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Polycyclic Aromatic Hydrocarbon (PAH)-DNA adducts and Breast Cancer: Modification by Gene Promoter Methylation in a Population-Based Study

Alexandra J. White[1,*], **Jia Chen**[2,3,4], **Lauren E. McCullough**[1], **Xinran Xu**[5], **Yoon Hee Cho**[6], **Susan L. Teitelbaum**[2], **Alfred I. Neugut**[7,8], **Mary Beth Terry**[7], **Hanina Hibshoosh**[9], **Regina M. Santella**[6], and **Marilie D. Gammon**[1]

[1]Department of Epidemiology, University of North Carolina, Chapel Hill, NC, USA

[2]Department of Preventive Medicine, Ichan School of Medicine at Mt. Sinai, New York, NY, USA

[3]Department of Oncological Science, Ichan School of Medicine at Mt. Sinai, New York, NY, USA

[4]Department of Pediatrics, Ichan School of Medicine at Mt. Sinai, New York, NY, USA

[5]Research Center for Translational Medicine, Shanghai East Hospital of Tongji University School of Medicine, Shanghai, China

[6]Department of Environmental Health Sciences, Columbia University, New York, NY, USA

[7]Department of Epidemiology, Columbia University, New York, NY, USA

[8]Department of Medicine, Columbia University, New York, NY, USA

[9]Department of Pathology, Columbia University, New York, NY, USA

Abstract

Purpose—Polycyclic aromatic hydrocarbons (PAH)-DNA adducts have been associated with breast cancer incidence. Aberrant changes in DNA methylation may be an early event in carcinogenesis. However, possible relations between PAH-DNA adducts, methylation and breast cancer are unknown. The objectives of this study were to (1) assess associations between PAH-DNA adducts and breast cancer, stratified by DNA methylation markers; and, (2) to examine interactions between adducts and DNA methylation in association with breast cancer and tumor subtype.

Methods—In a population-based case-control study, promoter methylation of 13 breast cancer-related genes was measured in tumor tissue (n=765-851 cases). Blood DNA from breast cancer cases (n=873) and controls (n=941) was used to assess PAH-DNA adducts and global methylation. Logistic regression was used to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CI); and the ratio of the OR (ROR) was used to assess heterogeneity.

[*]Corresponding Author: Alexandra J. White, MSPH; Department of Epidemiology, CB#7435, McGavran-Greenberg Hall, University of North Carolina, Chapel Hill, NC, 27599-7435 USA, Telephone: (919) 966-7421; Fax: (919) 966-2089; whitea@unc.edu.

Conflicts of interest: none.

Supplemental Material.
Polycyclic Aromatic Hydrocarbon (PAH)-DNA adducts and Breast Cancer: Global DNA Methylation Results.

Results—Women with detectable PAH-DNA adducts and methylated *RARβ* (ROR=2.69, 95%CI 1.02-7.12; p for interaction=0.03) or *APC* (ROR=1.76, 95%CI 0.87-3.58; p for interaction=0.09) genes were more likely to have hormone receptor-positive tumors than other subtypes. Interactions with other methylation markers were not apparent (p≥0.10). The association between adducts and breast cancer did not vary by methylation status of the tumor nor did adducts associate with global methylation in the controls.

Conclusions—Gene-specific methylation of *RARβ,* and perhaps *APC,* may interact with PAHDNA adducts to increase risk of hormone receptor-positive breast cancer. There was little evidence that adducts were associated with or interacted with other methylation markers of interest.

Keywords

polycyclic aromatic hydrocarbons; breast cancer; DNA methylation

Breast cancer is the most commonly diagnosed cancer among women in the United States (US), excluding non-melanoma skin cancer [1]. Polycyclic aromatic hydrocarbons (PAHs) are one of the few environmental exposures to be linked to breast cancer risk in several epidemiological studies [2-5] and induce mammary tumors in laboratory animals [6]. PAHs form during combustion of organic material [7]. Major ambient, PAH sources include tobacco smoke, diet and indoor and outdoor air pollution [7].

PAH-DNA adducts are a biomarker of PAH exposure, the measurement of which reflects the resulting level of DNA damage and thus represents the effective biological PAH dose [8]. PAH-DNA adducts generally represent exposures ranging in the preceding few months to few years due to high cell turnover rates [5]. However, PAHs are lipophilic compounds and can be stored in adipose tissue and consequently be released over time [3].

Aberrant DNA methylation, both global and gene-specific, has been shown previously to be relevant to breast carcinogenesis [9-11]. Decreased global methylation is hypothesized to be associated with increased genomic instability and mutation rates [12]. Previously, we reported an increase in risk with breast cancer for luminometric methylation assay (LUMA), but not for methylation of long interspersed elements-1 (LINE-1) in white blood cells [9]. In contrast, increased methylation of tumor suppressor genes, typically indicative of the gene being ‘silenced,’ has been considered to be relevant to cancer incidence [13] and has been demonstrated to be associated with breast cancer clinical/pathological factors and mortality in this study population [14]. Previous research has found that exposure to PAH may be associated with changes in DNA methylation [15-17].

While both PAH-DNA adducts and aberrant DNA methylation are known to be relevant to breast cancer, no previous research has investigated their potential interaction with breast cancer incidence. DNA methylation may promote spontaneous deamination, enhance DNA binding of carcinogens such as PAH, and increase ultraviolet absorption by DNA; all mechanisms that may result in increased DNA adduct formations and gene inactivation [18]. Thus, it is biologically plausible that PAH-DNA adducts and methylation may have a synergistic impact on breast carcinogenesis.

In this same study population, we have previously reported an OR of 1.29 (95%CI 1.05, 1.58) for the association between breast cancer incidence and detectable PAH-DNA adducts relative to women with non-detectable adducts [4]. For this current study, we first aimed to further examine the association between PAH-DNA adducts and breast cancer incidence by stratifying by the promoter methylation status of a panel of 13-breast cancer related genes (APC, BRCA1, CCND2, CDH1, DAPK1, ESR1, GSTP1, HIN1, CDKN2A, PGR, RARβ , RASSF1A and *TWIST1*), measured in the tumor tissue of cases. These 13 genes were chosen because of their established roles in breast carcinogenesis [11]. The promoters of steroid hormone genes (*ESR1,PGR, RARβ*) and tumor suppressors (*BRCA1, APC, CDKN2, HIN1, CDH1, RASSF1a, DAPK1*) are often hypermethylated in breast tumor tissues and methylation of these genes has been associated with malignancy and survival [19-28]. Hypermethylation at the promoter region is hypothesized to be the cause, or at least a marker, of loss of gene function [11]. We also investigated whether PAH-DNA adducts were associated with global methylation in controls, using two independent global methylation markers, LINE-1 and LUMA, measured in blood DNA. Further, we investigated the possibility of effect measure modification between PAH-DNA adducts and DNA methylation with breast cancer and tumor heterogeneity by hormone receptor status.

Materials and Methods

The investigation detailed here builds upon existing population-based resources from the Long Island Breast Cancer Study Project (LIBCSP). The parent LIBCSP methods have been previously reported in detail [29]. Institutional Review Board approval was obtained Institutional Review Board (IRB) approval was obtained from UNC-CH, Columbia University and Mt. Sinai Medical Center.

Study Population

For our DNA gene-specific methylation approach, our study draws primarily upon data collected from the case participants of the LIBCSP; and for global methylation, our study draws upon data from both the LIBCSP cases and controls. The LIBCSP cases and controls were English-speaking women residing in Nassau and Suffolk counties on Long Island, New York.

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, were eligible as cases. Cases were identified using rapid case ascertainment via daily/weekly contact with pathology departments of all 28 hospitals on Long Island and three tertiary care hospitals in New York City. Breast cancer diagnoses were confirmed by the physician or the medical record.

Controls were women with no previous history of breast cancer, and residents of either Nassau or Suffolk counties in 1996-1997, were eligible as controls. Controls were frequency matched in 5-year age groups to cases based on the expected age distribution of case women. Controls were identified 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.

Parent study participants included 1,508 cases and 1,556 controls (82% and 62.7%, respectively, of all eligible subjects), who completed the structured, epidemiologic questionnaires administered by trained interviewers, shortly after diagnosis or identification. Written informed consent was obtained from all study participants prior the interview and blood collection. Participants ranged in age from 20-98 years and 67% were postmenopausal at the time of diagnosis for cases, and time of identification for controls; and 94% reported their race as white, 4% as black, and 2% as other, which is consistent with the underlying racial/ethnic distribution in these two NY counties at the time of data collection.

Exposure Assessment

PAH-DNA adducts—The assessment of PAH-DNA adducts, and the modest positive association observed between these adducts and breast cancer risk has been previously published [5,4]. Briefly, 73.0% of cases and 73.3% of controls who completed the questionnaire donated nonfasting blood samples, approximately three months after diagnosis for cases (and thus prior to chemotherapy for 77.2%) and six months after identification for controls. These samples were then shipped overnight at room temperature to Columbia University in New York City. Samples were processed and stored at −80°C. DNA was extracted from blood samples and was used to assess PAHDNA adduct levels in mononuclear cells by competitive enzyme-linked immunosorbent assay (ELISA). Laboratory assays of the PAH-DNA adduct was successfully completed for 873 cases and 941 controls [4]. The limit of detection for the ELISA assay was defined as <15% inhibition [5]. PAH-DNA adducts were defined as detectable versus non-detectable as previous study findings [5,4] did not support a dose-response association.

Gene-specific promoter DNA methylation assessment—Promoter methylation status was measured in tumor tissue for a panel of 13 breast cancer-related genes (APC, BRCA1, CCND2, CDH1, DAPK1, ESR1, GSTP1, HIN1, CDKN2A, PGR, RARβ , RASSF1A and *TWIST1*). These genes are known to play an important role in breast carcinogenesis and their promoter regions are frequently methylated in breast tumor tissues [11].

The methods used to determine gene-specific promoter methylation levels have been previous published and are briefly described below [9,11]. Tumor blocks were acquired and DNA was extracted as described in previously published methods [30].To determine gene-specific promoter methylation levels for *ESR1*, *PGR* and *BRCA1*, methylation-specific PCR was used [30,31]. DNA was considered methylated if PCR product was yielded using the methylation-specific primers. This produces a dichotomous outcome, methylated or not methylated. Thus, *ESR1*, *PGR* and *BRCA1* are dichotomous variables (methylated vs. unmethylated) as determined by the assay. The MethyLight assay was used to measure the methylation status of the remaining genes [32,33]. Genomic bisulfite-converted DNA is amplified by fluorescence-based, real-time quantitative PCR which provides a quantitative assessment of percentage methylated [34,35]. Number of samples completed and percent methylated for each promoter in LIBCSP has been reported; number of samples ranges between n=765 and n=851 and percent methylation ranges between 3.6% and 62.9% [11]. Continuous values were dichotomized (<4%, ≥4% methylated) to be consistent with

previous published reports by our study team and others [11,33]. This methylation cutpoint has been found to best distinguish between malignant and normal tissues as well as to indicate repressed gene expression when using the MethyLight assay [36,37].

Global Methylation Assessment—As previously described [9], two independent, but complimentary, methods were used to assess global methylation levels in DNA extracted from blood samples: (1) the analysis of LINE-1, which is an approximate measure of methylation levels in repetitive elements or transposons; and, (2) LUMA, which measures levels of 5-mC in the 'CCGG' sequence frequently found in gene promoters. For both LINE-1 and LUMA, methylation assessment was completed for 1,055 cases and 1,101 controls.

The LINE-1 assay was completed using a prevalidated pyrosequencing assay to assess 4 CpG sites in the promoter of LINE-1 at EpigenDx (Worcester, MA, USA) as described previously [9]. Methylation status at each of the 4 CpG loci was analyzed individually as a T/C single nucleotide polymorphism (SNP) using QCpG software (Qiagen). Methylation status data at all 4 loci were averaged to provide an overall percent 5-^{m}C status. The methods used for the LUMA assay in LIBCSP has been previously described [9]. This method has been previously validated using samples with known DNA methylation levels [38]. LUMA methylation level is expressed as a percentage obtained using the following equation [38]: methylation (%) = ((1-(HpaII∑G/∑T)/ (MspI∑G/∑T) * 100)). For LUMA quality control, cases and controls were assayed at the same time and laboratory personnel were blinded. Randomly selected samples were replicated to examine potential batch effects and to determine any variation between different runs and the percent corresponding inter-individual variability (CV) was less than 1%.

Hormone receptor subtype—We abstracted data recorded on the medical record to ascertain breast cancer subtype defined by hormone receptor status [29]. Estrogen and progesterone receptor status (ER/PR) of the first primary breast cancer was available from the medical record for 990 cases (65.6%).

Statistical Analysis

All analyses were completed using SAS 9.3 (Cary, NC).

Gene-specific promoter methylation—To investigate whether PAH-DNA adducts (detectable/nondetectable) were associated with breast cancer, stratified by gene-specific promoter methylation levels measured in case tumor tissue, we used a case-control approach. Polytomous logistic regression [39] was used to estimate ORs and ratio of the odds ratios (RORs) with corresponding 95%CIs with the case groups determined by tumor methylation status.

To assess whether PAH-DNA adducts and gene-specific methylation interacted to influence the development of hormonally-defined breast cancer subtypes, we utilized both a case-case [40] and a case-control approach [39]. First, we assessed whether the association between PAH-DNA adducts and ERPR status varied across methylation group, by evaluating the multiplicative interaction between adducts and gene-specific promoter methylation using a

case-case approach. We estimated the association between detectable PAH-DNA adducts and tumor subtype within strata of gene-specific promoter methylation using a case-control approach to calculate ORs and 95%CI. We also estimated RORs and 95%CIs for the association between PAH-DNA adducts with breast cancer characterized by tumors subtype, stratified by gene-specific promoter methylation [41]. We considered two definitions for the outcome, tumor subtype: (1) hormone receptor positive (ER+PR+) tumors compared to all other subtypes (ER-PR-, ERPR+, ER+PR-) and; (2) hormone receptor negative (ER-PR-) tumors compared to all other subtypes (ER+PR+, ER-PR+, ER+PR-). When using a case-case approach in this scenario, the ROR is an estimation of the likelihood of a certain tumor subtype given the combined methylation status and PAHDNA adduct level and can be interpreted as a measure of heterogeneity [40] as well as a measurement of the interaction on the multiplicative scale [42]. If the gene-specific promoter sample size within strata was less than 5, it was no longer considered. Multiplicative interaction was assessed by comparing polytomous regression models (outcome defined as 0=controls, 1=unmethylated cases, 2=methylated cases) with and without cross-product terms to denote the interaction between adducts and the individual methylation marker using an *a priori* alpha level of 0.10 [41]. A significant interaction indicated that the odds of having a certain tumor type, given PAH-DNA adduct level, is statistically different across strata of tumor methylation.

Global Methylation—We used a controls-only approach to assess whether PAH-DNA adducts (detectable vs. nondetectable) are associated with global methylation levels measured in the blood of controls only. We limited to controls as cases may have lower global methylation levels [12]. We conducted unconditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CI) [41], with global methylation markers as the outcome. LINE-1 and LUMA levels were dichotomized based on the distribution among controls.

We use a case-control approach to assess whether PAH-DNA adducts interact with global methylation global methylation (LUMA and LINE-1) to influence breast cancer risk. We conducted unconditional logistic regression [41] among cases and controls. Multiplicative interaction was assessed by comparing multivariable models with and without cross-product terms to denote the interaction between adducts and the individual methylation marker using an *a prior* alpha level of 0.10 [41]. To explore heterogeneity of the estimates for the interaction between global methylation markers and PAHDNA adducts across breast cancer subtype we used a case-case approach [40].

Confounders—Potential confounders considered included age at diagnosis, age at menarche (continuous), body mass index (weight in kilograms/height in meters squared, continuous), lactation (yes, no), income (<$15,000, $15,000-$19,000, $20,000-$24,999, $25,000 - $34,999, $35,00-$49,999, $50,000-$69,999, $70,00-$89,000, ≥$90,000), active/passive smoking history (no active nor passive, active but no passive, passive but not active, active and passive), and average lifetime intake of grilled and smoked food (servings per year, quintiles). Confounders were included in the model if they were significantly associated with PAH-DNA adducts or the methylation marker and if they changed the

estimate by greater than 10%. Using this criterion, only 5-year age group remained in the models as a confounder; thus all models include 5-year age group.

Sensitivity Analyses

Approximately three-quarters of the cases had not undergone chemotherapy prior to blood sample. We conducted a sensitivity analysis restricting to cases who gave blood prior to chemotherapy.

Results

The age distribution of the LIBCSP study population by case status and PAH-DNA adduct levels is shown in Table I.

Gene-Specific Promoter Methylation

The association between detectable PAH-DNA adducts and breast cancer, stratified by the 13 gene-specific promoter methylation levels in case tumor tissue is displayed in Table II. Cases with detectable adducts were less likely to have a methylated *HIN1* gene(ROR=0.66, 95%CI 0.42-1.05), although the confidence intervals included the null value and the interaction was not statistically significant ($p \geq 0.10$). The remaining 12 gene-specific promoter methylation levels did not appear to impact the association between detectable PAH-DNA adducts and breast cancer incidence.

We hypothesized that PAH-DNA adducts and gene-specific promoter methylation levels may interact to influence the likelihood of developing hormonally-responsive breast cancer. While these associations are limited by the low prevalence of these methylated markers in our hormone-responsive case population, we did find evidence of a multiplicative interaction with methylation of *RARβ* (p=0.03), and *APC* (p=0.09). The association between PAH-DNA adducts and ER+PR+ tumor subtype by promoter methylation levels are shown in Table III. When compared to controls, cases with detectable PAH-DNA adducts and methylated *RARβ* or *APC* were more likely to have hormone receptor-positive breast cancer (*RARβ,* OR=2.15, 95%CI 1.03-4.47; *APC,* OR=1.59, 95%CI 0.98-2.58) than to have other tumor subtypes (*RARβ,* OR=0.80, 95%CI 0.41-1.56; *APC,* OR=0.91, 95%CI 0.52-1.58). Thus, the joint association of PAH-DNA adducts and methylation of these two genes varied across tumor subtypes (ER+PR+ vs all others, *RARβ,* ROR=2.69, 95%CI 1.02-7.12; *APC,* ROR=1.76; 95%CI 0.87-3.58). Conversely, women without a methylated *RARβ* or *APC* were less likely to develop ER+PR+ breast cancer compared to other subtypes (*RARβ,* ROR=0.79, 95%CI 0.42-1.46; *APC,* ROR=0.73, 95%CI 0.35-1.53).

The interaction between detectable adducts and methylation of *RARβ* was robust and remained significant when ER+PR+ tumors were compared to ER-PR- tumors alone (p interaction=0.05); however, the interaction with *APC* was no longer significant (p interaction =0.7). Further, no three-way interaction was observed between age, gene specific methylation and PAH-DNA adducts suggesting that age did not drive this association (*APC,* p interaction=0.7; *RARβ,* p for interaction=0.5). We did not observe interactions with the remaining six gene promoters when using a case-case approach ($p \geq 0.10$) (Table III). Although not a significant interaction as defined by our *a priori* criteria, we did observe a

similar trend with the gene promoter *GSTP1*; with an elevated association with ER+PR+ tumors among women with detectable adducts and a methylated *GSTP1* (Table III). When investigating potential interaction between adducts and methylated genes in association with ER-PR- breast cancer, we did not find any evidence of significant multiplicative interaction for the five gene promoters tested ($p \geq 0.10$) (Table IV).

Global Methylation

Using a control-only approach, detectable PAH-DNA adducts were modestly associated with LINE-1 methylation (OR=1.25, 95%CI 0.94-1.65), but not with LUMA methylation levels (OR=0.92, 95%CI 0.70-1.22); however, confidence intervals for both effect estimates included the null value (Table V). Using a case-control approach, we investigated potential interaction, between PAH-DNA adducts and global DNA methylation (either LINE-1 or LUMA), in association with breast cancer risk, but did not observe evidence of interactions on the multiplicative or additive scales (Supplemental Table I). We additionally investigated, using a case-case approach, interactions between PAH-DNA adducts and LUMA when considering breast cancer tumor subtype, and found no evidence of interaction (Supplemental Table II).

Sensitivity analyses

When we restricted our analyses to women who donated blood prior to chemotherapy, our results remained similar (data not shown).

Discussion

In our population-based study, we observed interactions between PAH-DNA adducts and select gene-specific promoter methylation levels with hormone-receptor positive breast cancer tumor subtype. Although our conclusions are limited by the number of subjects with positive PAH-DNA adducts and DNA methylation, we found that women with detectable adducts and methylated *RARβ* or *APC* genes were more likely to have ER+PR+ breast cancer compared to other subtypes. We also found that detectable PAH-DNA adducts were associated with *HIN1* methylation in tissue of cases and LINE-1 in blood of controls, although the confidence intervals included the null value. In contrast, we did not find evidence that the association between PAH-DNA adducts and breast cancer incidence was modified by global DNA methylation markers. Further, we found little evidence that the other methylation markers of interest interacted with PAH-DNA adducts to influence breast cancer incidence or tumor subtype. To our knowledge, this is the first study to investigate potential interactions of global and gene-specific methylation with the relationship between PAH-DNA adducts and breast cancer.

Findings from previous studies suggest that breast cancer may have distinct patterns of CpG island methylation according to subtypes [14]. *RARβ* is an established target for silencing by epigenetic modifications in tumor as part of an early event of carcinogenesis [43]. Our results suggest that epigenetic inactivation of tumor suppressor genes, in particular *RARβ* and *APC*, may interact with PAH-DNA adducts, to influence hormone receptor status of the tumor. Thus, DNA methylation patterns may result in an increased susceptibility to certain

breast cancer tumor subtypes given exposure to PAH. However, the mechanism for this relation is not elucidated and requires replication in other study populations. APC has been shown in cell lines to have a role in DNA repair [44]. Silencing of APC via DNA methylation may thus impact the efficiency of DNA repair processes. Functioning DNA repair is very important in the presence of PAH-DNA adducts and not correcting this damage could lead to breast carcinogenesis [6]. Additionally, methylation at APC has previously been found to correlated with ER positivity in tumor tissue [45] and methylation of *RARβ* has been correlated with PR positivity in the LIBCSP [14]. Additional information on the interplay between environmental exposures and epigenetic variation by breast tumor subtype may provide a better understanding of molecular differences. We did not observe interactions with the 11 remaining genes considered in this study, many of which, such as *GSTP1*, are known to be relevant to breast cancer and metabolism of PAHs [46]. The low prevalence of these methylation biomarkers in our study sample may have contributed to our inability to detect modest interactions.

In our LIBCSP cases detectable PAH-DNA adducts were found to be possibly associated with *HIN1* promoter methylation levels but not any of the other remaining 12 genes. Although limited, previous studies have reported that both hyper- and hypomethylation of tumor suppressor genes are associated with DNA adducts [47,17]. For example, one study reported *IL-6* hypermethylation and *p53* hypomethylation to be correlated with anti-benzo[a]pyrene diol epoxide-DNA adduct levels [48]. Therefore, any associations between adducts and gene-promoter methylation is likely site-specific.

Methylation frequencies may vary based on factors such as laboratory assay, progression of the tumor and demographics such as race [49]. Lewis et al. 2005 reported the frequency of methylation in tumor tissues to be about 57% for *APC* and about 59% for *RASSF1a* when using methylation-specific PCR in a small convenience sample of US women (n=38 breast cancer cases) [50]. Another clinic-based study of women in Turkey (n=40 breast cancer cases) measured methylation using MethyLight assay and found frequencies to be 18% for *BRCA1*, 75% for *HIN1*%, 83% for *RASSF1a*, 23% for *CDH1*, 25% for *RARB*, 53% for *APC*, 18% for *TWIST* and 30% for *CCND2* [51] . In LIBCSP (n=765-851 breast cancer cases), methylation frequencies were 48% for *APC*, 85% for *RASSF1a*, 59% for *BRCA1*, 63% for *HIN1*, 6% for *CDH1*, 28% for *RARB*, 15% for *TWIST* and 20% for *CCND2* [11]. So, the frequencies of certain methylation sites are similar across studies, including our own much larger population-based study, but there is some variability, which could be due to differences in the populations that were sampled as well as the laboratory methods employed.

Some promoter methylation is necessary in order to maintain normal cell function and studies that measure gene-specific methylation in tissue of women without breast cancer do report lower frequencies of methylation. One study found that women with breast cancer were more likely to have methylation at *RARβ2*, *RASSF1a*, *CCND2*, *APC* compared to normal breast tissue among women without breast cancer [50]. They also reported that the likelihood of having two or more methylated genes was significantly higher in cancer tissue compared to the normal breast tissue of women without breast cancer [50]. Similarly, Fackler and colleagues reported methylation levels of *RASSF1a*, *Cyclin D2*, *HIN1* and

TWIST were higher in malignant breast tissues compared to adjacent normal tissue among women with breast cancer [27]. It is generally accepted that the highest methylation levels occur in the malignant tissue, followed by normal tissue adjacent to the malignancy, and that women without breast cancer have the lowest levels. Thus, any case-control results or results from comparing malignant vs. adjacent tissue in the same woman, would yield underestimates of a true methylated vs. unmethylated tissue comparison. Consequently, the case-case comparisons may better reflect truth, because we actually have measured methylation levels in the tissue of all case women. Hence, we only present results from case-case comparisons for this study.

Hypothesized to represent decreased genomic integrity, LINE-1 hypomethylation may be associated with cancer, including breast cancer [12]. However, LINE-1 hypomethylation was not found to be significantly associated with breast cancer in the LIBCSP [9], and a recent meta-analysis concluded there was no association with LINE-1 and overall cancer risk [12]. As shown in our supplementary information, PAH-DNA adducts were imprecisely associated with LINE-1 hypermethylation in our population-based sample of ambiently exposed women, but not LUMA. A study of prolonged B[a]P exposure in mouse embryonic fibroblasts found global DNA hypermethylation was associated with DNA-methyltransferase overexpression [52]. DNMTs bind to DNA lesions with high affinity when there is high DNA damage [53], which might explain at least in part this result of increased methylation of LINE-1 in association with adduct levels reported here and in other studies [54,48,47].

A strength of our study is its population-based design, where we recruited women newly diagnosed with a first primary breast cancer in a single year who were residents of a prescribed geographic area. Additionally, it was conducted in women who were ambiently exposed to PAH [29,5], a group that is often understudied because of the challenges with identifying associations in lower exposed individuals. We were also able to utilize the extensive resources of the LIBCSP, including tumor subtype information, and were able to take into account both global and gene-specific DNA methylation. Despite being the largest study to investigate this research question to date, estimates, especially in the case-case analysis, had wide confidence intervals. Therefore, future studies would benefit from a larger sample size to more precisely estimate some potentially modest associations between the gene-specific promoter methylation with tumor subtype. Similarly, although we investigated more DNA methylation markers than any other study to date, future studies would benefit from including additional markers, which may play a role in the association between PAH-DNA adducts and breast cancer. Due the use of methylation-specific PCR for *ESR1*, *PCR* and *BRCA1*, which is less sensitive than the MethyLight assay, we may have not been able to detect very low levels of methylation for these genes. We did not adjust for multiple comparisons in this study; as such, we did not rely heavily on statistical significance in the interpretation of the results and all comparisons were driven by previous hypothesis and were considered to be biologically plausible [55]. However, we cannot rule out the possibility that due to the number of comparisons made, we may have some estimates that are false positives.

Ours is the first epidemiologic study to report an interaction between gene-specific promoter methylation levels in *RARβ* and PAH-DNA adducts in association with hormone receptor positive tumor subtype, suggesting that these may be important factors in tumor subtype differentiation. However, few other associations or interactions between adducts and methylation markers were observed. Better understanding of the factors that influence breast cancer subtype is crucial; tumor subtype is considered in treatment decisions and is associated with differences in survival. These results require replication and further investigation of the biological mechanism to better understand the implications to carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. American Cancer Society. Cancer Facts and Figures 2013. American Cancer Society; Atlanta, GA: 2013.
2. Perera FP, Estabook A, Hewer A, Channing K, Rundle A, Mooney LA, Whyatt R, Phillips DH. Carcinogen-DNA Adducts in Human Breast-Tissue. Cancer Epidemiology Biomarkers & Prevention. 1995; 4(3):233–238.
3. Rundle A, Tang DL, Hibshoosh H, Estabrook A, Schnabel F, Cao WF, Grumet S, Perera FP. The relationship between genetic damage from polycyclic aromatic hydrocarbons in breast tissue and breast cancer. Carcinogenesis. 2000; 21(7):1281–1289. doi:10.1093/carcin/21.7.1281. [PubMed: 10874004]
4. Gammon MD, Sagiv SK, Eng SM, Shantakumar S, Gaudet MM, Teitelbaum SL, Britton JA, Terry MB, Wang LW, Wang Q, Stellman SD, Beyea J, Hatch M, Kabat GC, Wolff MS, Levin B, Neugut AI, Santella RM. Polycyclic aromatic hydrocarbon-DNA adducts and breast cancer: a pooled analysis. Arch Environ Health. 2004; 59(12):640–649. doi:10.1080/00039890409602948. [PubMed: 16789472]
5. Gammon MD, Santella RM, Neugut AI, Eng SM, Teitelbaum SL, Paykin A, Levin B, Terry MB, Young TL, Wang LW, Wang Q, Britton JA, Wolff MS, Stellman SD, Hatch M, Kabat GC, Senie R, Garbowski G, Maffeo C, Montalvan P, Berkowitz G, Kemeny M, Citron M, Schnabel F, Schuss A, Hajdu S, Vinceguerra V. Environmental toxins and breast cancer on Long Island. I. Polycyclic aromatic hydrocarbon DNA adducts. Cancer Epidemiology Biomarkers & Prevention. 2002; 11(8): 677–685.
6. Hecht SS. Tobacco smoke carcinogens and breast cancer. Environ Mol Mutagen. 2002; 39(2-3): 119–126. doi:10.1002/em.10071. [PubMed: 11921179]
7. Boström CE, Gerde P, Hanberg A, Jernstrom B, Johansson C, Kyrklund T, Rannug A, Tornqvist M, Victorin K, Westerholm R. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ Health Perspect. 2002; 110(Suppl 3):451–488. [PubMed: 12060843]
8. Godschalk RW, Van Schooten FJ, Bartsch H. A critical evaluation of DNA adducts as biological markers for human exposure to polycyclic aromatic compounds. J Biochem Mol Biol. 2003; 36(1): 1–11. [PubMed: 12542969]
9. Xu X, Gammon MD, Hernandez-Vargas H, Herceg Z, Wetmur JG, Teitelbaum SL, Bradshaw PT, Neugut AI, Santella RM, Chen J. DNA methylation in peripheral blood measured by LUMA is

associated with breast cancer in a population-based study. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2012; 26(6):2657–2666. doi: 10.1096/fj.11-197251. [PubMed: 22371529]

10. Choi JY, James SR, Link PA, McCann SE, Hong CC, Davis W, Nesline MK, Ambrosone CB, Karpf AR. Association between global DNA hypomethylation in leukocytes and risk of breast cancer. Carcinogenesis. 2009; 30(11):1889–1897. doi:10.1093/carcin/bgp143. [PubMed: 19584139]
11. Xu X, Gammon MD, Jefferson E, Zhang Y, Cho YH, Wetmur JG, Teitelbaum SL, Bradshaw PT, Terry MB, Garbowski G, Hibshoosh H, Neugut AI, Santella RM, Chen J. The influence of one-carbon metabolism on gene promoter methylation in a population-based breast cancer study. Epigenetics : official journal of the DNA Methylation Society. 2011; 6(11):1276–1283. doi: 10.4161/epi.6.11.17744; 10.4161/epi.6.11.17744.
12. Brennan K, Flanagan JM. Is there a link between genome-wide hypomethylation in blood and cancer risk? Cancer Prev Res (Phila). 2012; 5(12):1345–1357. doi: 10.1158/1940-6207.capr-12-0316. [PubMed: 23135621]
13. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nature reviews Genetics. 2012; 13(7):484–492. doi:10.1038/nrg3230.
14. Cho YH, Shen J, Gammon MD, Zhang YJ, Wang Q, Gonzalez K, Xu X, Bradshaw PT, Teitelbaum SL, Garbowski G, Hibshoosh H, Neugut AI, Chen J, Santella RM. Prognostic significance of gene-specific promoter hypermethylation in breast cancer patients. Breast Cancer Res Treat. 2012; 131(1):197–205. doi:10.1007/s10549-011-1712-y. [PubMed: 21837480]
15. Tang WY, Levin L, Talaska G, Cheung YY, Herbstman J, Tang D, Miller RL, Perera F, Ho SM. Maternal Exposure to Polycyclic Aromatic Hydrocarbons and 5'-CpG Methylation of Interferon-gamma in Cord White Blood Cells. Environ Health Perspect. 2012; 120(8):1195–1200. doi: 10.1289/ehp.1103744. [PubMed: 22562770]
16. Shenker NS, Ueland PM, Polidoro S, van Veldhoven K, Ricceri F, Brown R, Flanagan JM, Vineis P. DNA Methylation as a Long-term Biomarker of Exposure to Tobacco Smoke. Epidemiology. 2013; 24(5):712–716. doi:10.1097/EDE.0b013e31829d5cb3. [PubMed: 23867811]
17. Duan H, He Z, Ma J, Zhang B, Sheng Z, Bin P, Cheng J, Niu Y, Dong H, Lin H, Dai Y, Zhu B, Chen W, Xiao Y, Zheng Y. Global and MGMT promoter hypomethylation independently associated with genomic instability of lymphocytes in subjects exposed to high-dose polycyclic aromatic hydrocarbon. Arch Toxicol. 2013; 87(11):2013–2022. doi:10.1007/s00204-013-1046-0. [PubMed: 23543013]
18. Baylin SB. DNA methylation and gene silencing in cancer. Nature Clinical Practice Oncology. 2005; 2:S4–S11.
19. Jin Z, Tamura G, Tsuchiya T, Sakata K, Kashiwaba M, Osakabe M, Motoyama T. Adenomatous polyposis coli (APC) gene promoter hypermethylation in primary breast cancers. Br J Cancer. 2001; 85(1):69–73. doi:10.1054/bjoc.2001.1853. [PubMed: 11437404]
20. Silva J, Dominguez G, Villanueva M, Gonzalez R, Garcia J, Corbacho C, Provencio M, Espana P, Bonilla F. Aberrant DNA methylation of the p16INK4a gene in plasma DNA of breast cancer patients. Br J Cancer. 1999; 80(8):1262. [PubMed: 10376981]
21. Agathanggelou A, Honorio S, Macartney DP, Martinez A, Dallol A, Rader J, Fullwood P, Chauhan A, Walker R, Shaw JA. Methylation associated inactivation of RASSF1A from region 3p21. 3 in lung, breast and ovarian tumours. Oncogene. 2001; 20(12):1509–1518. [PubMed: 11313894]
22. Dulaimi E, Hillinck J, Ibanez de Caceres I, Al-Saleem T, Cairns P. Tumor suppressor gene promoter hypermethylation in serum of breast cancer patients. Clin Cancer Res. 2004; 10(18 Pt 1): 6189–6193. doi:10.1158/1078-0432.ccr-04-0597. [PubMed: 15448006]
23. Nass SJ, Herman JG, Gabrielson E, Iversen PW, Parl FF, Davidson NE, Graff JR. Aberrant methylation of the estrogen receptor and E-cadherin 5′ CpG islands increases with malignant progression in human breast cancer. Cancer Res. 2000; 60(16):4346–4348. [PubMed: 10969774]
24. Sirchia SM, Ferguson AT, Sironi E, Subramanyan S, Orlandi R, Sukumar S, Sacchi N. Evidence of epigenetic changes affecting the chromatin state of the retinoic acid receptor beta2 promoter in breast cancer cells. Oncogene. 2000; 19(12):1556–1563. [PubMed: 10734315]

25. Esteller M, Corn PG, Urena JM, Gabrielson E, Baylin SB, Herman JG. Inactivation of glutathione S-transferase P1 gene by promoter hypermethylation in human neoplasia. Cancer Res. 1998; 58(20):4515–4518. [PubMed: 9788592]
26. Evron E, Umbricht CB, Korz D, Raman V, Loeb DM, Niranjan B, Buluwela L, Weitzman SA, Marks J, Sukumar S. Loss of cyclin D2 expression in the majority of breast cancers is associated with promoter hypermethylation. Cancer Res. 2001; 61(6):2782–2787. [PubMed: 11289162]
27. Fackler MJ, McVeigh M, Mehrotra J, Blum MA, Lange J, Lapides A, Garrett E, Argani P, Sukumar S. Quantitative multiplex methylation-specific PCR assay for the detection of promoter hypermethylation in multiple genes in breast cancer. Cancer Res. 2004; 64(13):4442–4452. doi: 10.1158/0008-5472.can-03-3341. [PubMed: 15231653]
28. Gort EH, Suijkerbuijk KP, Roothaan SM, Raman V, Vooijs M, van der Wall E, van Diest PJ. Methylation of the TWIST1 promoter, TWIST1 mRNA levels, and immunohistochemical expression of TWIST1 in breast cancer. Cancer Epidemiology Biomarkers & Prevention. 2008; 17(12):3325–3330.
29. Gammon MD, Neugut AI, Santella RM, Teitelbaum SL, Britton JA, Terry MB, Eng SM, Wolff MS, Stellman SD, Kabat GC, Levin B, Bradlow HL, Hatch M, Beyea J, Camann D, Trent M, Senie RT, Garbowski GC, Maffeo C, Montalvan P, Berkowitz GS, Kemeny M, Citron M, Schnabe F, Schuss A, Hajdu S, Vincguerra V, Collman GW, Obrams GI. The Long Island Breast Cancer Study Project: description of a multi-institutional collaboration to identify environmental risk factors for breast cancer. Breast Cancer Res Treat. 2002; 74(3):235–254. [PubMed: 12206514]
30. Xu X, Gammon MD, Zhang Y, Bestor TH, Zeisel SH, Wetmur JG, Wallenstein S, Bradshaw PT, Garbowski G, Teitelbaum SL, Neugut AI, Santella RM, Chen J. BRCA1 promoter methylation is associated with increased mortality among women with breast cancer. Breast Cancer Res Treat. 2009; 115(2):397–404. doi:10.1007/s10549-008-0075-5. [PubMed: 18521744]
31. Liu ZJ, Maekawa M, Horii T, Morita M. The multiple promoter methylation profile of PR gene and ERalpha gene in tumor cell lines. Life Sci. 2003; 73(15):1963–1972. [PubMed: 12899921]
32. Eads CA, Danenberg KD, Kawakami K, Saltz LB, Danenberg PV, Laird PW. CpG island hypermethylation in human colorectal tumors is not associated with DNA methyltransferase overexpression. Cancer Res. 1999; 59(10):2302–2306. [PubMed: 10344733]
33. Eads CA, Danenberg KD, Kawakami K, Saltz LB, Blake C, Shibata D, Danenberg PV, Laird PW. MethyLight: a high-throughput assay to measure DNA methylation. Nucleic Acids Res. 2000; 28(8):E32. [PubMed: 10734209]
34. Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. Genome Res. 1996; 6(10):995–1001. [PubMed: 8908519]
35. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987; 162(1):156–159. doi:10.1006/abio.1987.9999. [PubMed: 2440339]
36. Ogino S, Kawasaki T, Brahmandam M, Cantor M, Kirkner GJ, Spiegelman D, Makrigiorgos GM, Weisenberger DJ, Laird PW, Loda M, Fuchs CS. Precision and performance characteristics of bisulfite conversion and real-time PCR (MethyLight) for quantitative DNA methylation analysis. The Journal of molecular diagnostics : JMD. 2006; 8(2):209–217. doi:10.2353/jmoldx.2006.050135. [PubMed: 16645207]
37. Eads CA, Lord RV, Kurumboor SK, Wickramasinghe K, Skinner ML, Long TI, Peters JH, DeMeester TR, Danenberg KD, Danenberg PV, Laird PW, Skinner KA. Fields of aberrant CpG island hypermethylation in Barrett's esophagus and associated adenocarcinoma. Cancer Res. 2000; 60(18):5021–5026. [PubMed: 11016622]
38. Bjornsson HT, Sigurdsson MI, Fallin MD, Irizarry RA, Aspelund T, Cui H, Yu W, Rongione MA, Ekstrom TJ, Harris TB, Launer LJ, Eiriksdottir G, Leppert MF, Sapienza C, Gudnason V, Feinberg AP. Intra-individual change over time in DNA methylation with familial clustering. JAMA. 2008; 299(24):2877–2883. doi:10.1001/jama.299.24.2877. [PubMed: 18577732]
39. Hosmer, D.; Lemeshow, S. Applied Logsitic Regression. vol Book, Whole. John Wiley & Sons; New York: 1989.
40. Begg CB, Zhang ZF. Statistical analysis of molecular epidemiology studies employing case-series. Cancer Epidemiol Biomarkers Prev. 1994; 3(2):173–175. [PubMed: 8049640]

41. Kleinbaum, DG.; Klein, M. Logistic regression: a self-learning text. Springer; 2002.
42. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol. 1996; 144(3): 207–213. [PubMed: 8686689]
43. Chen KM, Stephen JK, Raju U, Worsham MJ. Delineating an epigenetic continuum for initiation, transformation and progression to breast cancer. Cancers (Basel). 2011; 3(2):1580–1592. doi: 10.3390/cancers3021580. [PubMed: 21776373]
44. Narayan S, Jaiswal AS, Balusu R. Tumor suppressor APC blocks DNA polymerase beta-dependent strand displacement synthesis during long patch but not short patch base excision repair and increases sensitivity to methylmethane sulfonate. J Biol Chem. 2005; 280(8):6942–6949. doi: 10.1074/jbc.M409200200. [PubMed: 15548520]
45. Widschwendter M, Siegmund KD, Muller HM, Fiegl H, Marth C, Muller-Holzner E, Jones PA, Laird PW. Association of breast cancer DNA methylation profiles with hormone receptor status and response to tamoxifen. Cancer Res. 2004; 64(11):3807–3813. doi: 10.1158/0008-5472.can-03-3852. [PubMed: 15172987]
46. McCarty KM, Santella RM, Steck SE, Cleveland RJ, Ahn J, Ambrosone CB, North K, Sagiv SK, Eng SM, Teitelbaum SL, Neugut AI, Gammon MD. PAH-DNA Adducts, Cigarette Smoking, GST Polymorphisms, and Breast Cancer Risk. Environ Health Perspect. 2009; 117(4):552–558. doi: 10.1289/ehp.0800119. [PubMed: 19440493]
47. Peluso M, Bollati V, Munnia A, Srivatanakul P, Jedpiyawongse A, Sangrajrang S, Piro S, Ceppi M, Bertazzi PA, Boffetta P, Baccarelli AA. DNA methylation differences in exposed workers and nearby residents of the Ma Ta Phut industrial estate, Rayong, Thailand. Int J Epidemiol. 2012; 41(6):1753–1760. discussion 1761-1753 doi:10.1093/ije/dys129. [PubMed: 23064502]
48. Pavanello S, Bollati V, Pesatori AC, Kapka L, Bolognesi C, Bertazzi PA, Baccarelli A. Global and gene-specific promoter methylation changes are related to anti-B[a]PDE-DNA adduct levels and influence micronuclei levels in polycyclic aromatic hydrocarbon-exposed individuals. Int J Cancer. 2009; 125(7):1692–1697. doi:10.1002/ijc.24492. [PubMed: 19521983]
49. Brooks J, Cairns P, Zeleniuch-Jacquotte A. Promoter methylation and the detection of breast cancer. Cancer Causes Control. 2009; 20(9):1539–1550. doi:10.1007/s10552-009-9415-y. [PubMed: 19768562]
50. Lewis CM, Cler LR, Bu DW, Zochbauer-Muller S, Milchgrub S, Naftalis EZ, Leitch AM, Minna JD, Euhus DM. Promoter hypermethylation in benign breast epithelium in relation to predicted breast cancer risk. Clin Cancer Res. 2005; 11(1):166–172. [PubMed: 15671542]
51. Cho YH, Yazici H, Wu HC, Terry MB, Gonzalez K, Qu M, Dalay N, Santella RM. Aberrant promoter hypermethylation and genomic hypomethylation in tumor, adjacent normal tissues and blood from breast cancer patients. Anticancer Res. 2010; 30(7):2489–2496. [PubMed: 20682973]
52. Yauk CL, Polyzos A, Rowan-Carroll A, Kortubash I, Williams A, Kovalchuk O. Tandem repeat mutation, global DNA methylation, and regulation of DNA methyltransferases in cultured mouse embryonic fibroblast cells chronically exposed to chemicals with different modes of action. Environ Mol Mutagen. 2008; 49(1):26–35. doi:10.1002/em.20359. [PubMed: 18172875]
53. James SJ, Pogribny IP, Pogribna M, Miller BJ, Jernigan S, Melnyk S. Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. J Nutr. 2003; 133(11 Suppl 1):3740S–3747S. [PubMed: 14608108]
54. Herbstman JB, Tang D, Zhu D, Qu L, Sjodin A, Li Z, Camann D, Perera FP. Prenatal exposure to polycyclic aromatic hydrocarbons, benzo[a]pyrene-DNA adducts, and genomic DNA methylation in cord blood. Environ Health Perspect. 2012; 120(5):733–738. doi:10.1289/ehp.1104056. [PubMed: 22256332]
55. Savitz DA, Olshan AF. Multiple comparisons and related issues in the interpretation of epidemiologic data. Am J Epidemiol. 1995; 142(9):904–908. [PubMed: 7572970]

Table I

Age distributions by PAH-DNA adducts (detectable versus nondetectable) and case status, LIBCSP 1996-1997.

	Cases				Controls			
	Detectable		Nondetectable		Detectable		Nondetectable	
Age Group	N	%	N	%	N	%	N	%
<40	12	5.3%	39	6.0%	26	8.9%	60	9.3%
40-50	53	23.2%	128	19.8%	80	27.3%	152	23.5%
50-60	49	21.5%	203	31.5%	81	27.6%	187	28.9%
60-70	55	24.1%	144	22.3%	68	23.2%	158	24.4%
70+	59	25.9%	131	20.3%	38	13.0%	91	14.0%

Table II

Age-adjusted ratio of the ORs and 95%CIs for the association between detectable PAH-DNA adducts and breast cancer, stratified by gene-specific methylation status (methylated and unmethylated breast tumor) using case-control (OR) and case-case (ROR) approaches where within-cell samples sizes ≥5 [a,b]

Genes[c]	PAH-DNA Adducts	Controls	Unmethylated Cases	Unmethylated cases versus controls OR (95%CI)	Methylated Cases	Methylated cases versus controls OR (95%CI)	Ratio of the Odds Ratio (ROR) (95%CI)
ESR1							
	Nondetectable	293	69	1.00 (reference)	54	1.00 (reference)	1.00 (reference)
	Detectable	648	187	1.21 (0.88, 1.65)	164	1.39 (0.98, 1.95)	1.16 (0.76, 1.75)
PR							
	Nondetectable	293	110	1.00 (reference)	14	1.00 (reference)	1.00 (reference)
	Detectable	648	315	1.30 (1.00, 1.69)	40	1.30 (0.69, 2.45)	1.00 (0.59, 1.92)
BRCA1							
	Nondetectable	293	49	1.00 (reference)	75	1.00 (reference)	1.00 (reference)
	Detectable	648	147	1.35 (0.94, 1.93)	208	1.25 (0.92, 1.69)	0.93 (0.61, 1.42)
APC							
	Nondetectable	293	56	1.00 (reference)	59	1.00 (reference)	1.00 (reference)
	Detectable	648	175	1.41 (1.01, 1.97)	163	1.24 (0.89, 1.72)	0.88 (0.57, 1.35)
HIN1							
	Nondetectable	293	33	1.00 (reference)	84	1.00 (reference)	1.00 (reference)
	Detectable	648	121	1.61 (1.06, 2.43)	201	1.09 (0.81, 1.46)	0.66 (0.42, 1.05)
RASSF1A							
	Nondetectable	293	17	1.00 (reference)	100	1.00 (reference)	1.00 (reference)
	Detectable	648	42	1.06 (0.59, 1.92)	280	1.27 (0.97, 1.67)	1.15 (0.62, 2.12)
DAPK							
	Nondetectable	293	96	1.00 (reference)	21	1.00 (reference)	1.00 (reference)
	Detectable	648	273	1.28 (0.97, 1.69)	49	1.03 (0.60, 1.76)	0.82 (0.46, 1.44)
GSTP1							
	Nondetectable	293	79	1.00 (reference)	38	1.00 (reference)	1.00 (reference)
	Detectable	648	235	1.35 (1.00, 1.81)	87	1.04 (0.69, 1.56)	0.80 (0.50, 1.26)
CYCLIND							
	Nondetectable	293	94	1.00 (reference)	23	1.00 (reference)	1.00 (reference)

Genes[c]	PAH-DNA Adducts	Controls	Unmethylated Cases	Unmethylated cases versus controls OR (95%CI)	Methylated Cases	Methylated cases versus controls OR (95%CI)	Ratio of the Odds Ratio (ROR) (95%CI)
	Detectable	648	265	1.27 (0.96, 1.67)	57	1.08 (0.64, 1.80)	0.89 (0.51, 1.53)
TWIST							
	Nondetectable	293	101	1.00 (reference)	16	1.00 (reference)	1.00 (reference)
	Detectable	648	269	1.22 (0.93, 1.60)	53	1.45 (0.80, 2.60)	1.20 (0.65, 2.21)
CDH1							
	Nondetectable	293	109	1.00 (reference)	8	1.00 (reference)	1.00 (reference)
	Detectable	648	305	1.28 (0.98, 1.66)	17	0.87 (0.37, 2.07)	0.72 (0.30, 1.72)
RARB							
	Nondetectable	293	84	1.00 (reference)	33	1.00 (reference)	1.00 (reference)
	Detectable	648	230	1.24 (0.93, 1.66)	92	1.24 (0.81, 1.90)	1.03(0.64, 1.66)

[a] among women on Long Island, NY, LIBCSP 1996-1997

[b] Multiplicative p for interaction for all genes ≥ 0.10; indicates that the odds of being a case, given PAH-DNA adduct level, is not statistically different across strata of methylated breast tumor.

[c] *CDKN2A* excluded as within cell sample size n <5.

Table III

Age-adjusted ORs and 95%CIs for the association between gene-specific methylation and PAH-DNA adducts with ER+PR+ breast cancer vs all others[a,b] using a case-control (RR) and case-case (ROR) approaches where within-cell samples sizes ≥5.

			ER+PR+ cases vs controls				All other (ER+PR−, ER-PR+, ER−PR−) cases vs. controls				ER+PR+ vs. all other (ER+PR−, ER−PR+, ER−PR−) cases			
			Methylated breast tumor		Unmethylated breast tumor		Methylated breast tumor		Unmethylated breast tumor		Methylated breast tumor		Unmethylated breast tumor	
Gene[c,d]	**PAH-DNA Adducts**	**Co**	**Ca**	**OR (95%CI)**	**Ca**	**OR (95% CI)**	**Ca**	**OR (95%CI)**	**Ca**	**OR (95% CI)**	**Ca**	**ROR (95%CI)**	**Ca**	**ROR (95% CI)**
RARβ														
	Nondetect Adducts	293	9	1.00 (reference)	48	1.00 (reference)	14	1.00 (reference)	19	1.00 (reference)	9/14	1.00 (reference)	48/19	1.00 (reference)
	Detectable Adducts	648	43	2.15 (1.03, 4.47)	111	1.05 (0.73, 1.51)	25	0.80 (0.41, 1.56)	56	1.33 (0.77, 2.28)	42/25	2.69 (1.02, 7.12)	111/56	0.79 (0.42, 1.46)
APC														
	Nondetect Adducts	293	23	1.00 (reference)	32	1.00 (reference)	20	1.00 (reference)	13	1.00 (reference)	23/20	1.00 (reference)	32/13	1.00 (reference)
	Detectable Adducts	648	81	1.59 (0.98, 2.58)	81	1.14 (0.74, 1.76)	40	0.91 (0.52, 1.58)	45	1.56 (0.83, 2.94)	81/40	1.76 (0.87, 3.58)	81/45	0.73 (0.35, 1.53)
ESR1														
	Nondetect Adducts	293	24	1.00 (reference)	36	1.00 (reference)	15	1.00 (reference)	18	1.00 (reference)	24/15	1.00 (reference)	36/18	1.00 (reference)
	Detectable Adducts	648	72	1.35 (0.83, 2.19)	95	1.20 (0.80, 1.80)	40	1.20 (0.65, 2.21)	49	1.24 (0.71, 2.17)	72/40	1.12 (0.53, 2.38)	95/49	0.97 (0.50, 1.87)
BRCA1														
	Nondetect Adducts	293	37	1.00 (reference)	23	1.00 (reference)	18	1.00 (reference)	15	1.00 (reference)	37/18	1.00 (reference)	23/15	1.00 (reference)
	Detectable Adducts	648	100	1.22 (0.82, 1.83)	70	1.38 (0.84, 2.25)	52	1.31 (0.75, 2.28)	37	1.12 (0.60, 2.07)	100/52	0.94 (0.49, 1.80)	70/37	1.23 (0.58, 2.65)
RASSF1a														
	Nondetect Adducts	293	51	1.00 (reference)	6	1.00 (reference)	28	1.00 (reference)	5	1.00 (reference)	51/28	1.00 (reference)	6/5	1.00 (reference)
	Detectable Adducts	648	138	1.23 (0.86, 1.74)	16	1.19 (0.46, 3.09)	64	1.04 (0.65, 1.66)	17	1.54 (0.56, 4.20)	138/64	1.18 (0.68, 2.04)	16/17	0.78 (0.20, 3.06)
DAPK														
	Nondetect Adducts	293	15	1.00 (reference)	42	1.00 (reference)	5	1.00 (reference)	28	1.00 (reference)	15/5	1.00 (reference)	43/28	1.00 (reference)
	Detectable Adducts	648	27	0.81 (0.43, 1.55)	127	1.36 (0.94, 1.99)	11	1.01 (0.34, 3.01)	70	1.13 (0.71, 1.79)	27/11	0.80 (0.23, 2.78)	127/70	1.20 (0.69, 2.12)
GSTP1														
	Nondetect Adducts	293	14	1.00 (reference)	43	1.00 (reference)	13	1.00 (reference)	20	1.00 (reference)	14/13	1.00 (reference)	43/20	1.00 (reference)
	Detectable Adducts	648	40	1.29 (0.69, 2.41)	114	1.21 (0.83, 1.76)	23	0.79 (0.40, 1.59)	58	1.32 (0.78, 2.24)	40/23	114/58	114/58	0.91 (0.49, 1.69)
TWIST														
	Nondetect Adducts	293	9	1.00 (reference)	48	1.00 (reference)	5	1.00 (reference)	28	1.00 (reference)	9/5	1.00 (reference)	48/28	1.00 (reference)
	Detectable Adducts	648	24	1.20 (0.55, 2.62)	130	1.22 (0.85, 1.75)	16	1.46 (0.53, 4.08)	65	1.05 (0.66, 1.67)	24/16	0.82 (0.23, 2.92)	130/65	1.17 (0.67, 2.03)

[a] All others= ER−PR−, ER−PR+, ER+PR−

[b] among women on Long Island, NY, LIBCSP 1996-1997. Co=controls, Ca=cases.

[c] *CCND2*, *CDH1*, *CDKN2A*, and *PGR* excluded as within cell sample size n <5.

[d] Multiplicative p for interaction for *RARβ*=0.03 and *APC*=0.09; indicates that the odds of having an ER+PR+ tumor, given PAH-DNA adduct level, is statistically different across strata of methylated breast tumor. Multiplicative p for interaction for all remaining genes ≥ 0.10; indicates that the odds of having an ER+PR+ tumor, given PAH-DNA adduct level, is not statistically different across strata of methylated breast tumor.

Table IV

Age-adjusted ORs and 95%CIs for the association between gene-specific methylation and PAH-DNA adducts with ER−PR− breast cancer vs all others[a,b] using case-control (OR) and case-case (ROR) approaches where within-cell samples sizes ≥5.

			ER−PR− cases vs controls				All other (ER+PR−, ER−PR+, ER+PR+) cases vs. controls				ER−PR− vs. all other (ER+PR−, ER−PR+, ER+PR+) cases			
			Methylated breast tumor		Unmethylated breast tumor		Methylated breast tumor		Unmethylated breast tumor		Methylated breast tumor		Unmethylated breast tumor	
Gene[c,d]	PAH-DNA Adducts	Co	Ca	OR (95%CI)	Ca	OR (95% CI)	Ca	OR (95%CI)	Ca	OR (95% CI)	Ca	ROR (95%CI)	Ca	ROR (95% CI)
ESR1	Nondetect Adducts	293	8	1.00 (reference)	9	1.00 (reference)	31	1.00 (reference)	45	1.00 (reference)	8/31	1.00 (reference)	9/45	1.00 (reference)
	Detectable Adducts	648	19	1.07 (0.46, 2.48)	28	1.41 (0.66, 3.02)	93	1.35 (0.87, 2.08)	116	1.17 (0.81, 1.70)	19/93	0.80 (0.32, 2.00)	28/116	1.20 (0.53, 2.75)
BRCA1	Nondetect Adducts	293	11	1.00 (reference)	6	1.00 (reference)	44	1.00 (reference)	32	1.00 (reference)	11/44	1.00 (reference)	6/32	1.00 (reference)
	Detectable Adducts	648	29	1.19 (0.59, 2.42)	18	1.36 (0.53, 3.45)	123	1.27 (0.87, 1.84)	89	1.26 (0.82, 1.93)	29/123	0.93 (0.43, 2.05)	18/89	1.08 (0.39, 2.95)
APC	Nondetect Adducts	293	8	1.00 (reference)	9	1.00 (reference)	35	1.00 (reference)	36	1.00 (reference)	8/35	1.00 (reference)	9/36	1.00 (reference)
	Detectable Adducts	648	24	1.36 (0.60, 3.05)	23	1.16 (0.53, 2.53)	97	1.25 (0.83, 1.89)	103	1.29 (0.86, 1.93)	24/97	1.08 (0.45, 2.63)	23/103	0.90 (0.38, 2.13)
GSTP1	Nondetect Adducts	293	6	1.00 (reference)	11	1.00 (reference)	21	1.00 (reference)	52	1.00 (reference)	6/21	1.00 (reference)	11/52	1.00 (reference)
	Detectable Adducts	648	13	0.98 (0.37, 2.61)	28	1.15 (0.57, 2.35)	50	1.07 (0.63, 1.82)	144	1.26 (0.89, 1.79)	13/50	0.92 (0.31, 2.74)	28/ 144	0.92 (0.43, 1.97)
RARβ	Nondetect Adducts	293	7	1.00 (reference)	10	1.00 (reference)	16	1.00 (reference)	57	1.00 (reference)	7/16	1.00 (reference)	10/57	1.00 (reference)
	Detectable Adducts	648	11	0.71 (0.27, 1.84)	30	1.36 (0.65, 2.81)	57	1.60 (0.90, 2.84)	137	1.09 (0.77, 1.53)	11/ 57	0.44 (0.15, 1.32)	30/137	1.25 (0.57, 2.72)

[a] All others=ER+PR+, ER−PR+, ER+PR−

[b] among women on Long Island, NY, LIBCSP 1996-1997. Co=controls, Ca=cases.

[c] *CCND2, CDH1, DAPK1 HIN1, CDKN2A, PGR, RASSF1A* and *TWIST1* excluded as within cell sample size n <5.

[d] Multiplicative p for interaction for all genes ≥ 0.10; indicates that the odds of having an ER−PR− tumor, given PAH-DNA adduct level, is not statistically different across strata of methylated breast tumor.

Table V

Age-adjusted ORs and 95%Cis for the association between detectable PAH-DNA adducts and global methylation markers using a control-only approach, LIBCSP 1996-1997.

Global Methylation	Nondetectable Adducts (N)	Detectable Adducts (N)	Age-Adjusted OR (95% CI)
LUMA			
<0.56	142	325	1.00 (reference)
≥0.56	149	318	0.92 (0.70, 1.22)
LINE-1			
<78.7	154	305	1.00 (reference)
≥78.7	137	336	1.25 (0.94, 1.65)