Environmental Research 158 (2017) 333–341

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Association between serum persistent organic pollutants and DNA methylation in Korean adults

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ARTICLE INFO

Keywords:
Persistent organic pollutants (POPs)
DNA methylation
Alu
LINE-1
Epigenetics

ABSTRACT

Background: Exposure to persistent organic pollutants (POPs) has been associated with epigenetic changes such as DNA methylation, which can influence human health. However, the association between POPs and DNA methylation by sex was not shown in previous studies.

Objectives: We investigated the association between POPs and DNA methylation in men and women using a larger population.

Methods: A cross-sectional study was conducted using the data of 444 Koreans (253 men and 191 women). Measurements for sixteen different POPs, including six organochlorine pesticides (OCPs) and ten polychlorinated biphenyls (PCBs) were taken in serum. DNA methylation via Alu and LINE-1 in peripheral leukocytes was measured by pyrosequencing. To evaluate the association between POPs and DNA methylation, the Pearson's correlation and multiple linear regression analyses were performed.

Results: Except for PCB52 and PCB101, we found significant inverse associations between p,p'-DDE, cis-Heptachlor epoxide, and PCBs and Alu assay in men after adjusting for age, BMI, smoking status, and alcohol consumption ($\beta = -0.67$ for p,p′-DDE; -0.28 for cis-Heptachlor epoxide; in the range from -0.43 to -1.60 for PCBs). In women, PCB153 and PCB180 showed statistically significant inverse association with Alu assay ($\beta = -0.22$ for PCB153; -0.22 for PCB180). Except for PCB101, p,p′-DDE and PCBs were positively associated with LINE-1 assay in women ($\beta = 0.48$ for p,p′-DDE; in the range from 0.40–0.89 for PCBs) while p,p′-DDE, PCB153, and PCB180 showed positive associations with LINE-1 assay in men ($\beta = 0.55$ for p,p′-DDE; 0.65 for PCB153; 1.02 for PCB180).

Conclusions: We found that several POPs were associated with global DNA hypomethylation in the Alu assay for men and global DNA hypermethylation in the LINE-1 assay for women.

1. Introduction

The International Agency for Research on Cancer (IARC) classified polychlorinated biphenyls (PCBs) under Group 1 and 4,4′-Dichlorodiphenyltrichloroethane under Group 2A. Group 1 means carcinogen and 2A means probable carcinogen to humans (International Agency for Research on Cancer, 2016). Numerous epidemiologic studies showed that Persistent organic pollutants (POPs) could be associated with the risk of cancers - such as breast cancer (Khanjani et al., 2007; Lopez-Cervantes et al., 2004), prostate cancer (Lim et al., 2017, 2015; Van Maele-Fabry and Willems, 2004), and colorectal cancer (Brustad et al., 2007).

Recently, epigenetics has been reported as one of the possible mechanisms for the association between POPs and various diseases (Collotta et al., 2013). Epigenetic mechanisms including histone modifications, microRNA expression, and DNA methylation are involved in the regulation of gene expression without DNA sequence modifications (Wolffe and Matzke, 1999). Among these mechanisms, DNA methylation is the addition of methyl group (CH_3) to 5-carbon position of cytosine (5-mC) by DNA methyltransferases (DMNTs) and S-adenosylmethionine (SAM), and that occurs in CpG dinucleotide (Miranda and Jones, 2007). DNA methylation includes hypomethylation and hypermethylation. In cancer, the expression of oncogenes is increased by DNA hypomethylation and the expression of tumor suppressor genes is inactivated by DNA hypermethylation (Lopez et al., 2009).

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; β-HCH, beta-Hexachlorocyclohexane; p, p'-DDE, *p,p′*-dichlorodiphenyltrichloroethane; p, p'-DDD, *p,p'*-dichlorodiphenyldichloroethane; p, p′-DDT, *p,p′*-dichlorodiphenyltrichloroethane

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http://dx.doi.org/10.1016/j.envres.2017.06.017
Received 30 January 2017; Received in revised form 17 June 2017; Accepted 19 June 2017
Available online 30 June 2017
0013-9351/

Alu and Long Interspersed Nucleotide Element-1 (LINE-1) are major repetitive DNA elements, composing approximately 11% and 17% of the mass of human genome, respectively (Lander et al., 2001). Alu and LINE-1 are useful surrogate markers for estimating the global DNA methylation status due to their high occurrence.

Based on associations such as between POPs and cancers and between DNA methylation and cancers, some cross-sectional studies have demonstrated associations between serum concentrations of POPs and DNA methylation level (Huen et al., 2014; Itoh et al., 2014; Kim et al., 2010; Lind et al., 2013; Rusiecki et al., 2008). However, the results of previous studies were not consistent. Two studies observed inverse associations between POPs and Alu assay (Kim et al., 2010; Rusiecki et al., 2008), and positive associations between POPs and global DNA methylation were observed in the elderly in Sweden (Lind et al., 2013). Moreover, previous studies did not show any difference in sex because of their small sample size, with n = 70 (Rusiecki et al., 2008) and 86 (Kim et al., 2010). Although there were studies using a larger sample, findings have been reported only regarding the elderly (Lind et al., 2013), children (Huen et al., 2014), and Japanese women (Itoh et al., 2014).

Therefore, in this study, we conducted a cross-sectional study to evaluate the association between serum concentrations of POPs and DNA methylation level by sex in a large healthy Korean adult population living in urban area of South Korea (Seoul and Gyeong-gi province).

2. Materials and methods

2.1. Study population

Subjects were Korean adults, ≥ 20 years of age, from the Korean Cancer Prevention Study (KCPS) -II (KCPS-II). The KCPS-II aims at examining the risk factors of chronic diseases such as cancers and heart diseases, and preventing such diseases. The population included 270,514 individual who visited 11 health promotion centers in Seoul and Kyung-gi province in South Korea from April 2004 to December 2011. Among the total sample population, 1050 subjects had POPs and DNA methylation measurements. Among them, 601 subjects who were diagnosed with diseases at the time of enrollment were excluded. In addition, 5 subjects with missing anthropometric measurements were excluded. Therefore, the final subject group consisted of 444 healthy Korean men and women adults (men: 253; women: 191).

All subjects were asked a paper-based questionnaire which included questions on socio-demographic characteristics, smoking habit, alcohol consumption status, and physical activity. The study protocol was approved by the institutional review board of Yonsei University. Additionally, all subjects provided informed written consent before they participated in the study.

2.2. POPs analyses

Serum concentration of POPs was obtained from each participant after a minimum fasting period of 12 h. Analysis of POPs in 0.5–1 ml serum was performed using an isotopic dilution method with gas chromatography (Agilent 6890 Series, Palo Alto, CA, USA) / high-resolution mass spectrometry (JMS-800D, Jeol, Tokyo, Japan) (Kang et al., 2008; H. A. Lee et al., 2016). The analytical method has been reported elsewhere (Park et al., 2016). A total of 51 POPs were measured: 32 polychlorinated biphenyls (PCBs) and 19 organochlorine pesticides (OCPs). Among 51 POPs measured, we selected 10 PCBs and 6 OCPs with detection rates ≥ 70% (Salihovic et al., 2012). All POPs concentrations were adjusted for lipids. Total lipids were calculated using the following calculation: total lipid (mg/dL) = 2.27 × total cholesterol + triglycerides + 62.3 (Phillips et al., 1989). POPs levels below the limit of detection (LOD) were assigned the value of LOD/2 (Huen et al., 2014). 15 serum samples were used for quality control (QC) and prepared by another laboratory. The recovery rate of congeners compounds with internal standards was calculated from 50% to 120%. All quality assurance/quality control(QA/QC) congeners' compounds' relative standard deviation (SD) was below 15%.

2.3. Sodium Bisulfite modification

Genomic DNA (gDNA) was extracted from the peripheral blood. The bisulfite conversion of gDNA (200 ng) was performed using EZ DNA Methylation-Lighting™ kit (Zymo Research, USA) according to the manufacturer's protocol. Final elution was performed with 20ul of M-Elution Buffer. DNA samples were stored at −20 ℃ for further analysis.

2.4. Pyrosequencing analysis

We performed DNA methylation analysis of the Alu and LINE-1 assay based on polymerase chain reaction (PCR) pyrosequencing method of bisulfite-treated DNA with the same PCR primers and conditions as described previously (Bollati et al., 2007; Oh et al., 2015). The bisulfite-treated DNA samples were amplified by PCR. Confirmation of the PCR reaction (2ul) was done by electrophoresis in a 2% Agarose gel and visualized by ethidium bromide staining. Sequencing was carried out using a PyroMark ID system with the Pyro Gold reagents kit (Qiagen) according to the manufacturer's protocol. The methylation level was expressed as 5-mC% divided by the sum of methylated and unmethylated cytosines. The methylation percentage was calculated from the average of the degree of methylation at 3 or 4 CpG sites.

To avoid DNA cross-contamination between sample batches, we used thoroughly washed water, and the water was washed with the Vacuum Prep Tool. And also, we used PyroMark Software (Qiagen) for a quality assessment of the sequence context as well as each sites.

2.5. Statistical analysis

Student's *t*-test and chi-square test used to test for the differences in sex. Also, Student's *t*-test and analysis of variance (ANOVA) were performed to test for the differences in DNA methylation levels by categorical variables. Since serum POPs concentrations were highly skewed, they were log-transformed naturally in order to achieve the approximate normal distribution before being included in Pearson's correlation analysis and regression models. Pearson's correlation analysis was used to evaluate correlation of variables, and multiple linear regression modeling was used to evaluate the relationships between two outcome variables (Alu and LINE-1 assay) and exposure variables (serum of POPs concentrations) in each sex group after adjusting for age, BMI, smoking status, and alcohol consumption. Confounding variables were selected based on the results of simple regression analysis, stepwise regression analysis, and the previous studies. The results showed not only the individual POPs but also the sum of OCPs, PCBs, and POPs. In case of PCBs, the sum of dioxin-like PCBs (PCB105 + PCB118 + PCB156 + PCB157 + PCB167) and non-dioxin-like PCBs (PCB52 + PCB101 + PCB138 + PCB153 + PCB180) was calculated according to the property of PCB congener (Baars et al., 2004). All statistical tests were two-sided, and statistical significance was determined as $P < 0.05$. SAS statistical software, version 9.2 (SAS Institute, Cary, NC) and STATA 13.1 (Stata Corporation, College Station, Tx, USA) were used for all analyses.

3. Results

3.1. Basic characteristics and DNA methylation distribution

The basic characteristics of all subjects (253 men and 191 women) are shown in Table 1. The mean age was 41.54 years for men and 39.14 years for women.