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Indoor air pollution from solid fuels and peripheral Blood DNA methylation: Findings from a population study in Warsaw, Poland

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ABSTRACT

DNA methylation is a potential mechanism linking indoor air pollution to adverse health effects. Fetal and early-life environmental exposures have been associated with altered DNA methylation and play a critical role in progress of diseases in adulthood. We investigated whether exposure to indoor air pollution from solid fuels at different lifetime periods was associated with global DNA methylation and methylation at the *IFG2/H19* imprinting control region (ICR) in a population-based sample of non-smoking women from Warsaw, Poland. Global methylation and *IFG2/H19* ICR methylation were assessed in peripheral blood DNA from 42 non-smoking women with Luminometric Methylation Assay (LUMA) and quantitative pyrosequencing, respectively. Linear regression models were applied to estimate associations between indoor air pollution and DNA methylation in the blood. Compared to women without exposure, the levels of LUMA methylation for women who had ever exposed to both coal and wood were reduced 6.70% (95% CI: −13.36, −0.04). Using both coal and wood before age 20 was associated with 6.95% decreased LUMA methylation (95% CI: −13.79, −0.11). Further, the negative correlations were more significant with exposure to solid fuels for cooking before age 20. There were no clear associations between indoor solid fuels exposure before age 20 and through the lifetime and *IFG2/H19* ICR methylation. Our study of non-smoking women supports the hypothesis that exposure to indoor air pollution from solid fuels, even early-life exposure, has the capacity to modify DNA methylation that can be detected in peripheral blood.

1. Introduction

Approximately half of the world's population uses solid fuels including coal, wood, dung, and crop residues for cooking or home heating (Torres-Duque et al., 2008). Usage distributions vary among countries with the largest prevalence in rural areas of developing countries, while a number of households in developed countries still use wood or other biomass products for heating (Naeher et al., 2007; Torres-Duque et al., 2008). The fuels are typically burned inefficiently in open fires, leading to high emission rates and high levels of indoor air pollution (Lewtas, 2007; Torres-Duque et al., 2008). Products of incomplete combustion contain particulate matter (PM), carbon monoxide, nitrogen and sulfur oxides, and many organic compounds such as carcinogens, e.g. (or including) polycyclic hydrocarbons (Lewtas, 2007; Torres-Duque et al., 2008). In light of the high concentrations of the many pollutants, exposure to indoor air pollution presents a huge global public health concern (Straif et al., 2006; Naeher et al., 2007). The Global Burden of Disease project (GBD-2010) has estimated that household air pollution due to the use of solid cookfuels is responsible for about 3.9 million premature deaths each year, and about 4.8% of the global

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burden of disease (Smith et al., 2014). Based on evidence in both humans and experimental animals, exposure to indoor emissions from household combustion of coal has been concluded as a carcinogen to human, while biomass-smoke exposure (mainly wood) is a probable carcinogen to human (Straif et al., 2006). Although the amount of indoor air pollution exposure depends on the type of fuel used, the nature of the combustion, the location and the length of time exposed to polluted environment, women and children who are at home for most of the day are the most vulnerable (IARC, 2010). Further, there has been evidence linked to early-life (prenatal, infants, children and adolescents) periods exposure to air pollution with adulthood health effects in both animal and human studies (Soto-Martinez and Sly, 2010; Weldy et al., 2013). Although the underlying biological mechanisms are not clear, one possible mechanism is that there are epigenetic alterations resulting from these exposures, which play a role in the development of adverse health effects.

It is now widely acknowledged that epigenetic alterations can have various impacts on disease susceptibility and development. As an important mechanism of epigenetic changes, aberrant DNA methylation, including loss of genome-wide (global) methylation and site-specific hypermethylation, has been attributed to carcinogenesis and many other diseases (Baylin and Jones, 2011; Brennan and Flanagan, 2012). The level of DNA methylation is sensitive to various types of environmental exposures including benzene, arsenic, lead, persistent organic pollutants, as well as air pollutants, especially the exposures during early-life period (Bernal and Jirtle, 2010; Lambrou et al., 2012; Tang et al., 2012; Janssen et al., 2013). There is a large literature on DNA methylation alterations at the tissue level; more recently, peripheral blood DNA methylation changes in white blood cells have also been investigated as risk factor or potential cancer risk markers in some studies (Terry et al., 2011). However, data on how exposure to indoor air pollution both over the lifetime and during different periods of the lifetime is related to global methylation in blood DNA are still very limited.

Insulin-like growth factor 2 (IGF2) is one of two ligands within the genome's insulin network, and is necessary for the proper control of somatic growth, especially throughout early life (Werner and Le Roith, 2000; Livingstone, 2013). The *IGF2/H19* gene locus is involved in early development through DNA methylation, and is one of the best studied imprinted genomic loci (Ollikainen and Craig, 2011). Genomic imprinting is a form of gene silencing that is regulated by gene-specific imprinting control regions (ICRs) through parent-of-origin differential methylation (Bell and Felsenfeld, 2000). Both animal models and human studies have shown that adverse prenatal environmental exposure is associated with subsequent *IGF2* ICR methylation disruption (Wu et al., 2004; Heijmans et al., 2008), suggesting this may be an "environmental sensor" or biomarker of environment exposure. There are a few studies of the associations of early-life exposure to indoor air pollution with methylation of *IGF2/H19* ICR in blood DNA from women.

To better understand the relationship of exposure to indoor air pollution from solid fuels at different lifetime periods with blood DNA methylation levels, we evaluated global DNA methylation and methylation levels of *IGF2/H19* ICR in peripheral blood in population-based women who had never smoked cigarettes.

2. Materials and methods

2.1. Study population

The study population was derived from the population-based controls of a case-control study of lung cancer conducted in Warsaw, Poland during 1998–2001. The population-based controls were randomly selected from the electronic registry of residents of Warsaw, Poland, and were frequency-matched to cases by age group (± 3 years), geographic area, and gender. Details of the recruitment methods have been published elsewhere (Lissowska et al., 2005; Zeka et al., 2006). Interviews were completed for 289 eligible female controls from Warsaw, Poland. For present study, we limited the subset of never-smoking Warsaw women defined by participants who reported having ever smoked less than 100 cigarettes in their lifetime ($n=82$). All participants provided informed consent, and the Institutional Review Boards of all the participating institutions approved the study protocol.

All study participants were in-person interviewed by trained interviewers. The standardized and structured questionnaire used for the study covered demographics, tobacco and alcohol use, second-hand smoking, history of lung-related disease, family history of cancer, habitual diet history, occupational history, as well as residential history. More details of the interview procedure on lifetime indoor air pollution exposure were provided elsewhere (Lissowska et al., 2005). Briefly, participants were first asked to report their ages at starting and leaving each residence where they had lived in for at least two years, and the principal fuel types there were used for cooking and heating in each residence. Modern nonsolid fuels for heating or cooking, such as gas, kerosene, and air-conditioning, were distinguished from traditional solid fuels, including coal and wood. From these reports, we calculated the total duration of being exposed to solid fuels as the sum of the differences between the ages at starting and leaving each residence in which solid fuels were used as principle means for cooking or heating. Current body weight and height were measured by trained interviewers according to a standardized protocol at the time of interviewing. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters (weight (kg)/height $(m)^2$).

2.2. Determination of peripheral blood DNA methylation

Of the never-smoking female participants from Warsaw, Poland, we were able to collect blood sample for about 80% of them. Of those we obtained, genomic DNA was extracted from blood samples, and only those with enough volume of blood DNA were analyzed their methylation levels ($n=42$). Blood DNA was modified by treatment with sodium bisulfite using the Qiagen EpiTect Plus Bisulfite Kit according to manufacturer's instruction. Global methylation was measured using Luminometric Methylation Assay (LUMA) as previously described (Karimi et al., 2006), which is based on the ability of two isoschizomers to differentially digest sequences depending on the methylation status of the CpG site within the sequence. We ran a modified version of the assay (Xu et al., 2012). Briefly, 300 ng genomic DNA was digested for 4 h by *Hpa*II+*Eco*RI or *Msp*I+*Eco*RI in two separate reactions, which were set up in a 96-well plate format. Then, 15 μl annealing buffer (20 mM Tris acetate and 2 mM Mg-acetate, pH 7.6) was added to the digestion product, and samples were pyrosequenced using a Pyromark Q24 sequencer (Qiagen). The LUMA methylation level was expressed as a percentage obtained from the following equation: methylation (%)$=[1-(HpaII\ \Sigma G/\Sigma T)/(MspI\ \Sigma G/\Sigma T)]\times 100$ (Xu et al., 2012).

There are six CpG sites within *IGF2/H19* ICR (CpG 1–6), among which a C > T polymorphism exists at CpG 5 that abolishes the methylation site and thus was excluded from methylation analysis. Pyrosequencing was applied to quantitatively measure the level of methylation at the remaining five CpG sites. The Pyromark Assay Design Software (Qiagen) was used to query a reliable reading sequence around the ICR, and PCR primers were designed to amplify (and later sequence) the region ((F) TTGTTGAATTAGTTGTGGGGTTTA; (R) Biotin–ATTCCAATACCAAAAATAAAAAAACTCT; Sequencing Primer: GAATTAGTTGTGGGGTTTATA). PCR cycling started with an initial 15 min of denaturation at 95 °C, followed by 50 cycles of 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 30 s, and ended with a final 10-min extension at 72 °C. Percent methylation for each CpG was determined using Pyro Q-CpG Software (Qiagen). In addition, for each assay, methylated DNA was used for a positive control along with a water blank as an unmethylated negative control. The laboratory staffs were blinded to epidemiologic exposure information and quality control status.

2.3. Statistical analysis

The linear regression model was used to estimate the association of exposure to indoor air pollution with the percentage of global methylation and *IGF2/H19* ICR methylation. Individuals who reported never using any solid fuels for cooking or heating comprised the referent group. We examined the association of DNA methylation and the use of solid fuels (cooking, heating, and both), the types of solid fuels used (coal, wood, and both) both before age of 20 and in lifetime. We also examined the effect of duration of exposure to solid fuels. In addition, the effects of switching from solid fuels to modern fuels were also investigated. All analyses were adjusted by age, education level (primary, secondary, and university), BMI (< 25, 25–30, and > 30), alcohol drinking status (yes, and no), dietary folate intake, and exposure to environmental tobacco smoke at work or from partners (yes, and no). The models for exposure before age of 20 were additionally adjusted for exposure to solid fuel after age of 20 (yes, and no). Tests for trend were performed by assigning the median value for each category and modeling this variable as a continuous variable using linear regression models. The major occupational categories for study participants were clerical and related workers, service workers, general public service activities, or education, composing