (\( i = 1, \ldots, 358 \)) at the \( j \)-th CpG position (\( j = 1, \ldots, 4 \)), and the \( k \)-th replicate (\( k = 1, \ldots, 3 \)). \( X_2, \ldots, X_n \) and \( b_2, \ldots, b_n \) represent the covariates and their corresponding slopes and for various models including variables like sex and cell count. The sum of \( b_0 \) and \( z_{ij} \) represents the random intercept for the subject \( i \) and the sum of \( b_1 \) and \( z_{ij} \) are the random slope for the \( j \)-th subject and the \( j \)-th CpG position. \( \epsilon_{ijk} \) is the residual error term.

To assess the differences in DNA methylation by age, we used mixed modeling to compare methylation levels in fetal and nine-year-old DNA among children with methylation measures at both time points (\( n = 134 \)). We also modeled the difference by age in all children, including those that did not have matched data at both time points (\( n = 358 \) children). To examine the relationship of sex with DNA methylation, we also performed mixed modeling for Alu and LINE-1 methylation (dependent variables) while including sex as a covariate in the model. Each age group (birth and nine years) was modeled separately. We repeated these models in a subset of newborns that also had differential cell counts available to adjust for cell type distribution. In these models, we included percent lymphocytes, monocytes, basophils, and eosinophils in the models and used the percent neutrophils as the baseline.

Mixed modeling was also used to determine the association of DDT/E and PBDE exposure measurements with repeat element DNA methylation (Alu and LINE-1). Separate models were performed for each of the three organochlorine compounds (\( \mu \)-DDT, \( \alpha \)-DDT, and \( \mu \)-DDE), the four penta-BDE congeners (BDE -47, -99, -100, and -153), and the sum of the four penta-BDE congeners at each time point separately (birth and nine years). Extreme outliers (>3 SD from mean) for \( \mu \)-DDT, \( \alpha \)-DDT, and \( \mu \)-DDE, PBDE congeners were not included in the models. Sex was included as a covariate in the model. We also used univariate models to identify other potential covariates associated with Alu or LINE-1 methylation (i.e., maternal age, maternal smoking during pregnancy, etc.); however, none were statistically significant. In additional models, we included interaction terms for sex and exposure (DDT/E or PBDEs). To consider the potential interaction between DDT/E and PBDEs, we also constructed models including one of the three DDT/E compounds with the sum of the PBDEs and an interaction term (DDT/E compound X sum of PBDEs). Interaction terms remained in the model if the f-test comparing the full model with interaction terms to the nested model with no interaction terms was statistically significant (\( P < 0.20 \)). To examine the significant interaction between \( \mu \)-DDE and the sum of PBDEs, we created dichotomous variables for each exposure. Concentrations above the median were considered high and those below the median were considered low. Then separate models were performed examining the association of PBDE exposure (high versus low) with LINE-1 methylation in newborns with high and low prenatal \( \mu \)-DDE. Similarly, we also ran models looking at the association of \( \mu \)-DDE exposure (high versus low) with LINE-1 methylation in newborns with high and low prenatal PBDEs (sum). As was described for the age analysis, models examining associations of methylation with exposure (including looking at the interaction of DDT/E with PBDEs) were also run in the subset of newborns with differential cell count data adjusting for percent lymphocytes, monocytes, basophils, and eosinophils.

All statistical analyses were carried out using STATA software, version 12.0 (College Station, TX). P-values less than 0.05 were considered significant and \( P \)-values less than 0.10 were reported as marginally significant.

### RESULTS

Maternal and child characteristics are presented in Table I. Mothers were primarily low-income, and Mexican-born. The majority of them did not smoke or drink alcohol during pregnancy but either worked in agriculture (41%) or lived with someone who worked in agriculture (82%). There was a relatively even distribution of CHAMACOS boys (50 and 47% at birth and age 9) and girls included in this analysis.

### DNA Methylation

Table II shows the means and standard deviations for Alu and LINE-1 methylation in fetal cord blood and blood from nine-year-old children. For Alu, methylation at each of the four positions was positively correlated at birth and age 9 (Supporting Information Table I) (\( r \approx 0.30–0.67 \) and 0.38–0.67 at birth and nine years, respectively). Similarly, LINE-1 methylation at all four positions was also positively correlated (Supporting Information Table II) (\( r \approx 0.16–0.69 \) and 0.22–0.74 in fetal and nine-year old’s blood, respectively); however, methylation at position 3 was consistently lower compared to the other positions. LINE-1 and Alu methylation levels were not correlated with each other at either time point. Furthermore, methylation levels at birth were not correlated with those measured at age 9 for either LINE-1 or Alu repeats.

Supporting Information Table III shows the relationship between differential cell count and Alu and LINE-1 methylation. The majority of the white blood cell population was composed of neutrophils (mean %: 60.5) followed by lymphocytes (29.3%), monocytes (7.0%), eosinophils...
(2.9%), and basophils (0.3%). For Alu, there were suggestive trends of higher methylation in samples with more lymphocytes and monocytes compared to neutrophils; however, this was not statistically significant for lymphocytes ($P = 0.21$) and only marginally significant for monocytes ($P = 0.08$). For LINE-1, we observed a suggestive trend of higher methylation in samples with more eosinophils and basophils compared to neutrophils; however, neither trend was statistically significant, and these two cell types represented only a small portion of the white blood cell population.

**Effects of Age**

Among the children who had measures in both fetal and nine-year-old blood ($n = 134$), methylation of both Alu and LINE-1 repeats was lower at nine years than in fetal blood (0.02%5mC, $P = 0.004$ and 0.05%5mC, $P < 0.005$ respectively). We found similar trends when including all children ($n = 358$, not all matched at both time points) in the model (0.01%5mC, $P = 0.05$ and 0.05%5mC, $P < 0.005$ for Alu and LINE-1, respectively).

**Effects of Sex**

At birth, mean DNA methylation was lower in girls compared to boys (Fig. 2) for both assays (0.42 and 0.15%5mC for LINE-1 and Alu, respectively). This difference was statistically significant for LINE-1 methylation ($P = 0.017$) and only marginally significant for Alu ($P = 0.07$). In nine-year-old children, we found a similar trend for LINE-1 with mean methylation 0.71%5mC lower in girls than boys ($P < 0.005$), but no significant differences between girls and boys for mean Alu methylation.

Since it has been demonstrated that sex can affect immune profiles in children [Uekert et al., 2006; Casimir et al., 2010], we also looked at the effect of sex after adjusting for cell composition in a subset of 103 newborns. The effect of sex on Alu methylation was similar before and after adjusting for cell composition in this subset of children ($β(95\%CI)$: $-0.18 (-0.46, 0.010)$ and $-0.19 (-0.47, 0.09)$, respectively). The effect of sex on LINE-1 methylation was no longer statistically significant in this smaller subset of newborns ($β(95\%CI)$: $-0.04 (-0.55, 0.46)$) and did not change appreciably after adjusting for cell type composition.

### TABLE II. DNA Methylation (%5mC) in Blood Among CHAMACOS Newborns (Cord Blood) and 9-Year Olds ($N = 358$)

<table>
<thead>
<tr>
<th>Repeat</th>
<th>Position 1</th>
<th>Position 2</th>
<th>Position 3</th>
<th>Position 4</th>
<th>Average over 4 CpG sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LINE-1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>84.7 ± 1.7</td>
<td>80.8 ± 1.4</td>
<td>76.1 ± 2.6</td>
<td>73.7 ± 1.8</td>
<td>78.8 ± 1.4</td>
</tr>
<tr>
<td>9 years</td>
<td>83.7 ± 2.0</td>
<td>80.5 ± 1.6</td>
<td>75.8 ± 2.6</td>
<td>73.4 ± 2.0</td>
<td>78.4 ± 1.6</td>
</tr>
<tr>
<td><strong>Alu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>32.2 ± 1.0</td>
<td>28.3 ± 0.9</td>
<td>16.5 ± 0.7</td>
<td>24.2 ± 0.7</td>
<td>25.3 ± 0.7</td>
</tr>
<tr>
<td>9 years</td>
<td>32.2 ± 1.0</td>
<td>28.3 ± 1.0</td>
<td>16.4 ± 0.7</td>
<td>24.1 ± 0.7</td>
<td>25.3 ± 0.7</td>
</tr>
</tbody>
</table>

### TABLE III. Differences in Repeat Element Methylation in Newborns (Cord Blood) Associated with a 10-Fold Increase in Prenatal DDT, DDE, and PBDE Exposure

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Alu</th>
<th>Line-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>β (95%CI)</td>
<td>p-value</td>
<td>β (95%CI)</td>
</tr>
<tr>
<td>DDT/E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log o,p'-DDT</td>
<td>-0.13 (-0.30, 0.03)</td>
<td>0.11</td>
</tr>
<tr>
<td>log p,p'-DDT</td>
<td>-0.08 (-0.21, 0.05)</td>
<td>0.21</td>
</tr>
<tr>
<td>log p,p'-DDE</td>
<td>-0.13 (-0.30, 0.05)</td>
<td>0.15</td>
</tr>
<tr>
<td>PBDEs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log BDE SUM</td>
<td>-0.00 (-0.20, 0.20)</td>
<td>0.97</td>
</tr>
<tr>
<td>Log BDE-153</td>
<td>0.04 (-0.16, 0.25)</td>
<td>0.67</td>
</tr>
<tr>
<td>Log BDE-100</td>
<td>-0.02 (-0.22, 0.17)</td>
<td>0.83</td>
</tr>
<tr>
<td>Log BDE-99</td>
<td>-0.00 (-0.19, 0.19)</td>
<td>0.99</td>
</tr>
<tr>
<td>Log BDE-47</td>
<td>-0.01 (-0.20, 0.18)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

DDT, dichlorodiphenyl trichloroethane; DDE, dichlorodiphenyldichloroethylene; PBDEs, polybrominated diphenyl ether; BDE, polybrominated diphenyl ether

*aEach row represents one mixed effects regression model examining the association of log10 OC or log10 PBDE exposure with Alu or LINE-1 methylation controlling for sex.

*bThe magnitude of the beta coefficients labeled as 0.00 were lower than 0.005.
Prenatal DDT/E and PBDE Serum Levels and DNA Methylation

We found weak yet consistent inverse relationships between prenatal DDT/E exposure and Alu and LINE-1 methylation in cord blood DNA; however, these associations did not reach statistical significance (Table III). For instance, for each 10-fold increase in prenatal o,p'-DDT, we observed a 0.13%5mC lower level of Alu methylation ($P=0.11$) after adjusting for sex. Similar trends were observed with repeat element methylation in nine-year-old children (Supporting Information Table IV).

We saw very little evidence of an association of prenatal PBDE exposure and Alu methylation in fetal cord blood DNA. Although there was a trend of lower levels of LINE-1 methylation with prenatal PBDE exposures for all four congeners and the sum of all congeners, these relationships were also not statistically significant. Among nine-year olds, there was a slight suggestion of lower Alu and higher LINE-1 methylation with prenatal PBDE exposure but again they were not statistically significant. We did not observe significant interaction between sex and prenatal exposure to DDT/E or PBDEs in any of the models at either age.

Since some previous data indicate that cell composition may be associated with environmental exposure [Peltier et al., 2012] and DNA methylation, we also examined the relationship of prenatal DDT/E and PBDE exposures with cell composition in a subset of 103 newborns with differential cell count data (89 had DDT/E data and 94 had PBDE data). When we first examined the relationship between exposure and cell composition, we found suggestive trends of lower basophils ($P=0.11$) and higher neutrophils ($P=0.24$) with increased DDT/E exposure that did not reach statistical significance. We also found a statistically significant association between increased PBDE exposures and higher percent eosinophils and basophils ($P<0.05$ for both). When we adjusted for cell type composition in our models of DDT/E exposure with DNA methylation, we found similar trends in the subset of newborns as we did in the larger dataset. Lower levels of Alu methylation were observed with higher levels of prenatal DDT/E in this subset of newborns. Furthermore, after adjusting for cell type composition, the magnitudes of the beta coefficients increased (12–14%) and the 95% confidence intervals were narrower. In fact the association became significant for o,p'-DDT ($P=0.02$, $\beta$(95%CI): $-0.37(-0.69, -0.05)$ Fig. 3) and $p,p'$-DDE($P=0.04$, $\beta$(95%CI): $-0.33(-0.64, -0.01)$) and marginally significant for $p,p'$-DDT($P=0.08$, $\beta$(95%CI): $-0.24(-0.50, 0.02)$). Adjustment for cell type did not appreciably change results for the relationship of prenatal DDT/E with LINE-1 methylation or for prenatal PBDEs with both Alu and LINE-1 methylation.

Co-exposures to DDT/E and PBDE Serum Level and DNA Methylation

Since mothers were exposed to both PBDEs and DDT/E during pregnancy, we also examined the potential
When we stratified by and ran models for one compound stratified by the other.

$log BDE \sum 0.31 (p,p') DDT, dichlorodiphenyl trichloroethane; DDE, dichlorodiphenyldichloroethylene; BDE, polybrominated diphenyl ether.

To better understand this interaction, we classified children as having high or low exposure to $p,p'$ DDT in the same models. Covariates included log$_{10}$ DDT/E concentrations, the sum of log$_{10}$ PBDE exposure, an interaction term (DDT/E X sum PBDE) and sex.

The magnitude of the beta coefficients labeled as 0.00 were lower than 0.005.

### Table IV. Associations of Prenatal Co-exposure to DDT/E and PBDEs in Newborns (Cord Blood)

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta$ (95%CI)</th>
<th>p-value</th>
<th>$\beta$ (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log $o,p'$-DDT</td>
<td>$-0.10 (-0.73, 0.53)$</td>
<td>0.76</td>
<td>$-1.26 (-2.52, -0.01)$</td>
<td>0.05</td>
</tr>
<tr>
<td>log BDE SUM</td>
<td>$-0.00 (-0.22, 0.22)$</td>
<td>1.00</td>
<td>$-0.24 (-0.68, 0.21)$</td>
<td>0.29</td>
</tr>
<tr>
<td>$o,p'$-DDT*BDE SUM</td>
<td>$-0.03 (-0.47, 0.42)$</td>
<td>0.91</td>
<td>$0.70 (-0.19, 1.59)$</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log $p,p'$-DDT</td>
<td>$-0.03 (-0.51, 0.45)$</td>
<td>0.91</td>
<td>$-0.74 (-1.70, 0.21)$</td>
<td>0.13</td>
</tr>
<tr>
<td>log BDE SUM</td>
<td>$0.01 (-0.43, 0.44)$</td>
<td>0.97</td>
<td>$-0.58 (-1.46, 0.29)$</td>
<td>0.19</td>
</tr>
<tr>
<td>$p,p'$-DDT*BDE SUM</td>
<td>$-0.04 (-0.38, 0.29)$</td>
<td>0.80</td>
<td>$0.42 (-0.25, 1.09)$</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log $p,p'$-DDE</td>
<td>$0.03 (-0.58, 0.64)$</td>
<td>0.93</td>
<td>$-1.18 (-2.40, 0.03)$</td>
<td>0.06</td>
</tr>
<tr>
<td>log BDE SUM</td>
<td>$0.31 (-0.97, 1.60)$</td>
<td>0.63</td>
<td>$-2.51 (-5.09, 0.07)$</td>
<td>0.06</td>
</tr>
<tr>
<td>$p,p'$-DDE*BDE SUM</td>
<td>$-0.12 (-0.54, 0.31)$</td>
<td>0.59</td>
<td>$0.82 (-0.03, 1.67)$</td>
<td>0.06</td>
</tr>
</tbody>
</table>

DDT, dichlorodiphenyl trichloroethane; DDE, dichlorodiphenyldichloroethylene; BDE, polybrominated diphenyl ether.

Each model represents one mixed effects regression model of Alu or LINE-1 methylation (outcome) with prenatal exposure to both DDT/E and PBDEs in the same models. Covariates included log$_{10}$ DDT/E concentrations, the sum of log$_{10}$ PBDE exposure, an interaction term (DDT/E X sum PBDE) and sex.

The magnitude of the beta coefficients labeled as 0.00 were lower than 0.005.

### DISCUSSION

In this study, we examined the relationship of age, sex, and exposure to two classes of POPs, DDT/E, and PBDEs, with methylation of Alu and LINE-1 repetitive elements in Mexican-American children living in California. These children had relatively high DDT/E exposure.

The magnitude of the beta coefficients labeled as 0.00 were lower than 0.005.

The trends were similar for $o,p'$-DDT and $p,p'$-DDT; however, these interaction terms were no longer statistically significant.

Among nine-year olds, we found no evidence of an association between prenatal DDT/E or PBDE exposures and DNA methylation (data not shown).

Higher prenatal exposure to $o,p'$-DDT was associated with lower levels of methylation ($p = 0.02$, b(95%CI): $-0.37 (-0.69, -0.05)$).
in utero [Bradman et al., 2007] and high childhood exposure to PBDEs [Eskenazi et al., 2013]. Specifically, DNA methylation of Alu and LINE-1 repeats was lower in nine-year-old blood compared to fetal cord blood. Furthermore, girls had lower levels of methylation than boys at both time points. We observed a consistent trend of lower Alu methylation in fetal blood with higher prenatal DDT/E exposure, particularly after adjusting for cell type composition. Furthermore, associations of prenatal exposure with levels of LINE-1 methylation were only
identified after examining the co-exposure of DDT/E with PBDEs simultaneously. These results suggest that host factors such as age, sex, and prenatal exposure are potential predictors of repetitive element DNA methylation in children.

Although the effect of age has been examined in adults in several studies, few studies have reported on the effects of age on DNA methylation in children. Among adults, several studies have reported an inverse relationship between age and Alu methylation with weak or no association with LINE-1 methylation [Bollati et al., 2009; Jintaridth and Mutirangura, 2010; Zhu et al., 2012]. A number of studies focusing solely on LINE-1 methylation also found no relationship between age and LINE-1 methylation [Jintaridth and Mutirangura, 2010; El-Maarri et al., 2011]. Similar to the findings in adults, we observed (non-significantly) lower Alu methylation in nine-year-old CHAMACOS children compared to fetal blood. However, in contrast to the adults, older children in our study also had significantly lower LINE-1 methylation compared to fetal blood. One other study in children (n = 51, ages 6–17 years) found no relationship between age and methylation of Alu repeats and only a weak correlation (r = −0.19) between age and LINE-1 methylation that was not statistically significant (P = 0.18) [Wu et al., 2011]. However, they used a different assay to characterize methylation of Alu repeats (MethyLight) and the weak relationship with LINE-1 may have been due to the small sample size. It should also be noted that the absolute differences in percent methylation in fetal blood and nine-year-old blood were quite small (0.01–0.05 %5mC); however, they are similar in range to other studies reporting differences in methylation by age in adults [Bollati et al., 2009; Zhu et al., 2012]. Furthermore, several studies have demonstrated that even small differences in methylation can be biologically relevant [Michels et al., 2011; Lambrou et al., 2012].

We also found significantly lower levels of methylation in girls compared to boys at both ages, particularly for LINE-1 repeats. Our data corroborate with Peng et al. [2012] who reported the same trend for LINE-1 in school-aged children and Burris et al. [2012] who reported similar findings in cord blood. They are also consistent with several studies in adults, finding lower methylation in women than men [Wilhelm et al., 2010; El-Maarri et al., 2011; Zhang et al., 2011; Burris et al., 2012]. This phenomenon is likely due to the relationship of LINE-1 with X chromosome inactivation. A recent study examined methylation at individual LINE-1 loci located on the X chromosome as well as some in autosomes and found that differential methylation in males and females was primarily located in the X chromosome [Singer et al., 2012]. Although the study does not show causality, they provide preliminary evidence supporting the theory first introduced by Lyon that hypomethylation of LINE-1 could lead to increased retrotransposon activity of LINE-1 towards X-linked loci that can be inactivated [Lyon, 2003].

Only a few studies have examined the relationship between POP exposure and repetitive element methylation in humans. They were primarily cross-sectional studies performed in adults and none have examined the potential interaction between different classes of compounds. Furthermore, to our knowledge, ours is the first study to examine the link between prenatal POP exposure and DNA methylation. Rusiecki et al. [2008] reported that exposure to p,p′-DDT, p,p′-DDE, the sum of PCBs, and other organochlorines, was associated with global DNA hypomethylation in a cross sectional study of Greenlandic Inuits ages 19–67 (N = 70). They were highly exposed to POPs with DDT exposure ranging from 4 to 373 ng/g-lipid and DDE levels between 264 and 5969 ng/g-lipid; these ranges were somewhat higher than those found in pregnant CHAMACOS mothers. This association was significant for Alu methylation and a similar but nonsignificant trend was also seen for LINE-1. Comparable results were reported in a cross-sectional study of healthy adult Koreans (N = 86) with relatively low levels of environmental exposure to organochlorine pesticides and PBDEs [Kim et al., 2010]. Another study found lower levels of LINE-1 methylation among PCB-exposed individuals who had a maternal 15q11-q13 duplication (Dup15q), but these levels may have been confounded by year of birth [Mitchell et al., 2012]. Among CHAMACOS children, we observed trends similar to those reported by Rusiecki et al. [2008] and Kim et al. [2010]. In fact, the magnitude of the effect of organochlorine compounds on Alu methylation in the subset of newborns with cell count data was quite similar in range to those two studies.

We also identified a significant interaction between PBDE and DDE exposure in which the direction of the association of PBDE exposure on fetal cord LINE-1 methylation was dependent on DDE exposure and vice versa. It is important to note that we only observed significant associations of prenatal exposures with LINE-1 methylation when we considered both classes of compounds (DDT/E and PBDEs) in the same model. Only a few other studies have examined the potential interaction of different classes of compounds and to our knowledge none have been reported in relation to DNA methylation and POPs exposure. Two studies have shown that co-exposure to PBDEs (BDE-47 and −99) and PCBs (PCB −126 and −123) can have a synergistic effect in rats and human neuroblastoma cell lines [He et al., 2011; Pellacani et al., 2012]. Co-exposure to PBDEs (−47,−99, −100, and −209) and DDT/E was reported in one in vivo study of steroid secretion and also found that the effect of PBDEs was dependent on the concentration of DDT in the mixture [Gregoraszczuk et al., 2008]. Humans are generally exposed to multiple chemicals.
present in the environment and our data suggest that taking into account multiple exposures may significantly impact findings in relation to DNA methylation.

Although some meaningful relationships of repetitive element DNA methylation with host factors such as age and sex in children were identified, this study has some limitations. First, we focused specifically on prenatal exposure to POPs; however, it is possible that postnatal exposures may also affect DNA methylation. In fact postnatal exposures to PBDEs tend to be higher in CHAMACOS children compared to prenatal exposures [Eskenazi et al., 2013] and we plan to examine their relationship with DNA methylation in future studies. Second, although many different sub-families of Alu and LINE-1 repeats exist, we examined only the AluSx and LINE-1 H sub-families. A recent study demonstrated that the methylation response to exposure can vary among different sub-families of repetitive elements and may be dependent on the evolutionary age of the sub-family [Byun et al., 2013]. Future studies may benefit from exploring other LINE-1 and Alu sub-families, including the younger sub-families that have higher mean methylation levels. Third, epigenetic modifications are tissue specific. In our study, we used whole blood as a surrogate matrix that we believe is appropriate for examining host factors but may not be the most relevant target tissue for prenatal exposures to DDT/E and PBDEs. Finally, different types of blood cells may have varying levels of methylation [Adalsteinsson et al., 2012]. It is well established that immune profiles are quite different at birth than they are as a child grows older, which may bias our analyses [Delspesse et al., 1998]. Additionally, blood cell composition can be affected by sex and exposure [Uekert et al., 2006; Casimir et al., 2010; Peltier et al., 2012]. Thus it may be difficult to determine whether the methylation differences observed in our study were truly due to sex and exposure or if they were confounded by differences in cell type distribution. Interestingly, when we adjusted for cell type composition in a subset of newborns we found similar trends and in many cases observed stronger associations with repeat element methylation. We did not however have these data for nine-year old children and thus could not determine whether the differences in methylation by age were confounded by the differences in cell type distributions in newborns and nine-year olds. Recently, new statistical methodologies have been applied to epigenome wide DNA methylation data (Illumina Infinium 450K) to estimate cell type distribution in blood DNA [Houseman et al., 2012; Koestler et al., 2013]. In the future, as this data becomes available for our study, we will apply these methodologies to adjust for cell type composition.

In summary, we found a significant relationship of Alu and LINE-1 DNA methylation with host factors (age and sex) in children. Furthermore, we observed a significant association of prenatal exposure to DDT/E with hypomethylation of Alu repeats in newborns and identified a relationship between prenatal exposures and differences in LINE-1 methylation only after considering co-exposure to both DDE and PBDE. Our data underscore the importance of taking age, sex, and other host factors as well as multiple exposures into account in population studies examining relationships between DNA methylation, exposure, and health.

ACKNOWLEDGEMENTS

We are grateful to the laboratory and field staff and participants of the CHAMACOS study for their contributions. We are especially thankful to Dr. Dana Barr who performed the measurement of organochlorine compounds, Dr. Andreas Sjödin who performed the measurement of PBDEs, and Michael Ha and Girish Motwani who helped with differential cell counts in this study. This publication was made possible by grants from the National Institute of Environmental Health Science (NIEHS) [PO1 ES009605, RO1 ES015572], from the US Environmental Protection Agency (EPA)[R82670901, RD3171001, and RD3451301], and from the National Institute for Occupational Safety and Health (NIOSH) [RO1 OH007400]. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIEHS, EPA, or NIOSH.

AUTHOR CONTRIBUTIONS

Drs. Holland and Huen and Mr. Yousefi conceived and designed the study. Drs. Eskenazi, Harley, and Bradman and Ms. Kogut were involved in recruitment of subjects and data collection for the study cohort. Mr. Yousefi, Dr. Huen, and Dr. Yang performed the experiments. Mr. Yousefi and Dr. Huen performed the data analysis and prepared the manuscript draft with important intellectual input from Drs. Holland and Eskenazi. All authors approved the final manuscript.

CONFLICT OF INTEREST

Liying Yan is the President and chief scientist of EpigenDx and owns more than 5% shares in EpigenDx. There are a number of patent applications related to cancer biomarkers discovery in filing process.

REFERENCES


MolMutagen 53:589–598.

Mol Mutagen 53:589–598.


