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Mutagen sensitivity, tobacco smoking and breast cancer risk: a case–control study

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Given the high incidence of breast cancer and that more than half of cases remain unexplained, the need to identify risk factors for breast cancer remains. Deficiencies in DNA repair capacity have been associated with cancer risk. The mutagen sensitivity assay (MSA), a phenotypic marker of DNA damage response and repair capacity, has been consistently shown to associate with the risk of tobacco-related cancers. ***Methods:*** **In a case–control study of 164 women with breast cancer and 165 women without the disease, we investigated the association between mutagen sensitivity and risk of breast cancer using bleomycin as the mutagen.** ***Results:*** **High bleomycin sensitivity (>0.65 breaks per cell) was associated with an increased risk of breast cancer, with an adjusted odds ratio of 2.8 [95% confidence interval (CI) = 1.7–4.5]. Risk increased with greater number of bleomycin-induced chromosomal breaks (P_{trend} = 0.01). The association between bleomycin sensitivity and breast cancer risk was greater for women who were black, premenopausal and ever smokers. Our data also suggest that bleomycin sensitivity may modulate the effect of tobacco smoking on breast cancer risk. Among women with hypersensitivity to bleomycin, ever smokers had a 1.6-fold increased risk of breast cancer (95% CI = 0.6–3.9, *P* for interaction between tobacco smoking and bleomycin sensitivity = 0.32).** ***Conclusions:*** **Increased bleomycin sensitivity is significantly associated with an increased risk of breast cancer in both pre- and postmenopausal women. Our observation that the effect of tobacco smoking on breast cancer risk may differ based on mutagen sensitivity status warrants further investigation.**

Introduction

Breast cancer is the most common malignancy in women (1). In the USA, breast cancer incidence rates have been rising slowly for the past two decades and breast cancer is the second leading cause of cancer-related death in women (2,3). However, there is currently no method available that predicts which individual woman is most probably to develop the disease in the general population with high discriminatory ability. Of the nearly 241 000 women diagnosed each year, ∼90% are sporadic cases in women without a significant family history of breast cancer and no other strong identifiable risk factors other than age and reproductive or hormonal risk factors (4).

Breast cancer is a disease of mixed etiology. The best-documented and most common risk factors are related to endogenous and exogenous hormonal exposure. Women who carry mutations in *BRCA1* or *BRCA2* genes have a very high risk of breast cancer. However, breast cancer cases caused by highly penetrant genes (*BRCA1* and *BRCA2)* account for only ∼5% of all cases (5). There are other moderately penetrant genes, namely *p53*, *pTEN*, *STK11*, *CHEK2*, *CDH1*, *ATM* and *PALB2*, which are associated with ∼20 to 25% of familial breast cancer cases and 1–3% of all cases in the general population (6). Recent genome-wide association studies have identified at least eight genetic variants that are common genetic susceptibility loci associated with modest increases in breast cancer risk (7) and more are probably to be identified in the future. These recent discoveries are shedding light on important mechanisms in breast carcinogenesis and provide evidence for the presence of etiologic heterogeneity across breast cancer subtypes (8). Noticeably, many of these breast cancer susceptibility genes, i.e. *p53*, *pTEN*, *CHEK2*, *ATM*, *BRCA1* and *MAP3K1*, regulate cell cycle control and DNA repair functions, suggesting that subtle flaws in these pathways may play a significant role in individual susceptibility to breast cancer in the general population (9). Mutagen sensitivity assay, a phenotypic assay that accounts for the net results of several genetic pathways and the cumulative effects of low-risk genetic variants, has been proposed as a potentially useful biomarker to explore individual susceptibility to breast cancer (10).

The mutagen sensitivity assay (MSA) is a promising method for cancer risk assessment (11). The MSA is a measure of the frequency of chromosomal breaks induced by mutagens in short-term peripheral blood cultures and serves as a phenotypic marker of the combined effects of sensitivity to the carcinogen exposure, the individual's DNA damage response and repair capacity. Individuals with suboptimal DNA repair capacity accumulate higher levels of chromosomal breaks compared with individuals with efficient DNA repair function (11). The MSA has been tested as a biological marker of cancer susceptibility in several case–control studies with mutagen sensitivity shown to be an independent risk factor for a variety of tobacco-related cancers, including lung, head and neck and liver cancers (12–18). In breast cancer, only a few small case–control studies (typically with <100 cases) examined the association between the MSA and breast cancer risk (19–21). These previous studies suggest that the MSA may be a promising biomarker for breast cancer risk in premenopausal women and African-American women. We conducted a case–control study to further investigate whether bleomycin sensitivity is associated with breast cancer risk in both pre- and postmenopausal women in a predominately white population.

Abbreviations: CI, confidence interval; HRT, hormonal replacement therapy; MSA, mutagen sensitivity assay; OR, odds ratio.

Materials and methods

Study population

The study population has been described previously (22). Breast cancer cases (n = 178) were recruited at the Georgetown University Hospital clinics (Lombardi Comprehensive Cancer Center's Division of Medical Oncology, Department of Surgery and the Betty Lou Ourisman Breast Cancer Clinic). The inclusion criteria for cases included a diagnosis of breast cancer within the prior 6 months, among women who have not yet received chemotherapy and radiotherapy treatment and were able to provide informed consent in English. Exclusion criteria included having a prior history of cancer, chemotherapy and radiation treatment or an active infection or immunological disorder that needed treatment with antibiotics or immunosuppressive medication within 1 month prior to enrollment. From 2006 through 2008, a total of 254 newly diagnosed breast cancer patients were identified to be eligible and 178 (70%) participated in our study. Common reasons for non-participation were too busy or not interested (21%), overwhelmed by cancer diagnosis (5%) and not responsive to phone call or e-mail contact (4%). Four cases (2%) did not provide a blood sample, six blood cultures failed (3%) and blood culture was not performed on four blood samples (2%). Therefore, the final number of cases with bleomycin sensitivity data for analysis was 164.

Between 2006 and 2008, a total of 380 controls were recruited by random selection from healthy women who visited the mammography screening clinic at Georgetown University Hospital and each control donated a blood sample for the mutagen sensitivity assay. The inclusion and exclusion criteria for controls were the same as for cases. Additionally, controls who had a breast biopsy within the past 6 months or were currently pregnant or breast-feeding

were not eligible. The overall participation rate among the eligible women was 60% for controls. The major reasons for non-participation were being too busy (19%) or not interested (20%). Blood cultures failed in 10 samples (3%); thus, the final number of controls with mutagen sensitivity data was 370. For this analysis, 165 controls were selected from the pool of 370 controls and matched to enrolled cases on age (2 year interval), race and state of residency (District of Columbia, Maryland or Virginia).

After providing informed consent, subjects completed a structured in-person interview assessing prior medical history, tobacco smoke exposures, alcohol use, current medications, family medical history, reproductive history and socioeconomic characteristics. Trained phlebotomists obtained venous blood using heparinized tubes. The study was approved by the MedStar Research Institute-Georgetown University Oncology Institutional Review Board.

Mutagen sensitivity assay

Lymphocyte cultures were set up within 48 h of blood collection using fresh whole blood, following a protocol described previously (15). Briefly, 1 ml of fresh blood was added to 9 ml of RPMI-1640 medium supplemented with 15% bovine serum, 1.5% phytohemagglutinin (Invitrogen, Rockville, MD), 2 mM L-glutamine and 100 U/ml each of penicillin and streptomycin. After the cells were cultured for 90 h at 37°C, 200 µl of 1.5 U/ml bleomycin (Mead Johnson Oncology Products, Princeton, NJ) was added into the culture and the culture was incubated at 37°C for an additional 5 h. To arrest the cells at metaphase, 0.2 µg/ml colcemid was added to the culture 1 h before harvest. The cells were treated in hypotonic solution (0.06 M HCl) and fixed in fixative (methanol: acetic acid = 3:1). The cells were then dropped onto clean microscopic slides, air dried and stained with 4% Gurr's Giemsa solution (BDH Laboratory Supplies, Poole, Dorset, UK). Fifty well-spread metaphase cells per subject were examined to visually score the chromosomal breaks. Details of the criteria for the scoring of chromosomal breaks were described previously (15). The slides were coded and scored without the knowledge of case–control status. The overall assay success rate was 97%. In order to assess the reproducibility of the MSA, blood samples from ~10% ($n = 34$) randomly selected subjects were assayed in duplicates. The results indicated that MSA score in assay 1 was very similar to that in assay 2 (mean ± SD = 0.57 ± 0.3 and 0.58 ± 0.2, respectively, $P = 0.71$). The MSA scores were significantly correlated between assay 1 and assay 2 [Pearson correlation coefficiency (r) = 0.83, $P < 0.01$] and the average coefficient of variation for the 34 pairs of duplicates was 12.8%.

Statistical analysis

The chi-square goodness-of-fit test or Student's t test was used to test differences in the distributions by age, gender, race, smoking status and other subject characteristics between cases and controls. Smoking status was stratified into two categories: never smokers—individuals who had never smoked >100 cigarettes in their life and ever (former/current) smokers—individuals who had smoked >100 cigarettes in their life. Family history of female cancers was defined as having breast or ovarian cancer in first- or second-degree biological relatives. Physical activity was defined as any physical activity on a regular basis (at least once a week on average) for at least 20 min at a time that either made the subjects sweat or increased their heart rate. Alcohol consumption was defined as whether the subjects had consumed a total of ≥12 alcoholic beverages over the course of their lifetime. Wilcoxon rank-sum test was used to determine the statistical significance of case–control comparisons of bleomycin-induced chromosomal breaks. Multivariate logistic regression was used to estimate the association between bleomycin sensitivity and breast cancer risk, adjusting for known breast cancer risk factors (age, race and menopausal status) and other potential confounders (physical activity during teenage years and environmental tobacco exposure at work). If inclusion of a factor altered the odds ratio (OR) estimation by >15%, that factor was retained in the final model. An individual was considered to have high bleomycin sensitivity if the MSA score was equal to or greater than the 50th percentile value in controls (0.64 breaks per cell). To assess for the presence of a dose-response trend between breast cancer risk and the degree of bleomycin sensitivity, women were categorized to hyposensitive (lowest quartile), sensitive (two middle quartile categories) and hypersensitive (highest quartile) based on their MSA scores and hyposensitive women (the lowest quartile) were used as the reference. The above-mentioned categories were also used to examine the combined effect of tobacco smoking and mutagen sensitivity. All P-values were two sided. All analyses were performed using SAS software, version 9 (SAS Institute, Cary, NC).

Results

Study population

Demographic characteristics of the study subjects are presented in Table I. The mean age was 52.3 for cases and 52.6 for controls. There were no significant differences in the distribution of race, tobacco smoking status, alcohol use, reproductive characteristics or family history of female cancers (breast and ovarian) between cases and controls. Controls were significantly more probably to be physically active and less probably to be exposed to environmental tobacco at work (Table I). Forty-two percent of cases and 48% of controls had completed college or higher education and 54% of cases and controls had median family income >$100k, reflecting the high socioeconomic characteristics of patients seen at the Lombardi Comprehensive Cancer Center.

Table I. Characteristics of study population by case–control status

Descriptive	Cases ($n = 164$)	Controls ($n = 165$)	P
Age (years), mean (SD)	52.31 (10.67)	52.62 (9.86)	0.79
Race, n (%)			
White	125 (76)	126 (76)	
Black	29 (18)	31 (19)	
Other	10 (6)	8 (5)	0.94
BMI, mean (SD)	26.89 (6.15)	26.66 (6.48)	0.75
Active smoking, n (%)			
Ever	58 (37)	70 (43)	
Never	101 (64)	93 (57)	0.25
ETS at work, n (%)[a]			
Yes	35 (38)	19 (21)	0.01
No	57 (62)	72 (79)	
Alcohol consumption, n (%)			
Ever	136 (88)	152 (94)	
Never	18 (12)	10 (6)	0.08
Number of children, mean (SD)	1.77 (1.30)	2.00 (1.39)	0.14
Age at menarche (years), mean (SD)	12.57 (1.41)	12.45 (1.48)	0.48
Menopausal status, n (%)			
Premenopausal	68 (44)	74 (45)	
Postmenopausal	87 (56)	89 (55)	0.78
Use of HRT (%)[b]			
Ever	42 (52)	55 (62)	
Never	40 (48)	34 (38)	0.19
Physical activity at age 13–19, n (%)			
Yes	99 (62)	123 (75)	
No	60 (38)	40 (25)	0.01
Family history of female cancers, n (%)			
0 relatives affected	141 (94)	151 (94)	
1 relative affected	8 (5)	9 (6)	
2 relatives affected	1 (1)	0 (0)	0.59
Educational level (%)			
Below college	89 (58)	85 (52)	
Equal/above college	65 (42)	77 (48)	0.34
Household income (%)			
Below $100K	53 (46)	56 (46)	
Equal/above $100K	63 (54)	67 (54)	0.98

Physical activity was defined as any weekly physical activity, longer than 20 min at a time that would make the subject sweat or increase their heart rate. Family history of female cancers was defined as any breast or ovarian cases among first- and second-degree blood relatives. ETS, environmental tobacco smoking.
[a]ETS among never smokers.
[b]HRT among postmenopausal women.

Correlations of bleomycin sensitivity and host factors

We assessed the relationship between bleomycin sensitivity and selected host factors among controls and cases separately. Among controls, mutagen sensitivity tended to increase with age [Pearson correlation coefficient (r) = 0.17, $P < 0.03$], was weakly inversely correlated with income ($r = -0.16$, $P = 0.07$) and was not affected by race, menopausal status, family history of female cancers, physical activity during teenage years, smoking status or environmental tobacco exposure at work and educational level. Among cases, none of the host factors examined seemed to significantly correlate with mutagen sensitivity.

Bleomycin sensitivity and breast cancer risk

Table II presents case–control comparisons of the mean number of bleomycin-induced breaks per cell. Overall, the mean breaks per cell were significantly higher in cases (mean = 0.86) than in controls (mean = 0.72, $P < 0.01$). When the case–control comparison was stratified by age, race, menopausal status, tobacco smoking and physical activity, we observed similar patterns of case–control differences across all subgroups of women (Table II).

We assessed the association between bleomycin sensitivity and breast cancer risk using multivariate logistic regression. Using the median (0.64 breaks per cell) in controls as a cut point, subjects were dichotomized into high (equal or above median) or low (below median) bleomycin-sensitivity groups. Women who had high bleomycin sensitivity had significantly increased breast cancer risk compared with women with low bleomycin sensitivity [adjusted OR = 2.8, 95% confidence interval (CI) = 1.7–4.5] in the overall study population (Table III). ORs were adjusted for age, race, menopausal status, physical activity during teenage years and environmental tobacco exposure at work. When stratified by menopausal status, the ORs were 3.3 (95% CI = 1.6–6.9) and 2.5 (95% CI = 1.3–5.0) for pre- and postmenopausal women, respectively. Among postmenopausal women, the association between MSA and breast cancer risk was stronger (OR = 2.6, 95% CI = 1.0–6.5) in those who reported ever use of hormonal replacement therapy (HRT) compared with women who never used HRT (OR = 1.9, 95% CI = 0.6–6.0). To assess for the presence of a dose-response trend between breast cancer risk and the degree of bleomycin sensitivity, women were categorized to hyposensitive (lowest quartile), sensitive (two middle quartile categories) and hypersensitive (highest quartile) based on their MSA scores and hyposensitive women (the lowest quartile) were used as the reference. A significant dose–response relationship was observed ($P_{trend} < 0.01$), and the lowest versus highest quartile OR was 3.8 (95% CI = 1.9–7.5) for all women. The lowest versus highest quartile ORs were 4.7 (95% CI = 1.7–13.3) and 3.7 (95% CI = 1.4–9.5) for pre- and postmenopausal women, respectively. Among postmenopausal women, the lowest versus highest quartile ORs were 4.2 (95% CI = 1.1–16.0) and 2.2 (95% CI = 0.5–10.5), respectively, for ever versus never HRT users. The associations between mutagen sensitivity and breast cancer risk were stronger among black women with a 6.9-fold increase, whereas white women had a 2.4-fold increase. The racial difference persisted across quartile comparisons (Table III).

Table II. Case–control comparison of mean bleomycin-induced breaks per cell

Host factors	Cases		Controls		P^*
	N	Mean (SD)	*N*	Mean (SD)	
All subjects	164	0.86 (0.37)	165	0.72 (0.34)	<0.01
Age					
<55	101	0.85 (0.36)	102	0.68 (0.33)	<0.01
≥55	63	0.88 (0.38)	63	0.77 (0.34)	0.06
Race					
White	125	0.85 (0.38)	126	0.71 (0.36)	<0.01
African-American	29	0.88 (0.33)	31	0.74 (0.27)	0.06
Active smoking					
Ever	101	0.83 (0.36)	93	0.72 (0.35)	<0.01
Never	58	0.92 (0.39)	70	0.71 (0.33)	<0.01
ETS at work[a]					
No	57	0.87 (0.41)	72	0.72 (0.35)	<0.01
Yes	35	0.79 (0.29)	19	0.73 (0.37)	0.42
Menopausal status					
Premenopausal	68	0.83 (0.36)	74	0.69 (0.37)	<0.01
Postmenopausal	87	0.88 (0.37)	89	0.74 (0.32)	<0.01
Physical activity at teenage years					
No	60	0.86 (0.41)	40	0.72 (0.39)	0.05
Yes	99	0.86 (0.35)	123	0.71 (0.33)	<0.01

ETS, environmental tobacco smoking.
[a]ETS among non-smokers.
*P-values are from Wilcoxon rank-sum test.

Joint effect of bleomycin sensitivity and tobacco smoking on breast cancer risk

To test the hypothesis that women who are more sensitive to carcinogens (as defined by high bleomycin sensitivity) are more susceptible to tobacco smoking-induced breast cancer, we performed various stratified analyses. When stratified by smoking status, the association between mutagen sensitivity and breast cancer risk was stronger for ever smokers (OR = 3.5, 95% CI = 1.5–8.0) than never smokers (OR = 2.4, 95% CI = 1.3–4.6, Table III). Overall, tobacco smoking was not significantly associated with breast cancer risk in our study population (age and race adjusted OR = 0.8, 95% CI = 0.3–2.3). When the subjects were stratified by the three categories of bleomycin sensitivity (hyposensitive, sensitive and hypersensitive), bleomycin sensitivity seems to modulate the effect of cigarette smoking on breast cancer risk. Tobacco smoking was not significantly associated with breast cancer risk among bleomycin-hyposensitive women (OR = 0.8, 95% CI = 0.3–2.8), was significantly associated with a decreased breast cancer risk among bleomycin sensitive women (OR = 0.4, 95% CI = 0.2–0.9) and was associated with a non-significant increased breast cancer risk among bleomycin hypersensitive women (OR = 1.6, 95% CI = 0.6–3.9). However, there was no significant interaction between tobacco smoking and bleomycin sensitivity when the interaction was formally tested in the logistic model ($P = 0.32$). We further examined the combined effect of tobacco smoking and bleomycin sensitivity on breast cancer risk, using women who were hyposensitive and never smokers as the referent group. Women who had bleomycin hypersensitive phenotypes and were smokers were at 3.8-fold increased risk of breast cancer compared with women who had a bleomycin-hyposensitive phenotype and were never smokers (Table IV).

Discussion

In this report, we demonstrated that, after adjusting for known breast cancer risk factors, high bleomycin sensitivity is significantly associated with an increased risk of breast cancer in both pre- and postmenopausal women. This finding supports our hypothesis that deficiencies in DNA repair and cell cycle control pathways, as measured by mutagen sensitivity assay, contribute to breast cancer susceptibility.

The failure to maintain genome integrity is central to the problem of carcinogenesis. Increased genetic instability, either spontaneous or mutagen-induced, has been considered a predisposing factor for neoplastic transformation. Epidemiological studies of markers of DNA repair and cancer susceptibility in humans have consistently revealed positive associations between DNA repair capacity and cancer occurrence (23). In a study of 36 familial cases, their 85 first or second degree female relatives, 36 sporadic cases and 40 unrelated female controls, Jyothish *et al.* (20) reported that bleomycin-induced chromosomal breaks were significantly higher in both familial and sporadic breast cancer patients compared with unrelated female controls. Bleomycin-induced chromosomal breaks were also significantly higher in female relatives of familial breast cancer cases compared with unrelated female controls (20). We also chose bleomycin as the mutagen for the MSA. The main reason is because bleomycin is a radiomimetic drug and ionizing radiation is an established cause for breast cancer (24). Different mutagens may act on cells through different molecular mechanisms and may activate different repair mechanisms. Bleomycin for example can induce single-stranded and double-stranded DNA damages that require base excision or recombinant DNA repair (25,26). Benzo[*a*]pyrene diol-epoxide is a metabolic product of benzo[*a*])pyrene, a major constituent of tobacco smoke, and forms covalent 'bulky' adducts upon interaction with DNA that requires the nucleotide excision repair pathway (27). Therefore, it may be necessary to use a panel of mutagens to assess multiple repair pathways. Xiong *et al.* (19) investigated benzo[*a*]pyrene diol-epoxide sensitivity and breast cancer risk in a case–control study (100 cases and 105 controls) of predominantly white women and reported that benzo[*a*]pyrene diol-epoxide sensitivity was

Table III. Logistic regression examining the association between bleomycin sensitivity and breast cancer risk

MSA categories (b/c)	Cases–controls	OR[a] (95% CI)	Cases–controls	OR[a] (95% CI)
	All subjects			
Low (≤0.64)	51/85	1.00		
High (>0.65)	113/80	2.8 (1.7–4.5)		
Hyposensitive (≤0.48)	21/43	1.00		
Sensitive (0.49–0.86)	73/80	2.0 (1.1–3.9)		
Hypersensitive (≥0.87)	70/42	3.8 (1.9–7.5)		
P trend		<0.01		
	White women		Black women	
Low (≤0.64)	41/65	1.00	6/17	1.00
High (>0.65)	84/61	2.4 (1.4–4.2)	23/14	6.9 (1.7–28.1)
Hyposensitive (≤0.48)	17/36	1.00	2/6	1.00
Sensitive (0.49–0.86)	54/58	2.0 (1.0–4.2)	15/17	3.8 (0.6–27.0)
Hypersensitive (≥0.87)	54/32	3.8 (1.7–8.1)	12/8	6.9 (0.9–54.3)
P trend		<0.01		0.07
	Premenopausal women		Postmenopausal women	
Low (≤0.64)	25/47	1.00	22/38	1.00
High (>0.65)	43/27	3.3 (1.6–6.9)	65/51	2.5 (1.3–5.0)
Hyposensitive (≤0.48)	10/23	1.00	11/20	1.00
Sensitive (0.49–0.86)	33/35	2.7 (1.1–7.1)	35/44	1.8 (0.7–4.7)
Hypersensitive (≥0.87)	25/16	4.7 (1.7–13.3)	41/25	3.7 (1.4–9.5)
P trend		<0.01		<0.01
	Postmenopausal women only		Postmenopausal women only	
	Ever user of HRT		Never user of HRT	
Low (≤0.64)	11/26	1.00	9/12	1.00
High (>0.65)	31/29	2.6 (1.0–6.5)	31/22	1.9 (0.6–6.0)
Hyposensitive (≤0.48)	5/14	1.00	5/6	1.00
Sensitive (0.49–0.86)	19/26	2.7 (0.7–10.0)	15/18	1.2 (0.3–5.1)
Hypersensitive (≥0.87)	18/15	4.2 (1.1–16.0)	20/10	2.2 (0.5–10.5)
P trend		0.03		0.23
	Ever smokers		Never smokers	
Low (≤0.64)	18/38	1.00	30/47	1.00
High (>0.65)	40/32	3.5 (1.5–8.0)	71/46	2.4 (1.3–4.6)
Hyposensitive (≤0.48)	8/19	1.00	12/24	1.00
Sensitive (0.49–0.86)	19/33	1.6 (0.6–4.7)	51/46	2.3 (1.0–5.4)
Hypersensitive (≥0.87)	31/18	6.4 (2.0–20.2)	38/23	2.7 (1.1–6.8)
P trend		<0.01		0.04

b/c, breaks per cell.
[a]Adjusted for age at interview, race (when appropriate), physical activity at teens, environmental tobacco smoking at work and menopausal status (when appropriate).

Table IV. Combined effect of mutagen sensitivity and cigarette smoking on breast cancer risk

Level of mutagen sensitivity	Smoking status	Cases–controls	OR[a] (95% CI)
Hyposensitive (≤0.48 b/c)	Never	12/24	1.00
Sensitive (0.49–0.86 b/c)	Never	51/46	2.4 (1.0–5.5)
Hypersensitive (≥0.87 b/c)	Never	38/23	3.0 (1.2–7.4)
Hyposensitive (≤0.48 b/c)	Ever	8/19	0.7 (0.3–2.3)
Sensitive (0.49–0.86 b/c)	Ever	19/33	1.2 (0.5–2.9)
Hypersensitive (≥0.87 b/c)	Ever	31/18	3.8 (1.5–10.0)

b/c = breaks per cell.
[a]Adjusted for age at interview, race, physical activity at teens, environmental tobacco smoking at work and menopausal status.

associated with a 3-fold increased risk of breast cancer in premenopausal women. More recently, a study by our group examined the MSA using gamma radiation as the mutagen and breast cancer risk in a case–control study of African-American women (61 cases and 86 controls) and gamma radiation sensitivity was found to be associated with an increased breast cancer risk (OR = 4.5, 95% CI = 2.2–9.1) (21). Our results are in agreement with these previous reports and provide further evidence that bleomycin sensitivity is significantly associated with breast cancer risk in both pre- and postmenopausal women. The observed association was somewhat stronger in premenopausal women than in postmenopausal women, which leads to the hypothesis that female hormone exposure may modulate the effect of MSA on breast cancer risk. This hypothesis was supported by data from further stratified analysis by HRT use in postmenopausal women. In postmenopausal women, the association between MSA and breast cancer risk was stronger in women who ever used HRT compared with women who never used HRT (Table III). We recommend caution when interpreting these data due to the small sample size. Since the publication of the Women's Health Initiative study where a significant increase in postmenopausal breast cancer risk with HRT use was demonstrated (28), the use of HRT for treating menopausal symptoms has become a hotly debated issue (29). The potential joint effect of mutagen sensitivity and HRT on breast cancer risk warrants further investigation.

Our data also suggest that bleomycin sensitivity may modulate the effect of tobacco smoking on breast cancer susceptibility. Stratified analysis suggested that bleomycin sensitivity is a stronger risk factor in smokers than in non-smokers. Among bleomycin hypersensitive women, having ever-smoked cigarettes is associated with a 1.6-fold increased risk of breast cancer, whereas among bleomycin less sensitive women, cigarette smoking is associated with a reduced risk of breast cancer. Despite considerable research, the relationship between tobacco smoke exposures and breast cancer risk remains controversial. Some epidemiological studies reported that there may be an increased breast cancer risk with smoking of long duration, smoking before a first full-term pregnancy and second-hand smoking (30,31).

Two prospective studies also showed that breast cancer risk is associated with active or second-hand smoking and tobacco exposure may increase risk as much as 60% among women diagnosed with breast cancer at a young age (32–34). However, the majority of epidemiology studies, including three large USA cohort studies that examined tobacco smoking alone as a breast cancer risk factor, do not support an overall association (35–40). Failure to detect an association in these studies may be due to the potentially opposing effects of tobacco smoking on breast cancer risk.

Cigarette smoking has been reported to have potential carcinogenic as well as antiestrogenic effects (41). Smoking has been associated with earlier age at menopause (42), higher rates of infertility (43), increased risk of osteoporosis and attenuated response to hormone replacement therapy (44). These opposing effects on risk may be variable, depending on certain host factors, both genetic and non-genetic. For example, the association between cigarette smoking and breast cancer risk is more evident in certain subgroups of women, i.e. premenopausal women (45,46), those with a family history of breast cancer (47), BRCA1/BRCA2 mutation carriers (48) or those who have certain genotypes. A pooled analysis suggested that the associations between cigarette smoking and breast cancer risk differ by *N*-acetyltransferase type 2 genotypes (49).

Five recent studies reported that defective DNA repair modestly increases tobacco-related breast cancer risk (50–54). Shore *et al.* (54) reported that in a nested case–control study of 612 cases and 612 controls, the XPC-PAT+/+ genotype increased breast cancer risk only among smokers, and a borderline significant ($P = 0.08$) interaction between the XPC-PAT+/+ polymorphism and cigarette smoking was observed. In the Carolina Breast Cancer Study, smoking duration was positively associated with breast cancer risk in women with the base excision gene XRCC1 codon 399 Arg/Arg genotype (52,55). Our study provided suggestive evidence that tobacco smoking may increase risk of breast cancer among women who are highly sensitive to carcinogens. We recommend caution when interpreting our data due to the small sample size. Nevertheless, this new finding is intriguing and warrants further investigation. Understanding the complex relationship between tobacco smoking and breast cancer risk has important public health impact because even a small increase in the risk of breast cancer among a subgroup of women may account for a substantial number of cases. Therefore, whether or not women should be warned about smoking as a possible cause of breast cancer needs to be studied with great care. Future studies should be designed to investigate the potential combined effect of smoking and mutagen sensitivity on breast cancer risk among women with different smoking patterns as judged by intensity, duration and age of smoking initiation.

Given that this is a case–control study, a theoretical concern is that bleomycin sensitivity is affected by case status (reverse causality). Results from previous studies indicated that mutagen sensitivity is a heritable trait (10,56) and bleomycin-induced chromosomal breaks were significantly higher in female relatives of familial breast cancer cases compared with unrelated female controls (20). Additionally, all the blood samples in our study were drawn before any chemotherapy and radiotherapy treatments. Thus, reverse causality may not be a plausible explanation for our results.

In conclusion, our data provide further evidence that bleomycin sensitivity is associated with breast cancer risk and is a promising biomarker for breast cancer risk assessment for both pre- and post-menopausal women. The observation that bleomycin sensitivity may modulate the effect of tobacco smoking on breast cancer risk warrants further investigation. Better understanding of the role of tobacco exposures in breast cancer development has significant public health impact, given the large number of women that are exposed to active and passive tobacco smoking.

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