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Sources of polycyclic aromatic hydrocarbons are associated with gene-specific promoter methylation in women with breast cancer

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ABSTRACT

Background: Tobacco smoke, diet and indoor/outdoor air pollution, all major sources of polycyclic aromatic hydrocarbons (PAHs), have been associated with breast cancer. Aberrant methylation may be an early event in carcinogenesis, but whether PAHs influence the epigenome is unclear, particularly in breast tissue where methylation may be most relevant. We aimed to evaluate the role of methylation in the association between PAHs and breast cancer.

Methods: In a population-based case-control study, we measured promoter methylation of 13 breast cancer-related genes in breast tumor tissue ($n=765$–851 cases) and global methylation in peripheral blood (1055 cases/1101 controls). PAH sources (current active smoking, residential environmental tobacco smoke (ETS), vehicular traffic, synthetic log burning, and grilled/smoked meat intake) were evaluated separately. Logistic regression was used to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs).

Results: When comparing methylated versus unmethylated genes, synthetic log use was associated with increased ORs for *CDH1* (OR=2.26, 95%CI=1.06–4.79), *HIN1* (OR=2.14, 95%CI=1.34–3.42) and *RARβ* (OR=1.80, 95%CI=1.16–2.78) and decreased ORs for *BRCA1* (OR=0.44, 95%CI=0.30–0.66). Residential ETS was associated with decreased ORs for *ESR1* (OR=0.74, 95%CI=0.56–0.99) and *CCND2* methylation (OR=0.65, 95%CI=0.44–0.96). Current smoking and vehicular traffic were associated with decreased ORs for *DAPK* (OR=0.53, 95%CI=0.28–0.99) and increased ORs for *TWIST1* methylation (OR=2.79, 95% CI=1.24–6.30), respectively. In controls, synthetic log use was inversely associated with LINE-1 (OR=0.59, 95%CI=0.41–0.86).

Discussion: PAH sources were associated with hypo- and hypermethylation at multiple promoter regions in breast tumors and LINE-1 hypomethylation in blood of controls. Methylation may be a potential biologic mechanism for the associations between PAHs and breast cancer incidence.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are established carcinogens to the lung (IARC, 2010), but their relationship with

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breast cancer is not as well studied. PAH biomarkers, which tend to reflect recent exposure, have been associated with breast cancer incidence (Gammon et al., 2004b). Additionally, PAHs induce mammary tumors in laboratory animals (Hecht, 2002). PAHs are ubiquitous pollutants that form as a combustion by-product of organic material (Boström et al., 2002). The major sources of PAH in the general population are indoor and outdoor air pollution, tobacco smoke and diet (Boström et al., 2002). Elevated associations with breast cancer incidence have been observed with the main sources of PAH exposure, including active cigarette smoking (Gaudet et al., 2013), long-term environmental tobacco smoke (ETS) (Gammon et al., 2004a; Morabia et al., 1996), indoor air pollution from burning synthetic logs (White et al., 2014), outdoor air pollution (Hystad et al., 2015; Mordukhovich et al., 2015; Nie et al., 2007), and intake of grilled and smoked foods (Fu et al., 2011; Steck et al., 2007).

Aberrant DNA methylation, an epigenetic modification, plays an important role in breast carcinogenesis (Xu et al., 2011; Xu et al., 2012). Higher levels of methylation at promoter regions can silence tumor suppressor genes and tumor tissue may be were methylation is most relevant (Jones, 2012). Our research team has reported that methylation at promoter regions of specific genes in breast tumor tissue is associated with breast cancer clinical/pathological factors and mortality in a population-based sample (Cho et al., 2012). In contrast, lower levels of global methylation may confer genomic instability and increased mutation rates (Brennan and Flanagan, 2012). Although the associations between cancer and global methylation have been inconclusive (Brennan and Flanagan, 2012), our research group has previously reported an association with breast cancer for luminometric methylation assay (LUMA), but not for methylation of long interspersed elements-1 (LINE-1) in white blood cells (Xu et al., 2012). DNA methylation may be altered in response to environment and lifestyle factors and may be a potential biologic mechanism for disease (Bollati and Baccarelli, 2010). Other investigators have found that exposure to the PAH sources of tobacco smoke and air pollution may be associated with changes in DNA methylation patterns (Duan et al., 2013; Shenker et al., 2013).

For the current study, we aimed to better understand the role methylation plays in the PAH and breast cancer association. We first aimed to examine whether five individual PAH sources, previously found to be modestly associated with breast cancer incidence (current active cigarette smoking (Gammon et al., 2004a), long-term residential ETS (Gammon et al., 2004a), total grilled/smoked food intake (Steck et al., 2007), residential burning of synthetic logs (White et al., 2014) and high vehicular traffic exposure (Mordukhovich et al., 2015)), were also associated with promoter methylation status in a panel of 13-breast cancer related genes measured in the tumor tissue of a population-based sample of women with breast cancer. We also aimed to investigate whether these same PAH sources were associated with global methylation in a population-based sample of women without breast cancer, using two independent global methylation markers, LINE-1 and LUMA, measured in peripheral blood DNA.

2. Materials and methods

Our study builds upon population-based resources from the Long Island Breast Cancer Study Project (LIBCSP). The parent LIBCSP methods have been previously published in detail (Gammon et al., 2002). Institutional Review Board approval was obtained from all relevant institutions.

2.1. Study population

Study participants included 1508 breast cancer cases and 1,556 controls who were English-speaking women residing in Nassau and Suffolk counties on Long Island, New York. Written informed consent was obtained from all study participants.

Cases were women who had been recently diagnosed with a first primary *in situ* or invasive breast cancer between August 1st, 1996 and July 31st, 1997, and were residents of Nassau or Suffolk counties on Long Island, New York (NY). There were no age or race restrictions for case eligibility. Cases were identified using rapid case ascertainment from the pathology departments of all 28 hospitals on Long Island and three tertiary care hospitals in New York City. Diagnoses were confirmed by the physician or the medical record.

Controls had no prior history of breast cancer and were frequency matched in 5-year age groups to cases based on the expected age distribution of case women. Controls were identified in 1996–1997 from among adult female residents of Nassau and Suffolk counties in NY using random digit dialing for those who were less than 65 years of age, and for those who were 65 years of age and greater, using the Health Care Finance Administration rosters.

Study participants ranged in age from 20–98 years, and most cases and controls were postmenopausal (68.1% and 66.3%, respectively) and identified themselves as white (93.8% and 91.8%, respectively); the racial distribution of our population-based sample reflects that of Nassau and Suffolk counties at the time of data collection (Gammon et al., 2002). Cases and controls had similar distributions of education and income (Gammon et al., 2002), and the median age at menarche was also similar (12.6 years, standard deviation (SD)=1.67; and 12.6 years, SD=1.65, respectively). On average, controls were more likely to be parous than cases (89.0% versus 86.9%, respectively) (Gammon et al. 2002). Some 10% of cases and 8% of controls reported drinking 1–2 glasses per day (15–30 g) of alcohol, on average across the life course (Terry et al. 2006).

2.2. PAH exposure sources assessment

Five PAH exposure sources were assessed. Current active smoking, residential ETS, grilled/smoked meat intake, and synthetic log burning were assessed by a trained interviewer using a structured questionnaire (Gammon et al., 2004a; Steck et al., 2007; White et al., 2014); and vehicular traffic exposure was assessed by a validated historical geographic model (Beyea et al., 2006; Mordukhovich et al., 2015). Detailed LIBCSP PAH source assessment methods have been previously published (Gammon et al., 2004a; Mordukhovich et al., 2015; Steck et al., 2007; White et al., 2014). The PAH variable definitions, based on previous published associations with breast cancer, and total sample sizes used in the study reported here are described below.

Current active smoking (yes, no) was defined as smoking within the 12 months prior to the reference date, which was date of diagnosis for cases and date of identification for controls (n=1553 controls/1508 cases) (Gammon et al. 2004a). Participants were asked if they had lived with a smoking spouse to determine residential ETS exposure (yes, no) (n=1515 controls/1468 cases) (Gammon et al., 2004a). Frequency of grilled/smoked meat intake was assessed for each of six decades across the life course, or fewer depending on age at diagnosis (Steck et al., 2007). Lifetime intake was defined as the average servings consumed per year based on quantile distributions in the controls ($<33\%$ vs $\geq 33\%$ percentile or <55 servings/year, 55+ servings/year) (n=1515 controls/1468 cases) (Steck et al., 2007). Residential stove and fireplace use was defined as using a stove/fireplace in a Long Island residence for at