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Residential environmental exposures and other characteristics associated with detectable PAH-DNA adducts in peripheral mononuclear cells in a population-based sample of adult females

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The detection of polycyclic aromatic hydrocarbon (PAH)-DNA adducts in human lymphocytes may be useful as a surrogate end point for individual cancer risk prediction. In this study, we examined the relationship between environmental sources of residential PAH, as well as other potential factors that may confound their association with cancer risk, and the detection of PAH-DNA adducts in a large population-based sample of adult women. Adult female residents of Long Island, New York, aged at least 20 years were identified from the general population between August 1996 and July 1997. Among 1556 women who completed a structured questionnaire, 941 donated sufficient blood (25+ ml) to allow use of a competitive ELISA for measurement of PAH-DNA adducts in peripheral blood mononuclear cells. Ambient PAH exposure at the current residence was estimated using geographic modeling ($n = 796$). Environmental home samples of dust ($n = 356$) and soil ($n = 360$) were collected on a random subset of long-term residents (15+ years). Multivariable regression was conducted to obtain the best-fitting predictive models. Three separate models were constructed based on data from : (A) the questionnaire, including a dietary history; (B) environmental home samples; and (C) geographic modeling. Women who donated blood in summer and fall had increased odds of detectable PAH-DNA adducts (OR = 2.65, 95% confidence interval (CI) = 1.69, 4.17; OR = 1.59, 95% CI = 1.08, 2.32, respectively), as did current and past smokers (OR = 1.50, 95% CI = 1.00, 2.24; OR = 1.46, 95% CI = 1.05, 2.02, respectively). There were inconsistent associations between detectable PAH-DNA adducts and other known sources of residential PAH, such as grilled and smoked foods, or a summary measure of total dietary benzo-[*a*]-pyrene (BaP) intake during the year prior to the interview. Detectable PAH-DNA adducts were inversely associated with increased BaP levels in dust in the home, but positively associated with BaP levels in soil outside of the home, although CIs were wide. Ambient BaP estimates from the geographic model were not associated with detectable PAH-DNA adducts. These data suggest that PAH-DNA adducts detected in a population-based sample of adult women with ambient exposure levels reflect some key residential PAH exposure sources assessed in this study, such as cigarette smoking.
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1. Abbreviations: PAH, polycylic aromatic hydrocarbons; SD, standard deviation; OR, odds ratio; CI, confidence interval; LIBCSP, Long Island Breast Cancer Study Project; BaP, benzo-(*a*)-pyrene; FFQ, food frequency questionnaire; BMI, body mass index.

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Introduction

Polycyclic aromatic hydrocarbons (PAH), ubiquitous chemical carcinogens (IARC, 1984), are prevalent in the ambient environment and are mainly formed by the incomplete combustion of fossil fuels (IARC, 1984). They have most frequently been studied in populations with high

occupational exposure, such as firefighters (Rothman et al., 1993a; Rothman et al., 1993b); and coke oven, aluminum, and foundry workers (Mastrangelo et al., 1996). Nonoccupational exposure sources include tobacco smoke (Besarati et al., 2002); foods such as charred, smoked, and broiled meats and leafy vegetables (Phillips, 1999); indoor contamination (Lewis et al., 1999); and air pollution (Lioy and Greenberg, 1990).

PAH bind to DNA forming PAH-DNA adducts, initiating mutagenesis and carcinogenesis (Lijinsky, 1991; Jeffy et al., 2002). Interindividual differences in DNA adduct formation of persons who are exposed to the same external levels suggest that differences in absorption, activation and detoxification mechanisms, DNA repair, cell turnover, cell-cycle control, as well as lifestyle or diet can modify individual response to exposure (Harris et al., 1982; Santella, 1999; Wiencke, 2002). Since the formation of PAH-DNA adducts is a function of exposure and genetic predisposition (Gorlewska-Roberts et al., 2002), it may provide a more objective measure of effective human exposure to environmental carcinogens (Wild and Pisani, 1998). PAH-DNA adducts have also been linked with a number of cancers, including cancers of the lung, head and neck, pancreas, urinary tract, cervix, and breast (Gammon et al., 2002a; Vineis et al., 2004).

In this study, we investigate the relationship between potential and known sources of residential PAH on the formation of PAH-DNA adducts in control women drawn from the largest population-based case–control study yet undertaken to examine the relationship between PAH-DNA adducts and breast cancer (Gammon et al., 2005). Our analysis differs from other published reports in that it is based on a large sample ($n = 941$) of nonoccupationally exposed subjects, drawn from the general population who encompass a wide age range. We also considered if known or suspected breast cancer risk factors are associated with PAH-DNA adducts; this information may be critical in understanding the role of confounding in epidemiologic studies undertaken to evaluate whether PAH-DNA adducts are associated with breast cancer risk.

Materials and methods

This study draws upon data collected as part of the Long Island Breast Cancer Study Project (LIBCSP), which was undertaken to assess whether breast cancer is associated with environmental factors, including PAH-DNA adducts (Gammon et al., 2002b).

Study Population

This analysis is based on the LIBCSP controls, a population-based sample of 1556 adult female residents of Nassau or Suffolk counties on Long Island, New York, who were enrolled between August 1996 and July 1997, spoke English, and did not have a personal history of breast cancer. These controls were frequency-matched to the expected age distribution of cases by 5-year age group. Women under 65 years of age were randomly selected by Waksberg's random digit dialing (RDD) method (Waksberg, 1978), while those aged 65 years and older were identified via Health Care Finance Administration (HCFA) rosters.

Data Collection

Prior to the interview, informed consent was obtained from the participants, with 63% ($n = 1556$) of the eligible women completing the interview. The main questionnaire (http://epi.grants.cancer.gov/LIBCSP/projects/Questionnaire.html), which queried each subject about her exposures prior to the reference date (date of identification), was administered by a trained interviewer at the subject's home and included questions about: reproductive and medical history, exogenous hormone use, family history of breast cancer, physical activity, body size, alcohol use, and questions on possible sources of PAH such as active cigarette smoking, exposure to environmental tobacco smoke in the home as an adult or child, residential history in Nassau and Suffolk counties, and lifetime history of grilled and smoked food consumption. Participants were also asked to self-administer a previously validated (Potischman et al., 1997) modified Block food-frequency questionnaire (FFQ), which asked about dietary intake in the year prior to interview.

Nonfasting blood samples were collected from 1141 (73.3%) controls with a higher number of younger rather than older women donating blood (Gammon et al., 2002b). Donors were also asked to complete a self-administered checklist, which queried each participant about her exposures in the few days prior to the interview. Samples were usually processed within 24 hours of donation and stored at -80°C.

A random sample of white respondents and all black respondents who were long-term residents (defined as living in their current home for at least 15 years) were invited to participate in the environmental home sampling component. Samples of outside residential soil ($n = 360$) and indoor carpet dust ($n = 356$) were collected for approximately 80% of eligible controls, and later used to develop geographical models to estimate past ambient PAH exposure levels (Beyea et al., 2002).

Laboratory Methods

The women in this analysis ($n = 941$) are a subset of all LIBCSP controls. These women donated enough blood to yield the 100 μg or more of DNA needed for the PAH-DNA adduct measurements. The measurements were conducted in two rounds. In round 1, the PAH-DNA adduct measurement was completed for a random sample of 424 controls, while in round 2, it was conducted for the remaining 517 with a sufficiently large blood donation.

Competitive ELISA (Poirier et al., 1980; Santella et al., 1992) was used to analyze the PAH diol epoxide-DNA adducts (Gammon et al., 2002a, 2005). Laboratory personnel were blinded to case–control status when they ran the samples in duplicate, and mean values were used to determine the percentage inhibition. Samples with $<15\%$ inhibition were considered nondetectable and assigned a value of $1/10^8$, an amount midway between the lowest positive value and zero. For quality control, 10% of the samples were reassayed after recoding.

Soil samples (approximately 2.0 g) were placed in scintillation vials and spiked with 50 μl of mixture of deuterated standards containing 50 ng each of phenanthrene-d10, fluoranthene-d10 and perylene d-10 (Community Bureau of Reference, Brussels, Belgium). Samples were sonicated with 10 ml of dichloromethane (B & J Corp., GC grade; four times for 5 min each). Extracts were then transferred into disposable centrifuge tubes (Fisher, 10 ml, #5182-0717) and evaporated under vacuum (SpeedVac Plus SC110 A, Savant) until 200–300 μl of the solution was left in the tube. The final volume was measured and 200 μl of the solution was transferred into a Hewlett-Packard Target DP vial. All soil samples were stored at -80°C until analyzed.

Soil samples were analyzed by gas chromatography with mass spectrometric detection, with the mass spectrometer (Hewlett-Packard Model 5973 Mass Selective Detector) operated in a selected ion detection mode. The analyses were performed on a DB-5 MS-fused silica capillary column (J&W) with the following temperature program: the injector port was kept at 250°C, the initial oven temperature of 70°C was kept for 1 min, then increased at 5°C/min to 300°C where it was kept for 30 min. The following compounds were quantified: naphthalene (monitoring ion of *m/z* 128), acenaphthylene (*m/z* 152), acenaphthene (*m/z* 154), fluorine (*m/z* 178), phenanthrene (*m/z* 178), anthracene (*m/z* 178), fluoranthene (*m/z* 202), pyrene (*m/z* 202), benz-[*a*]-anthracene (*m/z* 228), chrysene (*m/z* 228), benzo-[*b*]-fluoranthene (*m/z* 252), benzo-[*k*]-fluoranthene (*m/z* 252), benzo-[*a*]-pyrene (*m/z* 252), indeno (1,2,3-cd)pyrene (*m/z* 276), dibenz-[*a,h*]-anthracene (*m/z* 278), and benzo-[*ghi*]-perylene (*m/z* 276). Each was assessed quantitatively on the basis of standard curves relating their levels to their integrated peaks corresponding to the appropriate ions. Reported concentrations are corrected for recoveries, which ranged from 70 to 100%.

Carpet dust samples were vacuum collected with the HVS3 from the room in which the subject spent the most waking time. Up to 2.0 g of fine (<150 μm) sieved dust was soxhlet-extracted with 6% ether/hexanes, florisil-cleaned, and analyzed by GC/MS selected ion monitoring for 16 pesticides, three PAH (benz-[*a*]-anthracene, benzo-[*a*]-pyrene, and dibenz-[*a,h*]-anthracene), and 13 PCB congeners.

Statistical Analyses

The subset of women used in these analyses did not differ substantially from all control women who donated blood. However, women who donated blood were more likely to be younger, nonsmokers, white or other race (Asian or Native American), consume alcohol, ever breastfeed, ever use exogenous hormones, and ever have a mammogram (Gammon et al., 2002b).

Outcome Variable Analyses were conducted using PAH-DNA adducts as the outcome, considered as: (1) a binary variable that grouped the subjects into detectable ($n=648$) and nondetectable ($n=293$) levels; and (2) a log-transformed continuous variable. The decision to dichotomize the outcome was based on our observation that there was no dose-response detected between increasing PAH-DNA adduct levels and breast cancer risk (Gammon et al., 2002a, 2005).

Preliminary Analyses We examined the differences in mean PAH-DNA adduct levels among white ($n=870$) and black ($n=41$) control women who donated blood, but found no statistically significant differences between the two groups (data not shown). Thus, all further analyses were conducted by combining racial groups.

General Methods For the binary outcome, unconditional logistic regression was used to estimate odds ratios (OR) for detectable PAH-DNA adducts, adjusted by 5-year age groups, and corresponding 95% confidence intervals (CI) (Hosmer and Lemeshow, 1989). Where applicable, tests for trend across quantiles were calculated. For the continuous outcome, linear regression was used to obtain beta estimates and standard errors (Kleinbaum et al., 1998). Multivariable regression was conducted to obtain the best-fitting predictive models. Covariates were systematically removed from each full model using backwards elimination. We excluded covariates that did not improve the overall model fit. All analyses were conducted using SAS, version 8.1 (SAS Institute, Cary, NC, USA). Three separate prediction models were constructed, as described below.

Questionnaire Data (Model A) This model ($n=933$) included exposures derived from the LIBCSP questionnaire. We evaluated interactions between income and smoking status but did not find statistically significant deviations from additivity or multiplicativity (data not shown). The full model included the following factors as potential predictors: age, race, ethnicity, education, marital status, religion, total household income before taxes in the last year, length of residence in interview home, county of residence, age at menarche, parity status, age at first birth, breastfeeding, menopausal status, history of fertility problem, body mass index (BMI) at reference, BMI at age of 20 years, lifetime alcohol intake, family history of breast cancer, history of benign breast disease, oral contraceptive use, hormone replacement therapy use, active or passive cigarette smoke

exposure, use of mammography, season of blood donation, total fruit and vegetable consumption, cruciferous vegetable intake, intake of all types of grilled/bbq/smoked foods in most recent decade of life, and intake of all foods containing benzo-[*a*]-pyrene (BaP). Definitions for the cigarette smoking variables are based on self-reported data from the main questionnaire and the self-administered checklist (Gammon et al., 2002b). A current active cigarette smoker was defined as smoking within the 12 months prior to the reference date, while a former active smoker was defined as a smoker who reported quitting more than 12 months prior to the reference date (Gammon et al., 2003). A passive smoker was defined as a subject who reported ever living with an active smoker (Gammon et al., 2003). The 11.4% of controls in this analysis who had missing values for household income were given a predicted value for income using regression-based imputation (Gammon et al., 2005). Corrected CI were generated for imputed income by applying the standard errors obtained from the smaller sample size to the betas obtained from the imputed data set. The measure of total individual dietary BaP intake is an adaptation of a PAH food index developed by Kazerouni et al. (2001) that utilizes data from the FFQ; the estimate (Gammon et al., 2005) reflects a composite measure of the proportion of time that a woman reported using a specific cooking method for each meat item, doneness level, and portion size.

Environmental Home Samples (Model B) This model ($n = 167$) evaluated the association between BaP levels in environmental home samples and PAH-DNA adducts among long-term residents of Long Island. To normalize the observed distributions, soil and dust BaP levels were log transformed on a natural scale. The factors that were assessed in this full model were: BaP concentration in soil (expressed as ng/g), BaP concentration per gram of indoor dust (expressed as ng/g), BaP loading per square meter of vacuumed carpet (expressed as ng/m^2), and other covariates found to be important predictors in Model A.

Ambient BaP Estimate (Model C) The aim of this model ($n = 796$) was to determine if ambient, airborne BaP levels estimated at current residence predicted detectable PAH-DNA adducts. Along with the current ambient BaP level estimate (log-transformed), additional factors present in the full model included all variables considered in Model A. The estimation of individual PAH exposure at the current residence uses a general geographic extension modeling method based on traffic patterns (Beyea and Hatch, 1999; Beyea et al., 2002) that was applied to subjects whose residential addresses could be geocoded to the street level. There were 145 women with missing values for the resulting ambient BaP estimate. BaP was used as a proxy for total PAH exposure, since BaP is an accurate indicator of PAH exposure and carcinogenicity (Fertmann et al., 2002).

Results

Study Population

Women in this analysis ($n = 941$) were between 20 and 95 years of age, with a median age of 55 years (mean $= 56$ years, standard deviation (SD) $= 12.5$ years). About 75% of the participants were below 65 years of age, 92% were white, 87% had lived on Long Island for at least 15 years, 59% had some college education or higher, and about a quarter were obese (BMI $\geq 30\,kg/m^2$).

Questionnaire Data

Important predictors identified in the age-adjusted models included fruit and vegetable intake, cruciferous vegetable intake, age at initiation of cigarette smoking, and duration of smoking (data not shown). Women in the highest quintile of fruit and vegetable intake (47+ half-cup servings/week) had the lowest odds of detectable PAH-DNA adducts (age-adjusted OR = 0.67, 95% CI = 0.43, 1.04). For cruciferous vegetable intake, women who consumed three half-cup servings/week had a lower odds (OR = 0.57, 95% CI = 0.36,0.92) compared with women who consumed 6+ half-cup servings/week (OR = 0.88, 95% CI = 0.58, 1.33). Women 8–17 years of age when they first started smoking had an increased odds of PAH-DNA adducts (OR = 1.47, 95% CI = 1.06, 2.05) as did women who started smoking at age 18+ years (OR = 1.36, 95% CI = 0.96, 1.94). Also, women who smoked between 0.5 and 12 years had a comparably elevated odds (OR = 1.42, 95% CI = 0.94, 2.14) as women who smoked for more than 34 years (OR = 1.55, 95% CI = 0.96, 2.51).

Table 1 shows the results from the final predictive model (Model A, see methods) that was initially based on all the questionnaire data with detectable PAH-DNA adducts considered as the outcome (detectable/not detectable). Results from the initial, full model (data not shown) did not differ substantially from those shown in the more parsimonious model shown in Table 1. Women who donated blood in summer had over a two-fold increased odds of detectable PAH-DNA adducts (multivariable-adjusted OR = 2.65, 95% CI = 1.69, 4.18), while women who donated blood in fall had a 59% increased odds (OR = 1.59, 95% CI = 1.08, 2.32). However, odds were not elevated among those who donated blood in winter (OR = 1.05, 95% CI = 0.72, 1.54). Also, current and former active cigarette smokers had a modest increased odds (OR = 1.50, 95% CI = 1.00, 2.24; OR = 1.46, 95% CI = 1.05, 2.02, respectively). Higher income was associated with detectable adducts, with women in the highest income bracket having the highest OR of 1.94 (95% CI = 1.08, 3.49). When compared to women who reached menarche below 12 years of age, those with menarche at the age of 13 years had an OR of 1.89 (95% CI = 1.25, 2.87). Finally, breastfeeding was associated with detectable

Table 1. Multivariate adjusted[a] odds ratios(OR) and 95% confidence intervals (CI) for detectable PAH-DNA[b] adducts levels in relation to known and suspected risk factors among randomly sampled controls ($n = 933$) who donated at least 25 ml of blood, Long Island Breast Cancer Study Project, 1996–1999.

Factor	OR[c]	95% CI
Age at reference		
<45 years	1.00	
45–54 years	1.11	0.73, 1.69
55–64 years	1.41	0.90, 2.20
65+ years	1.31	0.81, 2.12
Season of blood donation (corresponding dates)		
Spring (3/21–6/20)	1.00	
Summer (6/21–9/20)	2.65	1.69, 4.18
Fall (9/21–12/20)	1.59	1.08, 2.32
Winter (12/21–3/20)	1.05	0.72, 1.54
Active smoking status		
Never smoked	1.00	
Current smoker(smoked within the past 12 months)	1.50	1.00, 2.24
Past/former smoker	1.46	1.05, 2.02
Total household income before taxes in last year[d]		
<$20,000	1.00	
$20,000–$49,999	1.45	0.85, 2.48
$50,000–$89,999	1.29	0.75, 2.21
$90,000+	1.94	1.08, 3.49
Age at menarche		
<12 years	1.00	
12 years	1.43	0.97, 2.10
13 years	1.89	1.25, 2.87
14+ years	0.87	0.59, 1.30
Lactation		
None	1.00	
<2 months	1.54	0.85, 2.79
2–5 months	1.93	1.06, 3.53
6–13 months	1.55	0.90, 2.69
14+ months	1.08	0.67, 1.73

[a]OR adjusted for all other factors listed in the table and parity.
[b]PAH-DNA adduct levels were log-transformed prior to logistic regression analyses.
[c]OR greater than 1.0 indicate that the factor is associated with a greater probability of PAH-DNA adduct formation in blood; less than 1.0 indicates the factor is associated with a lower probability of PAH-DNA adduct formation in blood.
[d]Includes values for missing income, which were imputed using age, race, and education. To calculate 95% CI for income, the standard errors (SE) obtained using imputed income were inflated back to the lower sample-size level in order to correct for the artificially minimized SE.

PAH-DNA adducts (OR = 1.93 for 2–5 months of lactation, CI = 1.06, 3.53).

Table 2 shows the results when PAH-DNA adducts were considered as a continuous variable. Factors associated with increasing levels of log-transformed adducts included season of blood donation, BMI at reference, and active cigarette smoking, while grilled/bbq/smoked food consumption in the most recent decade of life was associated with decreasing levels of log-transformed PAH-DNA adducts.

Table 2. Relationship of known and suspected risk factors to PAH-DNA adducts[a] among randomly sampled controls ($n = 933$) who donated at least 25 ml of blood, Long Island Breast Cancer Study Project, 1996–1999: linear regression analysis.

Factor	Beta estimate	Standard error	*P*-value
Intercept	1.21	0.11	<0.0001
Season of blood donation	0.11	0.04	0.003
BMI at reference	0.12	0.04	0.002
Active cigarette smoking exposure	0.08	0.05	0.08
Intake of all types of grilled/bbq/smoked foods in most recent decade of life	−0.05	0.03	0.06

[a]Levels were log-transformed prior to analyses.

Environmental Home Samples

For this analysis, we limited our consideration to measures derived from the environmental home samples collected among long-term residents. First, in the age-adjusted models (data not shown), the odds of detectable PAH-DNA adducts increased with increased quartiles of BaP in soil although all the point estimates included the null value (age-adjusted OR for quartile 2 = 1.37, 95% CI = 0.60, 3.11; OR for quartile 3 = 1.41, 95% CI = 0.60, 3.33; OR for quartile 4 = 1.46, 95% CI = 0.58, 3.67), but the test for trend was not statistically significant. Odds were lower in the highest vs. the lowest tertile of both BaP concentration per gram of indoor dust (OR for the highest tertile = 0.53, 95% CI = 0.25, 1.11) and BaP loading per square meter of vacuumed carpet (OR for the highest tertile = 0.77, 95% CI = 0.38, 1.58).

Table 3 shows the final multivariable environmental home sample model (Model B, see methods, with adjustments made for the covariates identified in Model A) with PAH-DNA adducts considered as a dichotomous variable. Factors that remained in the model were BaP concentration in soil and BaP concentration per gram of indoor dust. BaP concentration in soil was positively associated with a higher odds of detectable PAH-DNA adducts, although estimates were unstable given the wide and overlapping CI. However, increased BaP concentration per gram of indoor dust was inversely associated with detectable adducts. Finally, as shown in Table 4, when PAH-DNA adducts were considered as a continuous variable, BaP concentration per gram of soil was associated with increasing levels of log-transformed adducts, while BaP concentration per gram of indoor dust was associated with decreasing levels.

Table 3. Multivariate adjusted[a] odds ratios (OR) and 95% confidence intervals (CI) for detectable PAH-DNA[b] adducts levels in relation to soil and dust PAH among randomly sampled controls ($n = 167$), who were long term residents of Long Island, Long Island Breast Cancer Study Project, 1996–1997.

Factor	OR[c]	95% CI
Benzo-a-pyrene concentration in soil[b]		
Quartile 1 (<4.70)	1.00	
Quartile 2 (4.7–5.6)	1.84	0.71, 4.80
Quartile 3 (5.7–6.8)	2.07	0.76, 5.64
Quartile 4 (6.9+)	2.19	0.75, 6.44
Benzo-a-pyrene concentration per gram of indoor dust[b]		
Tertile 1 (<6.7)	1.00	
Tertile 2 (6.7–8.0)	0.41	0.16, 1.02
Tertile 3 (8.1+)	0.22	0.08, 0.59

[a]OR adjusted for all other factors listed in the table.
[b]Levels were log-transformed prior to logistic regression analyses.
[c]OR greater than 1.0 indicate that the factor is associated with a greater probability of PAH-DNA adduct formation in blood; less than 1.0 indicates that the factor is associated with a lower probability of PAH-DNA adduct formation in blood.

Table 4. Relationship of soil and dust PAH to PAH-DNA adducts[a] among controls who were long-term residents of Long Island ($n = 167$), Long Island Breast Cancer Study Project, 1996–1997: linear regression analysis.

Factor	Beta estimate	Standard error	*P*-value
Intercept	1.77	0.17	<0.0001
BaP concentration per gram of soil (ng/g)[a]	0.29	0.12	0.02
BaP concentration per gram of dust (ng/g)[a]	−0.44	0.12	0.0006

[a]Levels were log-transformed prior to analyses.

Ambient BaP Estimate

The ambient BaP estimate at current residence from the geographic modeling did not contribute to the overall predictivity of Model C (see Materials and methods), and was not considered further.

Discussion

To the best of our knowledge, this study is the first to identify an array of potential residential factors that may be associated with detectable PAH-DNA adducts in a large, population-based sample of ambiently exposed women. Determination of such factors will help in understanding the underlying exposures that contribute to PAH-DNA adduct formation in the general population. Also, it will help to identify variables that should be considered as potential confounders in studies of PAH-DNA adducts and breast cancer. Other studies that have examined contributors to PAH-DNA adduct formation have either been feeding studies (Rothman et al., 1990; Kang et al., 1995), or have used highly exposed occupational cohorts (Rothman et al., 1993b; Tuominen et al., 2002). In addition, the only other population-based investigations have been limited by small sample sizes (Scherer et al., 2000; Georgiadis et al., 2001).

Our findings show that season of blood donation and smoking status are the strongest predictors of detectable PAH-DNA adducts. Other predictors include increased age, higher income, earlier age at menarche, fewer months of breastfeeding, and soil BaP. We also observed an inverse relationship between increasing tertiles of indoor dust BaP and detectable PAH-DNA adducts. In addition, although total fruit and vegetable intake did not predict detectable PAH-DNA adducts when considered along with other factors, it was an important predictor of a lower odds in the age-adjusted analysis. Inconsistent associations were observed for certain other residential sources of PAH, such as smoked and grilled foods, dietary BaP intake, and the smoking variable that considered active and passive cigarette smoke simultaneously.

As compared with previous studies among highly exposed occupational cohorts, the results from our population-based study are more generalizable to the general population. However, the median age of our participants at 55 years, is higher than the median age of 39.9 and 37.6 years, respectively, of female residents residing on Nassau and Suffolk counties, Long Island (US Census Bureau, 2000). This higher median age of the participants is due to the matching constraint employed in our case–control study, where controls were sampled to ensure that their age distribution matched that of the cases. Older age, as confirmed in our results, is also related to higher adduct formation. The body's repair capability diminishes with age (Josyula et al., 2000), and, it is also possible that the relation between an exposure and adduct formation varies with age.

In our analysis, current and former active cigarette smokers, but not passive smokers, have similarly increased odds of PAH-DNA adducts. Some studies have reported that an earlier age at initiation of smoking, during adolescence, for example, may lead to physiological changes that increase the likelihood of adduct formation, such that even after they quit smoking, smokers who started at a younger age still have higher levels of adducts in comparison to those who started smoking in later life (Wiencke et al., 1999; Vineis, 2000; Wiencke, 2002). However, our findings do not support this hypothesis. Rather, the odds are equally elevated for those who started smoking in adolescence and for those who started at later ages. Furthermore, women who smoked for a shorter duration had a similar increase in the

odds of detectable adducts as women who smoked for a longer duration. These findings suggest that once cigarette smoking is initiated, it appears to compromise the body's ability to handle carcinogenic insult.

Diet is an important source of PAH intake (Kazerouni et al., 2001), but we did not find any consistent associations between detectable PAH-DNA adducts and various dietary sources of PAH, including smoked and grilled foods eaten in the most recent decade of life (assessed in the main questionnaire), and the BaP food index (assessed in the food frequency questionnaire (FFQ)). Bias in the estimation of PAH food intake from the FFQ may have arisen because of inaccuracies with recall of specific food items that contain PAH, or because of weaknesses in the FFQ instrument. Our BaP diet measure was a modification of the more detailed one used by Kazerouni et al. (2001), which may have resulted in a less accurate estimate of BaP food intake used in our analyses. The FFQ that we used did not query participants on several parameters that influence BaP levels in meat, such as distinguishing between beef and hamburger consumption when asking about intake of red meat. Cantwell et al. (2004) also found that the FFQ underestimates beefsteak, pork, sausage, and grilled/bbq chicken intake. Also, our results are based on a measure of PAH-DNA adducts assessed in peripheral mononuclear cells which have a short lifetime (Tuominen et al., 2002), yet our measure of grilled and smoked food reflected intake over the current decade of life. Thus, if there were fluctuations in diet over time, it may be understandable that we would not find associations with detectable PAH-DNA adducts.

Fruit and vegetable intake may lead to decreased levels of PAH-DNA adducts, with heavy consumption leading to a greater reduction (Palli et al., 2000; Peluso et al., 2000). In the age-adjusted analysis, there was some indication that adduct levels were inversely associated with fruit and vegetable intake (regardless of whether they are sources of PAH). Cruciferous vegetables contain isothiocyanates, which have been shown to be powerful inhibitors of carcinogenesis in laboratory animals (Hecht, 1999). Isothiocyanates may act as cancer chemopreventives by favorably modifying carcinogen metabolism, by selective inhibition of cytochrome P450 enzymes involved in carcinogen metabolic activation (Hecht, 1999, 2000). However, we observed inconsistent associations with cruciferous vegetable intake.

A number of studies in areas with high ambient PAH concentrations have found associations between season and PAH-DNA adducts, with higher levels found in winter than in summer months (Perera et al., 1992a, b; Grzybowska et al., 1993; Moller et al., 1996). Studies have shown that pollution, including that from heating sources contribute to higher airborne PAH concentrations in the winter (Moller et al., 1996; Topinka et al., 2000). However, in our study, blood donation in summer was found to be the strongest predictor of PAH-DNA adducts, regardless of whether the outcome was considered as a dichotomous or continuous variable. Summer may be associated with more time spent outdoors as well as higher consumption of grilled and smoked foods; and therefore season may be a better surrogate of dietary PAH intake and recent ambient PAH exposure than the measures used in our study. Part of the explanation for an increase in adduct formation in summer may not be associated with input PAH at all, but may be due to interaction effects between PAH input and enzymatic activation that could change with season. Seasonal changes occur in endocrine function (Holdaway et al., 1997; Walker et al., 1997; Hansen et al., 2001); immune response (Maes and De Meyer, 2000; Nelson and Drazen, 2000; Myrianthefs et al., 2003); breast biology (Paradiso et al., 2001); and in enzyme inducibility, such as aryl hydrocarbon hydroxylase (AHH), which is an enzyme responsible for the metabolism of PAH like BaP (Paigen et al., 1981; Peluso et al., 1998; Gupta and Singh, 2004; Kiyohara and Hirohata, 1997).

The short lifetime of peripheral mononuclear cells (Tuominen et al., 2002) might explain why ambient PAH exposure at current residence is not highly correlated with detectable PAH-DNA adducts. A publication on past smokers reported that the half-life of DNA adducts in total white blood cells (which are mainly short-lived granulocytes) was between 9 and 13 weeks (Mooney et al., 1995). However, this finding is in conflict with results from another study which reported that after a month long vacation, DNA adducts in total white blood cells of foundry workers were almost comparable to background levels seen in controls (Perera et al., 1988). Thus, due to the uncertainty in the literature, the half-life of DNA adducts is not absolutely clear, but appears to be short.

Soil measurements are likely to reflect airborne PAH concentrations because deposition of PAH is proportional to airborne concentrations above the soil (Odabasi et al., 1999). Soil BaP concentrations may be more stable and not vary as much as airborne exposures. Increased levels of dust BaP were associated with decreased adducts, which may be due to the effect of prolonged exposure on the body's biological response to PAH (Van Schooten et al., 1997; Vineis et al., 2000), or to the saturation of PAH-activating enzymes, or increased DNA repair of adducts.

The positive association between total household income and detectable PAH-DNA adducts in this study was unexpected. In addition, we did not anticipate an inverse association between increased adducts and either younger ages at menarche or shorter durations of breastfeeding. The reasons for these findings are unclear, and need confirmation in other populations.

An individual's response to PAH exposure may be affected by genetic susceptibility (Tuominen et al., 2002). PAH-DNA adduct formation may be a better biological parameter for assessing risk since levels reflect the net effect of competing

activation and detoxification pathways and DNA repair (Kriek et al., 1993; Rothman et al., 1995). Genetic variations in the cytochrome P450 pathway (activation) or the glutathione-*S*-transferase pathway (detoxification) may result in some individuals with greater susceptibility to adduct formation (Santella, 1999). PAH-DNA adducts are an integrated marker of exposure that reflect the amount of cumulative unrepaired DNA damage in an individual (Vineis, 2000). A limitation is that ELISA does not quantify a specific adduct. Rather, because of antibody crossreactivity with structurally related adducts, ELISA measures the class of PAH-diol epoxide adducts, and results do not provide an absolute quantitative value. Nonetheless, the whole class of adducts are biologically relevant.

A strength of our study is that we considered the specific contributions of many possible residential PAH exposure sources, including tobacco smoke, dietary PAH, and indoor and outdoor air pollution. Recruitment of women from the general population enhanced our likelihood of obtaining unbiased study results. Other studies examined predictors of adduct formation among cancer cases (as reviewed in Wiencke, 2002), but since DNA adducts are thought to be part of the causal pathway in a number of different cancers, our study design using women without breast cancer is more methodologically sound and interpretation is more straightforward.

Future large-scale studies are needed not only to further elucidate the influence of known sources of PAH and other factors on PAH-DNA adduct formation, but also to understand the multifaceted nature of gene–environment interactions. The understanding of the underlying mechanisms may enhance interpretation of the findings from epidemiologic studies (Poirier et al., 2000). Potential modulators of DNA adducts also need to be explored. For example, dietary intake of certain micronutrients like β-carotene and α-tocopherol may be beneficial in people with susceptible genotypes (Vineis, 2000). In addition, few studies have examined predictors of adduct levels specifically among former smokers (Wiencke, 2002).

In conclusion, our findings suggest that PAH-DNA adducts detected in a population-based sample of adult women with ambient exposure levels reflect some key residential PAH exposure sources, such as cigarette smoking, but not all expected sources. Our study is also the largest investigation of PAH-DNA adduct measurements in humans. As data on genetic polymorphisms become available, future analyses could consider how they influence PAH-DNA adduct formation.

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