Polycyclic Aromatic Hydrocarbon–DNA Adducts and Breast Cancer: A Pooled Analysis

MARILIE D. GAMMON
SHARON K. SAGIV
Department of Epidemiology
University of North Carolina School of Public Health
Chapel Hill, North Carolina
SYBIL M. ENG
Epidemiologic Resources
Safety Evaluation and Epidemiology
Pfizer, Inc.
New York, New York
SUMITRA SHANTAKUMAR
MIA M. GAUDET
Department of Epidemiology
University of North Carolina School of Public Health
Chapel Hill, North Carolina
SUSAN L. TEITELBAUM
JULIE A. BRITTON
Department of Community Medicine
Mt. Sinai School of Medicine
New York, New York
MARY BETH TERRY
Department of Epidemiology
Mailman School of Public Health
Columbia University
New York, New York
LIAN WEN WANG
QIAO WANG
Department of Environmental Health Sciences
Mailman School of Public Health
Columbia University
New York, New York
STEVE D. STELLMAN
Department of Epidemiology
Mailman School of Public Health
Columbia University
New York, New York
JAN BEYEA
Consulting in the Public Interest, Inc.
Lambertville, New Jersey
MAUREEN HATCH
Department of Community Medicine
Mt. Sinai School of Medicine
New York, New York
GEOFFREY C. KABAT
Department of Preventive Medicine
Stony Brook University
New York, New York
MARY S. WOLFF
Department of Community Medicine
Mt. Sinai School of Medicine
New York, New York
BRUCE LEVIN
Department of Biostatistics
Mailman School of Public Health
Columbia University
New York, New York
ALFRED I. NEUGUT
Department of Epidemiology
Mailman School of Public Health
Columbia University
New York, New York
and
Department of Medicine
College of Physicians and Surgeons
Columbia University
New York, New York
REGINA M. SANTELLA
Department of Environmental Health Sciences
Mailman School of Public Health
Columbia University
New York, New York

ABSTRACT. Polycyclic aromatic hydrocarbon (PAH)-DNA adducts have been associated with breast cancer in several small studies. The authors' pooled analysis included 873 cases and 941 controls from a population-based case-control study. Competitive enzyme-linked immunosorbent assay in peripheral mononuclear cells was conducted in 2 rounds, and results were pooled on the basis of round-specific quantiles. The odds ratio for breast cancer was elevated in relation to detectable PAH-DNA adducts (1.29 as compared with non-detectable adduct levels; 95% confidence interval = 1.05, 1.58), but there was no apparent dose-response relationship with increasing quantiles. No consistent pattern emerged when

the results were stratified by PAH sources (e.g., active cigarette smoking or PAH-containing foods), or when the cases were categorized by stage of disease or hormone receptor status. These data provide only modest support for an association between PAH-DNA adducts and breast cancer development.
<Key words: air pollution, breast cancer, cigarette smoking, cooked meat, DNA adducts, passive smoking, polycyclic aromatic hydrocarbons>

POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) have been shown, in laboratory-based studies, to induce mammary tumors,[1] but their role in human breast carcinogenesis has not been established.[2] PAH compounds are ubiquitous; major exposure sources include active and passive cigarette smoke, consumption of PAH-containing foods (e.g., grilled and smoked foods and leafy vegetables), and air pollutants (from industrial manufacturing, fires, and motor vehicle traffic).[3,4] PAH compounds are classified as probable or possible human carcinogens.[5] Cancer risk in humans (cancer of the lung, for example) in relationship to PAH exposure varies with an individual's ability to detoxify or eliminate the contaminant. PAH-DNA adduct lesions are formed during this multistep metabolic process, and their presence suggests either high levels of exposure to the contaminant or the body's inability to adequately respond to the exposure, or both.[6,7]

Using PAH-DNA adducts as a body burden measure of exposure and response, researchers in several epidemiologic studies observed a positive association with breast cancer among women.[8–11] Investigators who studied small, hospital-based series of patients reported at least a doubling of breast cancer risk in relation to the presence of DNA adducts.[9–11] In contrast, a relatively more modest 35% increase in breast cancer risk was noted in relation to detectable PAH-DNA adducts in the only investigation reported to date of a large ($N = 999$) population-based sample of cases and controls.[8] No increase in the odds ratios (ORs) for breast cancer was observed with increasing adduct levels in the larger study, nor was there any substantial variation associated with several sources of PAH, such as active cigarette smoking or consumption of grilled and smoked foods.[8] These results are consistent with the hypothesis that individual variation in response to the exposure may be a more important influence on breast carcinogenesis than the absolute exposure amount.[8] To clarify such an association, reports are needed from studies that include large numbers of women drawn from the general population, and that employ state-of-the-art exposure measurement and data collection methods.[2]

Materials and Method

The Long Island Breast Cancer Study Project (LIBCSP) is a multi-institutional collaboration to determine whether environmental factors are associated with breast cancer risk. Details of the study method are described elsewhere.[12] The study was undertaken in response to women's concerns about the high incidence rates of breast cancer in Nassau and Suffolk counties (117.8 and 113.6 per 100,000 women, respectively, from 1992–1996), and that breast cancer in this geographic area may be caused by exposure to environmental contaminants.[12] The primary aims of the LIBCSP were to examine whether breast cancer incidence was associated with PAH-DNA adducts or organochlorine compounds in peripheral blood. This study was conducted with approval from participating institutional review boards and in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services.[13]

The study reported here is a pooled analysis based on 1,814 women who participated in the LIBCSP and who donated a blood sample of sufficient volume to assess PAH-DNA adduct levels using competitive enzyme-linked immunosorbent assay (ELISA). Our previous research[8] was based on a sample of 999 of these Long Island respondents (i.e., Round 1). The present report provides results on the remaining 815 women who participated in the LIBCSP and who donated sufficient blood volume to conduct the assays (Round 2), as well as the results of pooled analyses combining the data from all 1,814 LIBCSP respondents (Rounds 1 + 2).

Study population. Eligible cases were defined as women newly diagnosed with in situ or invasive breast cancer between August 1, 1996, and July 31, 1997, who were aged 20 yr or older, spoke English, and resided in Nassau or Suffolk county on Long Island, New York, at the time of diagnosis. Eligible controls were defined as women who were aged 20 yr or older, spoke English, and resided in the same Long Island counties as the cases but had no personal history of breast cancer. (More than 97% of the population in these 2 counties speaks English.[12]) Study staff identified potentially eligible cases through frequent contact with pathology departments of regional Long Island and New York City hospitals; the physician was approached to confirm the patient's study eligibility and to obtain permission to contact her. Potentially eligible controls were frequency-matched to the expected age distribution of the cases and identified through random-digit dialing[14] for women aged under 65 yr, and through Health Care Finance Rosters for women aged 65 and older.

Data collection. Potentially eligible subjects were contacted by mail and telephone to confirm eligibility and arrange for in-home personal interviews. Signed informed consent was obtained from all women prior to the interview. We conducted interviews with 1,508 cases (82%) and 1,556 controls (63%). Response rate varied with age,[12] with higher rates among cases and controls under age 65 (88.9% and 76.1%, respectively) than among those aged 65 and older (71.6% and 43.3%, respectively). The main questionnaire,[14] which was administered by a trained interviewer, sought information on known and suspected risk factors for breast cancer, including PAH exposure sources over the life course (e.g., active cigarette smoking, exposure to passive smoking in the subject's home, and intake of grilled and smoked foods).[15] The LIBCSP food frequency questionnaire,[12] which gathered data on diet history during the 12 mo prior to the interview, was self-completed by 98% of respondents and included questions on food preparation methods. Nonfasting blood samples were collected from nearly three-quarters of study participants (73% of cases and 73% of controls).

As previously published,[12] an increase in breast cancer among women on Long Island was found to be associated with lower parity, late age at first birth, little or no breastfeeding, a family history of breast cancer, and increasing income and education. Results were similar when the analyses were restricted to respondents who donated blood, or to those with DNA available for these analyses (data not shown). Factors that were found to be associated with a decreased likelihood that a respondent, regardless of case-control status, would donate blood[12] included increasing age (1% decrease for each year increase in age) and past active smoking (25% decrease); factors associated with an increased probability included white or other race (65% and 74% increase, respectively, vs. black race), alcohol use (28% increase), ever breastfed (47% increase), ever used hormone replacement therapy (63% increase), ever used an oral contraceptive (21% increase), and ever had a mammogram (51% increase). Case-control status and intake of grilled and smoked foods were not predictors of blood donation.[8]

Laboratory methods for Round 2. In Round 2, the PAH-DNA adduct assays were conducted by the same laboratory (Dr. Regina M. Santella, Columbia University, New York, NY), using the same procedures that were used to analyze the samples in Round 1.[8] PAH diolepoxide-DNA adducts were analyzed by competitive ELISA, using a previously described method.[16,17] Samples were run with the lab blinded to case-control status, in duplicate, and mean values were used for determination of percentage inhibition. For analytical purposes, those 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. The corresponding mean value and standard deviation (*SD*) for the positive control run with multiple batches was 7.8 ($SD = 3.10$) ($n = 10$) in Round 2. As an additional quality control measure, 135 samples were assayed in duplicate; there was no significant difference in mean adduct levels (mean difference = –0.02; $SD = 3.24$), paired *t*-test; $p = 0.93$.

In Round 1, assays of PAH-DNA adducts were completed for a random sample of women with invasive breast cancer ($n = 446$) and for nearly all women with in situ disease ($n = 129$), as well as for a random sample of control women ($n = 424$).[8] For Round 2, PAH-DNA adduct assays were completed for the remaining subjects (276 with invasive breast cancer, 22 with in situ breast cancer, and 517 controls) who had donated a blood sample of adequate volume (about 25 ml) to yield sufficient DNA for the PAH analyses (100 μg or more of DNA). To enhance the stability of our estimates for women with in situ disease in our original report,[8] we included most of these women in Round 1. This sampling strategy, however, altered the case:control ratio in the 2 rounds. Nevertheless, risk factors among women with PAH-DNA adduct results in either Rounds 1 or 2 and the pooled group (Rounds 1 + 2) did not differ systematically from all LIBCSP participants who donated blood (data not shown).

Analysis of Round 2 samples was performed approximately 4.5 yr after Round 1. During the intervening time, several assay materials changed, including the lot of 96 microwell plates, the secondary antiserum, and the benzo(*a*)pyrene diolepoxide adducts to lymphocyte DNA (BPDE-DNA) standard. In Round 2, 50% inhibition in the competitive ELISA was approximately 3-fold lower than in Round 1 and resulted in sample values that were also lower than in Round 1. This difference could have been caused by errors in quantitation of the new standard, variations in dilution of primary or secondary antibodies, or plate differences. However, the percentage of nondetectable samples was similar in Round 1 (28.2%) and Round 2 (29.3%). This suggests that the assay was working at a similar level of sensitivity both times, and that the problem was in the conversion of percentage inhibition to absolute adduct level.

Statistical analysis for Round 2. We conducted all statistical analyses using SAS, version 8 (SAS Institute, Inc., Cary, NC). For statistical analysis of the data from Round 2, we used the same methods as described for Round 1.[8] Briefly, the raw lab data were log-transformed on a natural scale. We compared means and *SD*s of PAH-DNA adduct levels (expressed as per 10^8 nucleotides) of cases and controls using the unpaired Student's *t*-test. The ORs and corresponding 95% confidence intervals (CIs) for breast cancer in relation to PAH-DNA adducts were calculated using unconditional logistic regression,[18] with adjustment for the frequency-matching factor of age at reference (date of diagnosis for cases and date of

identification for controls). We considered PAH-DNA adduct levels as a continuous variable, dichotomized into a single binary variable (detectable levels vs. nondetectable levels), as well as categorized into quantiles. We constructed PAH-DNA adduct quantiles based on the distribution among the controls in Round 2, with subjects with nondetectable levels of adducts categorized in the lowest quantile of exposure; those with detectable levels were grouped on the basis of their rank order. Quantile ranges (per 10^8 nucleotides) for subjects in Round 2 ($n = 815$) were as follows: quantile 1, nondetects; quantile 2, $>$ 0–3.8355; quantile 3, $>$ 3.8355–5.9151; quantile 4, $>$ 5.9151–8.3407; quantile 5, $>$ 8.3407.

We assessed confounding by examining the percentage change in the OR. Covariates considered as potential confounders included age at menarche, parity, number of live births, lactation, months of lactation, age at first birth, number of miscarriages, history of fertility problems, body mass index (BMI, weight [kg]/height [m^2]) at reference, BMI at age 20, weight, height, first degree family history of breast cancer, history of benign breast disease, menopausal status, oral contraceptive use, hormone replacement use, race, ethnicity, education, religion, marital status, season of blood donation, total years of residence in the Long Island area, age first moved to Long Island, length of residence in the interview home, and income. Definitions of the covariates used for these analyses have been published,[8,12] except for income. For the 11.5% of subjects with missing information on household income in the year prior to the referent date, we assigned values derived from simple regression models for case and control subjects that included age, race, and education. Omission of subjects with missing income information from the logistic models did not materially alter the estimates of effect for PAH-DNA adducts. None of the covariates we evaluated changed the estimate by 7% or more when we considered them individually or in a backward-elimination process that began with a full model (data not shown). Thus, only the age-adjusted models are shown. We did not include PAH sources, such as smoking or consumption of grilled or smoked foods, in the models to evaluate confounding.

Statistical analysis of pooled data. We pooled the data from Rounds 1 and 2 to increase the stability and precision of the effect estimates for breast cancer associated with PAH-DNA adduct levels, and to facilitate subgroup analyses. We based the pooled analyses on 1,814 subjects who participated in the LIBCSP and provided a sufficiently large blood sample to conduct the adduct assay ($n = 873$ breast cancer cases and 941 controls).

For the pooled analyses, we combined the PAH-DNA adduct data from Rounds 1 and 2 into 2 exposure variables: (1) a single binary variable (detectable/nondetectable), and (2) round-specific quantiles (as described above). We then performed unconditional multivariate logistic regression using the same methods described previously, except that a term indicating round (1 or 2) was included. However, inclusion of this indicator term for round did not materially change the estimates, and thus only results without this indicator are shown. None of the previously mentioned covariates considered as potential confounders changed the estimate of effect by 5% or more (data not shown); therefore, we did not include these covariates in the results shown.

We used polytomous logistic regression[19] to determine whether the ORs varied when the breast cancer cases were partitioned on their tumor characteristics (e.g., stage of disease [in situ vs. invasive] or a joint measure of estrogen receptor [ER] and progesterone receptor [PR] status), as reported in the subjects' medical records. We evaluated effect modification on a multiplicative scale by comparing the log-likelihood estimates derived from unconditional logistic regression models with and without an interaction term. Covariates we considered as potential effect modifiers included menopausal status, season of the blood draw, length of residence in the interview home, age at diagnosis, active cigarette smoking, a combined measure of active cigarette smoking and exposure to passive smoking (from the parent or spouse) in the home, alcohol intake, and intake of grilled or smoked foods (as assessed in the main questionnaire).[8] An additional covariate we considered as a potential effect modifier was a measure of total dietary benzo[*a*]pyrene (BaP), which is one of the major components of PAH[20] and an established carcinogen.[5] The measure used in this analysis was a modification of a PAH food index developed recently by Kazerouni et al.[21] that uses data collected as part of the diet history. Our estimates were modified to reflect the assessment of cooking preparation methods for meat, which were obtained as part of the LIBCSP food frequency questionnaire. For each meat item reported, the percentage of time a woman reported using a specific cooking method was used to create weights. The BaP values unique to that cooking method and doneness level[21] were then weighted. For each meat item, weighted BaP levels for each cooking method were summed across cooking methods. We multiplied the resulting nanograms of BaP per gram for each meat item by the woman's intake (in grams) of that specific meat item. We then categorized the estimated total BaP index levels into quantiles based on the distribution in the control group.

Results

Round 2 analyses. The mean level of PAH-DNA adducts, expressed as per-10^8 nucleotides, among the 298 cases (5.48; $SD = 5.10$) was only slightly higher

than the corresponding adduct level in the 517 controls (5.37; *SD* = 6.90). The age-adjusted OR associated with adduct levels, dichotomized into detectable vs. nondetectable, was 1.23 (95% CI = 0.90, 1.70). As shown in Table 1, there was little evidence for a dose-response relationship; the OR for breast cancer in relation to the highest quantile of adduct levels, as compared with the lowest, was only modestly increased (age-adjusted OR = 1.24 for the highest quantile, as compared with the lowest; 95% CI = 0.80, 1.90). As found in Round 1,[8] the ORs for detectable adducts were higher among premenopausal (OR = 1.84; 95% CI = 1.04, 3.27) than among postmenopausal (OR = 1.01; 95% CI = 0.69, 1.50) women.

Pooled analyses. The mean levels of adducts for LIBCSP participants, obtained from the Round 2 assays, were appreciably lower for both cases and controls than the values reported previously for the LIBCSP participants in Round 1.[8] However, the proportion of control subjects with nondetectable adducts was comparable for Round 1 (28.2%) and Round 2 (29.3%), indicating that the standard curves for Rounds 1 and 2 were relatively different, but that the sensitivity of the method was nearly the same in both (see Materials and Method). In addition, the elevation in the ORs of 1.23 associated with the detectable adducts, vs. nondetectable adducts, observed in Round 2 is not substantially different from the Round 1 estimate of 1.35.[8] Further, there was no apparent dose-response relationship with increasing levels of adducts in either Round 1 or Round 2.

As shown in Table 1, the age-adjusted OR for the highest quantile of PAH-DNA adduct levels in the pooled data was 1.41 (95% CI = 1.07, 1.86). Again, there was no consistent increase in the OR with increasing quantiles of adduct levels. With the adduct levels dichotomized, the age-adjusted OR for detectable vs. nondetectable adducts was 1.29 (95% CI = 1.05, 1.58).

The effect of adduct levels on breast cancer appeared to be more pronounced among premenopausal women (age-adjusted OR = 1.56; 95% CI = 1.09, 2.23) than among postmenopausal women (corresponding OR = 1.14; 95% CI = 0.88, 1.47) (Table 2), although the heterogeneity was not statistically significant on a multiplicative scale ($p > 0.05$). Among postmenopausal women, there was some variability in the estimates with age (OR for 50–64 yr = 1.20 [95% CI = 0.83, 1.75]; for 65–74 yr = 0.98 [95% CI = 0.61, 1.56]; and for 75+ yr = 1.43 [95% CI = 0.71, 2.88]), suggesting that the increase in risk associated with detectable adducts is not limited to premenopausal women only. ORs varied little when cases were categorized by stage of disease or hormone receptor status.

As shown in Table 3, there was some variation in the ORs associated with PAH-DNA adduct levels when subjects were subgrouped on the basis of their self-reported exposure to PAH sources. For example, when we used a conventional smoking exposure measure that considers only active smoking status, the OR for adducts was increased by 48% among nonsmokers, but there was no increase among current smokers. In contrast, use of an exposure measure that takes into consideration active cigarette smoking as well as exposure to passive cigarette smoke in the residential home resulted in an OR for adducts that was decreased by 35% among those who reported no active or residential passive smoking exposure, but was increased by 82% among those reporting only passive smoke exposure in the home, by 67%

Table 1.—Age-Adjusted ORs for Breast Cancer and 95% CIs in Relation to PAH-DNA Adduct Levels for Subjects with Blood Analyzed in Round 2 and for All Subjects with Analyzed Blood

	Subjects with blood analyzed in Round 2 (n = 815)				All subjects with analyzed blood* (N = 1,814)			
			Age-adjusted				Age-adjusted	
Quantile of PAH DNA adduct level†	Cases n	Controls n	OR	95% CI	Cases n	Controls n	OR	95% CI
1‡	80	159	1.00		228	293	1.00	
2	51	90	1.18	0.76, 1.83	156	163	1.26	0.95, 1.67
3	66	89	1.51	0.99, 2.29	175	161	1.40	1.06, 1.85
4	46	90	1.02	0.65, 1.60	137	163	1.09	0.82, 1.45
5	55	89	1.24	0.80, 1.90	177	161	1.41	1.07, 1.86

Notes: OR = odds ratio, CI = confidence interval, and PAH = polycyclic aromatic hydrocarbon. Data from the Long Island Breast Cancer Study Project, 1996–1997.[13]

*Pooled analysis (n = 1,814) included all subjects with blood analyzed in Round 2 (n = 815), plus subjects with blood analyzed in Round 1 (n = 999; see Gammon et al.).[8]

†Quantile ranges for subjects with blood analyzed in Round 2 (n = 815): Quantile 1, nondetects; Quantile 2, > 0–3.8355; Quantile 3, > 3.8355–5.9151; Quantile 4, > 5.9151–8.3407; Quantile 5, > 8.3407. Quantiles were pooled for subjects with blood analyzed in Rounds 1 and 2; therefore, quantile ranges are specific to each round.

‡Includes all subjects with nondetectable DNA adduct levels.

among those reporting only active smoking, and by 6% among those reporting both exposures.

The relationship between adducts and breast cancer also varied with the season in which the blood was drawn (Table 3), with a more than 2-fold increase in the OR for adducts among those who donated blood during the winter (defined as December 21–March 20), in contrast to a 17% decrease in the OR among those who donated blood in the summer months (June 21–September 20). There was some heterogeneity in the relationship between adducts and breast cancer when the results were stratified by dietary sources of PAH, including grilled or smoked foods, in the most recent decade as assessed in the main questionnaire, or the index of total dietary BaP derived from the food frequency questionnaire. For example, the OR for adducts was reduced by 15% among those in the lowest quantile of BaP intake from food, but was elevated by 69% among those with the highest intake of BaP from food. However, none of the variations observed across subgroups was statistically significant. Further, there was no variation in the OR with alcohol intake.

Discussion

We based our analyses on data collected in 1996 and 1997 as part of the LIBCSP; however, the assays to determine PAH-DNA adduct levels were conducted in 2 separate rounds because of budgetary constraints. We reported the results from Round 1 previously,[8] and we report the results from Round 2 here. Analyses from Rounds 1 and 2 yielded similar results for the main effects of PAH-DNA adducts on breast cancer incidence: the magnitude of the association was less than 2.0, the magnitude was not appreciably confounded by known or suspected risk factors for breast cancer, and there was no apparent dose-response relationship.

We then pooled data from Rounds 1 and 2, and the results from these analyses were based on data for 1,814 women; however, the observed CIs from the pooled analyses were not directly comparable with those in either round alone. The pooled analyses revealed a 29% increase in the incidence of breast cancer in relation to detectable, as compared with nondetectable, PAH-DNA adducts. In subgroup analyses, the OR for adducts was more pronounced among premenopausal than postmenopausal women, but the interaction with menopausal status was not statistically significant. Also, the OR was higher among women who donated their blood sample in winter as compared with the other seasons, but again, the variation in the effect estimates was not significant. There was no variation in the ORs associated with PAH-DNA adduct

Table 2.—Age-Adjusted ORs for Breast Cancer and 95% CIs in Relation to Detectable PAH-DNA Adduct Levels for All Subjects with Analyzed Blood

Stratifying factor	Cases *n*	Controls *n*	Age-adjusted OR	Age-adjusted 95% CI
Main effect	873	941	1.29	1.05, 1.58
Menopausal status				
Premenopausal	279	319	1.56	1.09, 2.23
Postmenopausal	572	585	1.14	0.88, 1.47
Unknown	22	37		
Length of residence in interview home				
<15 yr	345	375	1.44	1.05, 1.99
15+ yr	511	544	1.27	0.97, 1.67
Unknown	17	22		
Age at diagnosis				
<65 yr	586	713	1.39	1.09, 1.77
65+ yr	287	228	1.08	0.73, 1.58
Stage*				
In situ	151	941	1.21	0.83, 1.78
Invasive	722	941	1.30	1.05, 1.62
ER and PR status*				
ER+/PR+	355	941	1.39	1.05, 1.83
ER+/PR−	79	941	1.29	0.76, 2.18
ER−/PR+	28	941	1.35	0.57, 3.21
ER−/PR−	106	941	1.33	0.84, 2.10

Notes: OR = odds ratio; CI = confidence interval; PAH = polycyclic aromatic hydrocarbon; ER = estrogen receptor; PR = progesterone receptor. Pooled analysis (*N* = 1,814) included all subjects with blood analyzed in Round 2 (*n* = 815) plus subjects with blood analyzed in Round 1 (*n* = 999; see Gammon et al.).[8] Data from the Long Island Breast Cancer Study Project, 1996–1997.[13]

*Modeled using polytomous regression analysis.

Table 3.—Age-Adjusted ORs for Breast Cancer and 95% CIs in Relation to Detectable PAH-DNA Adduct Levels for All Subjects with Analyzed Blood

Stratifying factor	Cases *n*	Controls *n*	Age-adjusted OR	Age-adjusted 95% CI
Main effect	873	941	1.29	1.05, 1.58
Active cigarette smoking exposure				
Never	388	429	1.48	1.10, 2.00
Former	309	336	1.23	0.86, 1.76
Current	176	174	0.99	0.62, 1.58
Unknown	0	2		
Active or passive cigarette smoking				
Never either	75	90	0.65	0.34, 1.24
Ever passive only	304	331	1.82	1.28, 2.57
Ever active only	68	80	1.67	0.79, 3.52
Ever both	406	417	1.06	0.77, 1.44
Unknown	20	23		
Alcohol intake				
Never	308	333	1.21	0.86, 1.70
Ever	565	608	1.34	1.04, 1.74
Intake of grilled or smoked foods in most recent decade of life*				
Quantile 1	186	209	0.79	0.51, 1.24
Quantile 2	165	182	1.45	0.90, 2.36
Quantile 3	174	173	1.51	0.95, 2.42
Quantile 4	146	174	1.21	0.73, 2.01
Quantile 5	167	166	1.52	0.94, 2.45
Unknown	35	37		
Total dietary BaP index (ng/g)*				
Quantile 1	173	180	0.85	0.53, 1.36
Quantile 2	178	172	1.33	0.83, 2.14
Quantile 3	182	180	1.37	0.87, 2.16
Quantile 4	163	183	1.80	1.10, 2.94
Quantile 5	147	191	1.69	1.05, 2.73
Unknown	30	35		
Season				
Winter	167	215	2.19	1.37, 3.48
Spring	223	279	1.33	0.91, 1.94
Summer	189	192	0.83	0.51, 1.37
Fall	294	255	1.06	0.73, 1.55

Notes: OR = odds ratio; CI = confidence interval; PAH = polycyclic aromatic hydrocarbon; BaP = benzo[*a*]pyrene. Pooled analysis (N = 1,814) included all subjects with blood analyzed in Round 2 (n = 815) plus subjects with blood analyzed in Round 1 (n = 999; see Gammon et al.).[8] Data from the Long Island Breast Cancer Study Project, 1996–1997.[13]
*Dietary quantiles were categorized using the distribution in the control group for the total population of subjects that responded to the main questionnaire, regardless of whether their blood was analyzed.

levels with the breast cancer cases' stage of disease or hormone receptor status.

These pooled results are based on the largest study published to date on the issue of PAH-DNA adducts and breast cancer incidence that also included comprehensive assessment of many known and suspected risk factors for breast cancer. The large sample size permitted further evaluation of results noted in previous reports. In contrast to our earlier report based on data from Round 1[8]—for which a higher OR for women with ER+PR+ tumors was noted—in our larger pooled analysis, we observed no variation in the OR for adducts when case women were stratified by the hormone receptor status of their tumor. Yasui and Potter[22] demonstrated that the age-incidence curve of hormone-receptor–positive tumors reflects the curve associated with the increasingly higher rates observed in Western societies, whereas the age-incidence curves for the other tumor types (hormone-negative or hormone-discordant) reflect those associated with the lower rates observed in Asia. Because of the potential public health importance of identifying factors that contribute to the development of ER+PR+ tumors, further research is needed on whether adducts are associated with hormone receptor status.

Concerns with the LIBCSP data collection methods include a lower response rate among controls than among cases, which was due primarily to the low participation among women aged 65 yr and older.[12] If the older respondents in the LIBCSP somehow differed systematically from nonrespondents, our results may not be

generalizable to older women. Also, blood donors differed from nondonors,[12] although there were no substantial differences in factors found to be associated with breast cancer in the 3 sets of data (i.e., all interviewed subjects, those with blood samples, and those with DNA assay). Nevertheless, there is the possibility that selection bias might have affected our study results. None of the known or suspected risk factors assessed as part of the LIBCSP substantially confounded or modified our pooled analyses, including those factors that were found to influence a subject's probability of donating a sample. Furthermore, the proportion of eligible subjects who were willing to donate blood was comparable with other population-based studies with a phlebotomy component.[23] Thus, it was unlikely that the factors that influence a subject's willingness to provide blood unduly affected our results.

A strength of the LIBCSP was the inclusion of a biomarker of exposure, rather than reliance on subject recall of past exposures. As reviewed by Santella et al.,[17] adducts reflect both PAH exposure and the body's response. PAHs are detoxified in the body through P450 and glutathione s-transferase (GST) pathways, and incomplete detoxification results in DNA damage, which can be reversed by normal DNA repair mechanisms. Susceptible persons for whom such exposure is very high, or those with genetic variations in the P450 and GST pathways or DNA repair mechanisms, yield detectable DNA adduct levels. With the use of such a biomarker, knowledge of the exposure source or the exact genetic susceptibility mechanism need not be characterized precisely. A drawback of this biomarker, however, is that it does not reflect long-term exposure. Instead, because PAH exposure is ubiquitous, it can perhaps be interpreted as an internal dose from recent exposures that reflects how the body would have been likely to respond to such exposures in the past.

Our results were based on a measure of PAH-DNA adducts assessed in peripheral mononuclear cells; other investigators have asserted that this approach may yield lower effect estimates than when adducts are measured in breast tissue.[24] The lower ORs reported here, and in our earlier report,[8] might have been caused by attenuation, which is frequently observed when studies with larger sample sizes yield more stable estimates of effect.[25,26] The stronger effects reported by others[10] were based on small numbers of subjects and were therefore more likely to result in less-stable effect estimates. Research based on large numbers of subjects and multiple exposure assessment methods would help to clarify this issue.

In our pooled analyses based on 1,814 subjects, premenopausal women had a higher OR for PAH-DNA adducts than postmenopausal women. In our previous Round 1 analysis[8] of 999 women, the variation in the OR with menopausal status was less pronounced than that of Round 2. However, in age-specific analyses among postmenopausal women, most age groups had elevated ORs for detectable adducts. Premenopausal women were not hypothesized a priori to be a high-risk subgroup. Other studies that have explored the association between adducts and breast cancer have had too few women to adequately conduct subgroup analyses. Additional research is needed to clarify this issue and, if the association is confirmed, to identify potential underlying mechanism(s) for the heterogeneity in risk by menopausal status.

Also in our pooled analyses, we observed inconsistent variation in ORs when the association between PAH-DNA adducts and breast cancer was stratified by various measures of PAH exposure. When we considered only active smoking, we noted the lowest OR among women who reported current active smoking, which has been associated with high levels of PAH exposure.[16] However, when we considered active smoking *and* exposure to passive smoke in the home together, the lowest OR was among those who reported neither exposure. Also, we noted a higher OR among women with blood collected during the winter months—a season that others have associated with high BaP air concentration measurements.[27] The OR for breast cancer and adducts also varied somewhat with intake of PAH-containing foods, regardless of the measure used, with a reduced OR for those in the lowest quantile of intake and an elevated OR for all other levels of intake. The inconsistent pattern of the ORs across varying levels of PAH exposure confirms that the detection of PAH-DNA adducts may not be associated with higher levels of exposure,[28] but may instead identify subjects particularly susceptible to the effects of PAH.

In a recent laboratory study,[29] researchers found that when human mammary epithelial cells were exposed to physiologically relevant concentrations of ethanol prior to dosing with BaP, adduct formation was enhanced. However, in our pooled sample of 1,814 women, the association between breast cancer and PAH-DNA adducts was not modified by alcohol intake.

Further research with sufficiently large sample sizes is needed to confirm our observation that PAH-DNA adducts are associated with a modest increase in breast cancer incidence, and to explore whether the effect differs with variations in genetic polymorphisms or other markers of susceptibility. In addition, although active cigarette smoking does not appear to be associated with breast cancer,[30] whether breast cancer is associated with other key sources of PAH exposures (e.g., ambient sources such as air pollution, environmental tobacco smoke, and food; or occupational sources[31]) would also be of interest. To advance research in this area, well-conducted studies with multiple exposure-assessment methods and based on large numbers of women are needed.[2]

* * * * * * * * * * *

For their valuable contributions to the Long Island Breast Cancer Study Project, the authors thank the members of the Long Island Breast Cancer Network; the 31 participating institutions on Long Island and in New York City, NY; our National Institutes of Health collaborators—Gwen Colman, Ph.D., National Institutes of Environmental Health Sciences; G. Iris Obrams, M.D., Ph.D. formerly of the National Cancer Institute; members of the External Advisory Committee to the population-based case-control study Leslie Bernstein, Ph.D. (committee chair); Gerald Akland, M.S.; Barbara Balaban, M.S.W.; Blake Cady, M.D.; Dale Sandler, Ph.D.; Roy Shore, Ph.D.; and Gerald Wogan, Ph.D., as well as other collaborators who assisted with various aspects of our data collection efforts, including Gail Garbowski, M.P.H.; H. Leon Bradlow, Ph.D.; David Camann, B.S.; Martin Trent, B.S.; Ruby Senie, Ph.D.; Carla Maffeo, Ph.D.; Pat Montalvan; Gertrud Berkowitz, Ph.D.; Margaret Kemeny, M.D.; Mark Citron, M.D.; Freya Schnabel, M.D.; Allen Schuss, M.D.; Steven Hajdu, M.D.; and Vincent Vinceguerra, M.D.

This work was supported in part by grants from the National Cancer Institute and the National Institutes of Environmental Health and Sciences (grants UO1CA/ES66572, UO1CA66572, P30ES09089, and P30ES10126), the Breast Cancer Research Foundation, and the Babylon Breast Cancer Coalition.

Submitted for publication October 28, 2003; revised; accepted for publication May 14, 2004.

Requests for reprints should be sent to Dr. Marilie D. Gammon, Department of Epidemiology, CB#7435 McGavran Greenberg Hall, University of North Carolina School of Public Health, Chapel Hill, NC 27599-7435.

E-mail: gammon@unc.edu

* * * * * * * * * * *

References

1. El-Bayoumy K, Chae YH, Upadhyaya P, et al. Comparative tumorigenicity of benzo[*a*]pyrene, 1-nitropyrene, and 2-amnio-1-methyl-6-phenylimidazo [4,5-*b*]pyridine administered by gavage to female CD rats. Carcinogenesis 1995; 16:431–4.
2. Gammon MD, Santella RM. Reply re: Environmental toxins and breast cancer on Long Island. I. Polycyclic aromatic hydrocarbons (PAH)-DNA adducts [Author response to Letter to the Editor]. Cancer Epidemiol Biomarkers Prev 2002; 12:75–6.
3. Menzie CA, Potocki BB, Santodonato J. Exposure to carcinogenic PAHs in the environment. Environ Sci Technol 1992; 26:1278–84.
4. Eder E. Intra-individual variations of DNA adduct levels in humans. Mutat Res 1999; 42:249–61.
5. U.S. Environmental Protection Agency (EPA). Federal Registry. Washington, DC: EPA, 1984.
6. Santella RM. Immunologic methods for detection of carcinogen-DNA damage in humans. Cancer Epidemiol Biomarkers Prev 1999; 8:733–9.
7. Poirier MC, Santella R, Weston A. Carcinogen macromolecular adducts and their measurement. Carcinogenesis 2000; 21:353–60.
8. Gammon MD, Santella RM, Neugut AI, et al. Environmental toxins and breast cancer on Long Island. I. Polycyclic aromatic hydrocarbons (PAH)-DNA adducts. Cancer Epidemiol Biomarkers Prev 2002; 11: 677–85.
9. Li D, Walcott FL, Chang P, et al. Genetic and environmental determinants on tissue response to in vitro carcinogen exposure and risk of breast cancer. Cancer Res 2002; 62:4566–70.
10. Rundle A, Tang D, Hibshoosh H, et al. The relationship between genetic damage from polycyclic aromatic hydrocarbons in breast tissue and breast cancer. Carcinogenesis 2000; 21:1281–9.
11. Li D, Wang M, Dhingra K, et al. Aromatic DNA adducts in adjacent tissues of breast cancer patients: clues to breast cancer etiology. Cancer Res 1996; 56:287–93.
12. Gammon MD, Neugut AI, Santella RM, et al. 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; 74:235–54.
13. U.S. National Institutes of Health, National Cancer Institute. Long Island Breast Cancer Study Project. <http://epi.grants.cancer.gov/LIBCSP/index.html> Accessed March 18, 2004.
14. Waksberg J. Sampling methods for random digit dialing. J Am Stat Assoc 1978; 73:40–6.
15. U.S. National Institutes of Health, National Cancer Institute. Long Island Breast Cancer Study Project. Breast Cancer and the Environment on Long Island: Main Questionnaire. <http://epi.grants.cancer.gov/LIBCSP/projects/Questionnaire.html> Accessed March 18, 2004.
16. Poirier MC, Santella R, Weinstein IB, et al. Quantitation of benzo(*a*)pyrene-deoxyguanosine adducts by radioimmunoassay. Cancer Res 1980; 40:412–6.
17. Santella RM, Gringberg-Funes RA, Young TL, et al. Cigarette smoking related polycyclic aromatic hydrocarbon-DNA adducts in peripheral mononuclear cells. Carcinogenesis 1992; 13:2041–5.
18. Selvin S. Statistical Analysis of Epidemiologic Data. 2nd ed. New York: Oxford University Press, 1996.
19. Hosmer DW, Lemeshow S. Applied Logistic Regression. New York: John Wiley & Sons, 1989.
20. Jeffy BD, Chirnomas RB, Romagnolo DF. Epigenetics of breast cancer: polycyclic aromatic hydrocarbons as risk factors. Environ Mol Mutagen 2002; 3:235–44.
21. Kazerouni N, Sinha R, Hsu CH, et al. Analysis of 200 food items for benzo[*a*]pyrene and estimation of its intake in an epidemiologic study. Food Chem Toxicol 2001; 39: 423–36.
22. Yasui Y, Potter JD. The shape of age-incidence curves of female breast cancer by hormone-receptor status. Cancer Causes Control 1999; 10:431–7.
23. Millikan RC, Pittman GS, Newman B, et al. Cigarette smoking, *N*-acetyltransferases 1 and 2, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998; 7:371–8.
24. Perera F, Rundle A. Re: environmental toxins and breast cancer on Long Island. I. Polycyclic aromatic hydrocarbon DNA adducts [Letter to the Editor]. Cancer Epidemiol Biomarkers Prev 2003; 12:75.
25. Hirschhorn JN, Lohmueller K, Byrne E, et al. A comprehensive review of genetic association studies. Genet Med 2002; 4:45–61.
26. Ioannidis JPA, Ntzani EE, Trikalinos TA, et al. Replication validity of genetic association studies. Nat Genet 2001; 3:306–9.
27. Flessel P, Wang YY, Chang KI, et al. Season variation and trends in concentrations of filter collected polycyclic aromatic hydrocarbons (PAH) and mutagenic activity in the San Francisco Bay area. J Air Waste Manag Assoc 1991; 41:276–81.
28. Boysen G, Hecht SS. Analysis of DNA and protein adducts of benzo[*a*]pyrene in human tissues using structure-specific methods. Mutat Res 2003; 543:17–30.

29. Barnes SL, Singletary KW, Frey R. Ethanol and acetaldehyde enhance benzo[*a*]pyrene-DNA adduct formation in human mammary epithelial cells. Carcinogenesis 2000; 21:2123–8.
30. Collaborative Group on Hormonal Factors. Alcohol, tobacco and breast cancer—collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer 2002; 87:1234–45.
31. Petralia SA, Vena JE, Freudenheim JL, et al. Risk of premenopausal breast cancer in association with occupational sources or polycyclic aromatic hydrocarbons and benzene. Scand J Work Environ Health 1999; 25:215–21.

Decision editor: Kaye H. Kilburn, MD