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ORIGINAL PAPER

Bleomycin-induced mutagen sensitivity, passive smoking, and risk of breast cancer in Chinese women: a case–control study

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Abstract
Background It is well recognized that genetic variation as well as environmental factors modulates breast cancer risk. Deficiencies in DNA repair capacity are thought to associate with breast cancer risk. The main aim of this study was to use the mutagen sensitivity assay as an indirect measure of DNA repair capacity to assess breast cancer risk and the relationship between passive smoking and breast cancer risk among women in China.
Methods We carried out a case–control study, involving 196 Chinese patients with breast cancer and 211 controls without the disease and with no history of cancer. We investigated the association between mutagen sensitivity and breast cancer risk using bleomycin as the mutagen. Mutagen sensitivity was measured by quantifying the chromatid breaks induced by mutagens in short-term cultures of peripheral blood lymphocytes. Nonparametric tests and the Fisher's exact test were used to determine the statistical significance of the crude case–control comparisons, followed by logistic regression to adjust for important covariates.
Results The mean number of bleomycin-induced breaks per cell was 0.81 for cases compared with 0.73 for the controls ($p = 0.016$). A greater number of bleomycin-induced chromosomal breaks per cell was associated with an increased risk of breast cancer (adjusted odds ratio of 1.82, p trend <0.01). The association between bleomycin sensitivity and breast cancer risk was greater for women who were exposed to tobacco smoke (passive smokers). The combination of bleomycin sensitivity and exposure to tobacco smoke increased risk further; women passive smokers with high sensitivity to bleomycin had a 2.77-fold increased risk of breast cancer.
Conclusions Our data indicate that increased bleomycin-induced mutagen sensitivity is significantly associated with an increased risk of breast cancer among Chinese women. Exposure to passive smoke is also associated with increased breast cancer risk, and the correlation is even greater for women with both longer passive exposure to tobacco smoke and high sensitivity to bleomycin.

Keywords Breast cancer · Passive smoke · MSA · Bleomycin · Epidemiology

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Introduction

Breast cancer is one of the most common types of cancer in women in China and the second only to lung cancer as a cause of cancer death. The incidence of breast cancer has increased steadily in China over the past few decades. Risk factors for the development of breast cancer can be grouped into categories including familial/genetic factors (family history, known and suspected BRCA1/2, TP53, PTEN, or other gene mutation associated with breast cancer risk), factors related to demographics (age, race), reproductive history (age at menarche, age at first live birth, age at menopause), environmental factors, etc. [1–6]. Women who are at high risk of breast cancer can be offered more intensive surveillance or prophylactic measures to lower the incidence of breast cancer. It is imperative to find novel candidate biomarker for breast cancer risk assessment.

DNA repair plays an essential role in the maintenance of DNA integrity. Deficient DNA repair capacity has been suggested as a predisposing factor in familial and sporadic breast cancer. The mutagen sensitivity assay (MSA) provides a phenotypic marker of DNA repair capacity and genomic stress response, which has been reported as a heritable trait that affects both familial and sporadic breast cancer risk [7–12]. This assay measures the number of chromosomal breaks in cultured lymphocytes following exposure to DNA-damaging agents which bleomycin was used in it. Using this assay, DNA from women in high-risk families and sporadic breast cancer cases exhibits a nearly twofold increase in the mean number of breaks per cell compared with DNA from women without cancer from low-risk families [6, 7, 11]. Epidemiologic studies have also suggested that mutagen sensitivity is a predisposing factor for other cancers [13–16]. Thus, mutagen sensitivity may specifically reflect differences in an individual's ability to repair DNA through the pathway of interest and homologous repair, and it may be a biomarker for cancer risk.

It is widely acknowledged that tobacco smoke is a human carcinogen [17]. In mainland China, 2010 data show that 26 % of the urban adult population and 30 % of the rural adult population are current smokers, with about 70 % of the adult population exposed to passive smoking in a typical week [18]. Accumulating evidence has implicated active smoking as a contributor to women's risk of breast cancer [19]. The evidence for a relationship between passive smoking and breast cancer, however, remains tenuous. To shed light on the potential roles of bleomycin-induced mutagen sensitivity in breast cancer susceptibility, we conducted a case–control study focused on the association between mutagen sensitivity, environment tobacco exposure, and breast cancer risk in Chinese women.

Materials and methods

Study population

Eligible cases were women with primary breast carcinoma who underwent mastectomy or breast-conserving surgery in two hospitals in Wuhan city, including Zhongnan Hospital and Renmin Hospital of Wuhan University during the period from January 2009 to February 2011. All of the cases were histopathologically confirmed, and the blood was drawn before any treatment. Controls were matched to cases from the same hospitals with no history of cancer, gynecological disease or endocrine disease, same residing area, and within ±3 years of age. Each participant donated 10 ml of blood after signing written informed consent. Of 219 breast cancer cases identified, in-person interviews were completed for 203 (93.1 %), three cases (1.4 %) did not provide a blood sample, and four blood cultures failed (1.8 %). In-person interviews were completed for 221 (91.7 %) of the 241 eligible controls. Four controls (1.8 %) did not provide a blood sample and six blood cultures failed (2.7 %). Therefore, data analysis for bleomycin sensitivity was performed on 196 cases and 211 controls.

Questionnaire

A thorough, structured questionnaire was completed by all subjects. Three experienced nurses (one interviewer, two collectors) were assigned to obtain information through face-to-face interviews conducted in hospital wards or in the subject's home. The baseline questionnaire focused on demographic and anthropometric parameters, dietary habits, menstrual and reproductive factors, hormone use, lifestyle, medical history, and family history. Postmenopausal women were defined as those having bilateral oophorectomy or having no menstrual cycle in the 12 months prior to blood sample collection. Passive smoking history was collected for two level of duration. First, the subject was asked whether her husband had ever smoked at home and/or she was exposed to the smoke of others in her workplace, then the subject was asked the average number of hours per day she was exposed to the smoke, and the total number of years she had been exposed to the smoke at home and/or workplace. The passive smoking index was calculated as the total number of years multiplies by hours per day (hour-year).

Mutagen sensitivity assays

The assay was described in detail previously [20]. Briefly, 1 ml of fresh whole blood was added to 9 ml of RPMI-1640 medium supplemented with 15 % bovine serum, 1.5 % of phytohemagglutinin (Wuhan Boster Bio-engineering Limited Co.), 2 mM L-glutamine, and 100 U/ml each of

penicillin and streptomycin. After the cells were cultured for 72–90 h at 37 °C, they were incubated for 5 h with 0.03 U/ml bleomycin (Hisun pharmaceutical Co.). To arrest the cells at metaphase, 0.2 μg/ml colcemid was added to the culture 1 h before the harvest. The cells were treated in hypotonic solution (0.06 M KCl) and fixed in fixative (three parts of methanol with one part of acetic acid). The cells were dropped onto clean microscopic slides, air dried and stained with 4 % Gurr's Giemsa solution (Wuhan Boster Bio-engineering Limited Co). Fifty well-spread metaphase cells per subject were examined to visually score the chromatid breaks. Only frank chromatid breaks or chromatid exchanges were scored. Criteria for a frank chromatid break were a discontinuity of a single chromatid in which the distance of discontinuity region was wider than the diameter of the chromatid, or there was a clear misalignment of one of the chromatids. A chromatid exchange is the result of two or more chromatid breaks and the subsequent rearrangement of chromatid material. Exchanges may be between chromatids of different chromosomes (interchanges), or between or within chromatids of one chromosome (intrachanges). The total number of breaks was divided by the number of the cells examined, and the mean number of breaks per cell was recorded for statistical analysis. Cells with more than 12 breaks were excluded from the calculation of mean breaks per cell to reduce the bias of the results by a very few severely damaged cells. In our study, the frequency of the cells with more than 12 breaks was rare. In the vast majority of the subjects, fifty cells were analyzed from one slide without seeing one cell with more than 12 breaks. The slides were coded and scored without the knowledge of case–control status. Twenty blinded quality control samples were included to assess variability, and each sample was run in triplicate. The coefficient of variation for repeats was 5.1 %.

Statistical analyses

The distribution of demographic information between cases and controls was examined using Fisher's exact test for categorical variables and Wilcoxon rank-sum test for the means of continuous variables. The number of bleomycin-induced chromatid breaks was analyzed as both continuous and categorical variables. 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 (0.75 breaks per cell). Bleomycin sensitivity was also categorized according to the quartiles in control subjects. Passive smoking status was stratified into two categories of tobacco smoke exposure (never: no exposure and ever: some exposure); and four categories (never, low: less than 10 h-year, medium: 10–20 h-year, and high: more than 20 h-year). Family history of female cancers was defined as having breast or ovarian cancer in first- or second-degree biological relatives. Postmenopausal women were defined as those having bilateral oophorectomy or having no menstrual cycle in the 12 months prior to blood sample collection. Alcohol consumption was defined as intake of at least 100 g alcoholic beverage per time per week. 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. Multivariate logistic regression was used to obtain the odds ratio (OR) and 95 % confidence intervals (CIs) for the strength of the association between breast cancer and mutagen sensitive phenotype, while controlling for known breast cancer risk factors and other potential confounders: age, physical activity, menopausal status, passive smoking, alcohol, body mass index (BMI), family history of female cancer (if inclusion of a factor altered the odds ratio (OR) estimation by 10 %, that factor was retained in the final model). Tests for a linear trend were done using quartile levels as continuous variables based on MSA scores and passive tobacco smoking exposure. We also explored the combined effect of passive smoking and mutagen sensitivity by their categories and the possibility of interactions of the two variables by logistic regression analysis. All p values were two-sided, and $p < 0.05$ was used as the threshold for statistical significance. All analyses were conducted using STATA/IC 11.2 (StataCorp, College Station, TX, USA).

Results

Characteristics of the study population

The characteristics of cases and controls are summarized in Table 1. The average age was 46.7 years for the cases (range 25–75 years) and 48.6 years for the controls (range 27–75 years). There were no significant differences in the distribution of alcohol use, oral contraceptive pill use, hormone replacement treat (HRT), and family history of female cancers (breast and ovarian) between cases and controls. There were significant case–control differences in passive smoke exposure, age at menopause, age at menarche, age at first full birth, total times of lactation, body mass index (BMI), and education.

Bleomycin sensitivity and breast cancer risk

Table 2 shows 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 0.81) than that in controls (mean 0.73, $p = 0.016$). When the case–control comparison was stratified by cancer risk, we observed significant differences between subjects over 50 years old, those exposed to passive smoke, those who

Table 1 Characteristics of cases and controls

Host characteristics	Controls ($n = 211$) mean (SD), or n (%)	Cases ($n = 196$) mean (SD), or n (%)	p
Age (year)	48.6 (10.70)	46.7 (10.33)	0.375
Education			0.047
<High school	110 (52.1 %)	82 (41.8 %)	
≥High school	101 (47.9 %)	114 (58.2 %)	
Smoking			0.815
Never	202 (95.7 %)	186 (94.9 %)	
Ever/current	9 (4.3 %)	10 (5.1 %)	
Passive smoking			0.032
No	173 (82 %)	143 (73 %)	
Yes	38 (18 %)	53 (27 %)	
Alcohol			0.594
No	195 (92.4 %)	178 (90.8 %)	
Yes	16 (7.6 %)	18 (9.2 %)	
Family history of female cancer			0.162
No	209 (99.1 %)	190 (96.9 %)	
Yes	2 (1 %)	6 (3.1 %)	
Menopause			0.001
Premenopause	82 (38.9 %)	112 (57.1 %)	
Postmenopause	129 (61.1 %)	84 (42.9 %)	
Age at menopause	47.1 (4.92)	52 (5.10)	0.001
Physical activity			0.069
No	94 (44.6 %)	105 (53.6 %)	
Yes	117 (55.5 %)	91 (46.4 %)	
Oral contraceptive			0.167
<3 month	176 (83.4 %)	152 (77.6 %)	
≥3 month	35 (16.6 %)	44 (22.5 %)	
HRT			1.000
No	203 (96.2 %)	188 (95.9 %)	
Yes	8 (3.8 %)	8 (4.1 %)	
BMI	21.9 (2.63)	22.5 (2.08)	0.012
Age at menarche	14.2 (1.87)	13.2 (1.71)	0.001
Pregnancies	3.15 (1.77)	2.91 (2.15)	0.064
Age at first full birth	26.62 (4.12)	27.71 (4.25)	0.011
Total times of lactation (year)	1.48 (0.73)	1.28 (0.95)	0.002

HRT hormonal replacement therapy, *BMI* body mass index

consumed alcohol, had a BMI <23, had family history of female cancer and those pre-menopause. The relationship between bleomycin sensitivity and breast cancer risk was estimated by calculating ORs and 95 % CIs using unconditional logistic regression analysis, with adjustment for age, physical activity, menopausal status, alcohol, body mass index (BMI), family history of female cancer. Defining bleomycin sensitive as ≥0.75 break/cell (median level in population controls), 59.2 % of the cases were bleomycin sensitive compared with 49.8 % of the controls with an adjusted OR of 1.23. Subjects were categorized into four groups (by quartiles) according to the bleomycin-induced breaks per cell in controls. A dose–response relationship was observed with the highest versus lowest quartile with an adjusted OR of 1.82, $p \leq 0.01$ (Table 3). We also stratified the bleomycin-induced chromatid breaks by passive smoking exposure and found that the breast cancer risk was associated with high bleomycin sensitivity in women who had been exposed to passive smoke (adjusted OR = 1.94). When the data were categorized by quartile distribution in the control group, the results showed that there is a significant correlation between bleomycin sensitivity and breast cancer risk in a dose-dependent manner for both women who had never been exposed to smoke, and those who had. The adjusted OR for the fourth quartile compared with the first quartile was 2.06 and 2.75, respectively (Table 5).

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

Table 4 shows the association between passive smoking and breast cancer risk. Overall, passive smoking had a positive relationship with breast cancer risk in our study population and had a significant dose–response relationship, adjusted OR 2.13, p trend = 0.01. However, there was no significant interaction between passive smoking and bleomycin sensitivity when the interaction was formally tested in the logistic model ($p = 0.471$). We further examined the combined effect of passive smoking and bleomycin sensitivity on breast cancer risk, using women who were low sensitivity and had never been exposed to passive smoke as the reference group. The combined effect of bleomycin sensitivity and passive smoking on the risk of breast cancer was significantly different from the single effect of either bleomycin sensitivity or passive smoking. Women who had high bleomycin sensitivity phenotype and passive smoking were at 2.77-fold increased risk of breast cancer compared with women who had a low bleomycin sensitivity phenotype and never passive smoking (Tables 5, 6).

Discussion

The failure to maintain genome integrity is central to the problem of carcinogenesis [21]. DNA repair capacity is a cellular defense system designed to protect genomic integrity. There is considerable interindividual variation in DNA repair capacity. Numerous epidemiologic studies have consistently yielded significant associations between reduced DNA repair capacity and increased cancer occurrence [22, 23], suggesting that DNA repair capacity is a cancer susceptibility factor. Mutagen sensitivity, measured

Table 2 Case–control comparison of mean bleomycin-induced breaks per cell

Factors	Controls		Cases		*p*
	n	Mean (SD)	*n*	Mean (SD)	
Overall MSA	211	0.73 (0.32)	196	0.81 (0.34)	0.016
Age					
<50	116	0.70 (0.23)	120	0.73 (0.25)	0.160
≥50	95	0.77 (0.41)	76	0.93 (0.42)	0.010
Passive smoking					
No	173	0.66 (0.25)	143	0.69 (0.24)	0.204
Yes	38	0.94 (0.3)	53	1.11 (0.35)	0.011
Alcohol					
No	195	0.74 (0.32)	178	0.78 (0.31)	0.052
Yes	16	0.67 (0.22)	18	0.99 (0.49)	0.031
BMI					
<23	120	0.73 (0.34)	93	0.78 (0.29)	0.033
≥23	91	0.74 (0.3)	103	0.83 (0.38)	0.425
Family history of female cancer					
No	209	0.73 (0.32)	190	0.81 (0.34)	0.021
Yes	2	0.76 (0.01)	6	0.85 (0.37)	0.505
Menopause					
Premenopause	82	0.68 (0.21)	112	0.75 (0.23)	0.016
Postmenopause	129	0.77 (0.37)	84	0.88 (0.43)	0.081
Age at menopause					
<50	84	0.66 (0.30)	30	0.49 (0.17)	0.006
≥50	45	0.84 (0.33)	54	1.09 (0.37)	0.001

by quantifying the chromatid breaks induced in short-term cultures of peripheral blood lymphocytes, has been used as an indirect measure of DNA repair capacity. Mutagens act on cells through different molecular mechanisms and may activate other repair mechanisms. In our study, we chose bleomycin as the mutagen for the MSA. The main reason is that bleomycin is a clastogenic agent that mimics the effects of radiation after formation of a complex with DNA, ferrous ions (Fe^{2+}), and oxygen, which releases oxygen radical [24]. The free oxygen radicals are capable of producing single- and double-strand breaks in DNA. Bleomycin is also relevant to tobacco use because it is similar to numerous compounds in tobacco smoke known to cause oxidative damage which can be repaired by the base excision and recombination repair systems [25, 26].

In this case–control study, we demonstrated that, after adjusting for known breast cancer risk factors, bleomycin sensitivity is positively related to the risk of breast cancer among Chinese women. For nearly two decades, mutagen sensitivity has been a commonly used phenotypic assay in cancer epidemiology. The epidemiologic evidence supporting its association with cancer risk is strong and consistent. Jyothish et al. [27] reported that bleomycin-induced chromosomal breaks were significantly higher in both familial and sporadic breast cancer patients compared with unrelated female controls. Xiong et al. [28] investigated benzo[a]pyrene diol-epoxide sensitivity and breast cancer risk in a case–control study of predominantly white women and reported that it was associated with a threefold increased risk of breast cancer in premenopausal women. A study examined the MSA using gamma radiation as the mutagen and breast cancer risk in a case–control study of African-American women, and gamma radiation sensitivity was found to be associated with an increased breast cancer risk (OR = 4.5) [8]. More recently, Wang et al. [29] reported that high radiosensitivity was related to risk of breast cancer in a case–control study of 515 young women with newly diagnosed sporadic breast cancer and 402 cancer-free controls. Our results are in agreement with these previous reports and provide further evidence (adjusted OR = 1.82).

Our results also suggest that accumulative exposure to tobacco smoke may increase risk for breast cancer among Chinese women, particularly in those who had high bleomycin sensitive phenotypes.

Table 3 Association of bleomycin sensitivity with breast cancer risk

MSA level (b/c)	Control (n = 211)	Case (n = 196)	OR (95 % CI)	ORadj (95 % CI)[a]
50th percentile, *n* (%)				
Low	106 (50.2)	80 (40.8)	1.00	1.00
High	105 (49.8)	116 (59.2)	1.46 (0.99–2.17)	1.23 (0.81–1.87)
By quartiles, *n* (%)				
1st quartile	53 (25.1)	45 (23)	1.00	1.00
2nd quartile	53 (25.1)	35 (17.9)	0.77 (0.43–1.39)	0.64 (0.32–1.21)
3rd quartile	52 (24.6)	50 (25.5)	1.13 (0.64–1.97)	1.07 (0.76–1.67)
4th quartile	53 (25.1)	66 (33.7)	1.47 (0.86–2.51)	1.82 (1.00–3.35)
p for trend			0.03	<0.01

MSA, categorized by mean breaks per cell in the controls

[a] Adjusted by age, physical activity, menopausal status, passive smoking, alcohol, BMI, family history of female cancer

Table 4 Association of passive smoking with breast cancer risk

Passive smoking	Control ($n = 211$)	Case ($n = 196$)	OR (95 % CI)	ORadj (95 % CI)[a]
Passive smoking, *n* (%)				
No	173 (82)	143 (73)	1.00	1.00
Yes	38 (18)	53 (27)	1.69 (1.05–2.70)	1.54 (0.94–2.52)
Passive smoking (ry), *n* (%)				
0	173 (82)	143 (73)	1.00	1.00
1–10	10 (4.7)	5 (2.6)	0.60 (0.20–1.81)	0.41 (0.13–1.29)
11–19	11 (5.2)	19 (9.7)	2.09 (0.96–4.54)	1.90 (0.84–4.28)
≥20	17 (8.1.0)	29 (14.8)	2.06 (1.09–3.91)	2.13 (1.09–4.15)
p for trend			0.01	0.01

Passive smoking: hour-year (ry)

[a] Adjusted by age, physical activity, menopausal status, alcohol, BMI, family history of female cancer

Table 5 Association of bleomycin sensitivity with breast cancer risk

MSA	No passive smoking			Passive smoking		
	Control/case	OR (95 % CI)	ORadj (95 % CI)*	Control/case	OR (95 %CI)	ORadj (95 %CI)[a]
50th percentile, *n* (%)						
Low	89/64	1.00	1.00	17/16	1.00	1.00
High	84/79	1.31 (0.84–2.04)	1.23 (0.78–1.94)	21/37	1.87 (0.79–4.46)	1.94 (1.07–3.98)
By quartiles, *n* (%)						
1st quartile	44/36	1.00	1.00	8/9	1.00	1.00
2nd quartile	45/28	0.76 (0.40–1.45)	0.79 (0.40–1.55)	9/7	0.69 (0.18–2.72)	0.71 (0.26–3.01)
3rd quartile	42/35	1.02 (0.54–1.91)	0.85 (0.46–1.58)	10/15	1.33 (0.38–4.62)	1.23 (0.42–3.93)
4th quartile	42/44	1.28 (0.70–2.36)	2.06 (1.05–4.02)	11/22	1.78 (0.54–5.88)	2.75 (1.26–8.72)
p for trend		0.31	0.05		0.24	0.04

MSA, categorized by mean breaks per cell in the controls

[a] Adjusted by age, physical activity, menopausal status, passive smoking, alcohol, BMI, family history of female cancer

Table 6 Combined effect of mutagen sensitivity and passive smoking on breast cancer risk

MSA level (b/c)	Passive smoking	Control/case	OR (95 % CI)	ORadj (95 % CI)[a]
Low	No	89/64	1.00	1.00
High	No	84/79	1.31 (0.84–2.04)	1.13 (0.71–1.80))
Low	Yes	17/16	1.31 (0.62–2.78)	1.49 (0.64–3.43)
High	Yes	21/37	2.45 (1.31–4.57)	2.77 (1.33–5.80)
p for trend			0.01	<0.01

MSA, categorized by mean breaks per cell in the controls

[a] Adjusted by age, physical activity, menopausal status, alcohol, BMI, family history of female cancer. The interaction between passive smoking and MSA, $p = 0.471$

We chose to investigate the effects of tobacco smoke exposure as a risk for female breast cancer because the vast majority of Chinese women have never smoked cigarettes, while the prevalence of smoking is high among adult men and most of them smoke at home. Smoking has not been restricted in public places, including work settings. This exposure pattern provides a unique opportunity to vigorously investigate the hypothesis related to passive smoking. The body of literature on this topic is still relatively small and findings to date have not been consistent. There have been ten prospective cohort studies and 17 case–control studies conducted to examine the relationship between passive smoking and breast cancer risk. Results have been mixed, with four of the ten cohort studies yielding positive results and 11 of the 17 case–control studies reporting positive findings [30]. The largest of these is the prospective Million Women Study from United Kingdom [31]. The authors reported an overall null association (OR = 0.98,

95 % CI = 0.93–1.05) for passive smoking and breast cancer, and the point estimate for risk in premenopausal women actually suggested an inverse association 0.54 (95 % CI = 0.33–0.99). However, our study shows a positive relationship between passive smoking and breast cancer risk and dose–response association with hours-years of exposure. We further investigate the combined effect of passive smoking and bleomycin sensitivity on breast cancer risk and suggest that women who had high bleomycin sensitivity phenotypes and longer passive smoking exposure have greater risk of breast cancer (adjusted OR = 2.77). In our study, stratified analysis suggests that bleomycin sensitivity is a stronger risk factor in women exposed to tobacco smoke than in those not exposed. Among high bleomycin sensitive women, having longer exposure to smoke is associated with a 2.77-fold increased risk of breast cancer. We suggest that while longer exposure to tobacco smoke may be associated with mutagen sensitivity, this needs further investigation.

Our study has several limitations and the results need to be interpreted with caution. We had limited sample size and thus limited power to detect an association. Another limitation of this study is the use of lymphocytes, the repair of which may not reflect that of breast epithelial cells. We recruited the controls in the hospital setting; they might not represent the general population, and the selection bias may influence the results.

In summary, our results showed that bleomycin sensitivity is associated with breast cancer risk and is a promising biomarker for breast cancer risk assessment for Chinese women. Our observation that passive smoking increases breast cancer risk especially for women with high sensitivity to bleomycin is intriguing and worthy of further investigation in large studies.

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Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Bray F, McCarron P, Parkin DM (2004) The changing global patterns of female breast cancer incidence and mortality. Breast Cancer Res 6(6):229–239
2. Newman B, Mu H, Butler LM et al (1998) Frequency of breast cancer attributable to BRCA1 in a population-based series of American women. JAMA 279(12):915–921
3. Turnbull C, Rahman N (2008) Genetic predisposition to breast cancer: past, present, and future. Annu Rev Genomics Hum Genet 9:321–345
4. Martin AM, Weber BL (2000) Genetic and hormonal risk factors in breast cancer. J Natl Cancer Inst 92(14):1126–1135
5. McPherson K, Steel CM, Dixon JM (2000) ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. BMJ 321(7261):624–628
6. Scully R (2000) Role of BRCA gene dysfunction in breast and ovarian cancer predisposition. Breast Cancer Res 2(5):324–330
7. Helzlsouer KJ, Harris EL, Parshad R et al (1996) DNA repair proficiency: potential susceptibility factor for breast cancer. J Natl Cancer Inst 88(11):754–755
8. Natarajan TG, Ganesan N, Carter-Nolan P et al (2006) gamma-Radiation-induced chromosomal mutagen sensitivity is associated with breast cancer risk in African-American women: caffeine modulates the outcome of mutagen sensitivity assay. Cancer Epidemiol Biomarkers Prev 15(3):437–442
9. Parshad R, Sanford KK (2001) Radiation-induced chromatid breaks and deficient DNA repair in cancer predisposition. Crit Rev Oncol Hematol 37(2):87–96
10. Parshad R, Price FM, Bohr VA et al (1996) Deficient DNA repair capacity, a predisposing factor in breast cancer. Br J Cancer 74(1):1–5
11. Patel RK, Trivedi AH, Arora DC, Bhatavdekar JM, Patel DD (1997) DNA repair proficiency in breast cancer patients and their first-degree relatives. Int J Cancer 73(1):20–24
12. Scott D, Spreadborough AR, Jones LA, Roberts SA, Moore CJ (1996) Chromosomal radiosensitivity in G2-phase lymphocytes as an indicator of cancer predisposition. Radiat Res 145(1):3–16
13. Zheng YL, Loffredo CA, Yu Z et al (2003) Bleomycin-induced chromosome breaks as a risk marker for lung cancer: a case–control study with population and hospital controls. Carcinogenesis 24(2):269–274
14. Cloos J, Spitz MR, Schantz SP et al (1996) Genetic susceptibility to head and neck squamous cell carcinoma. J Natl Cancer Inst 88(8):530–535
15. Spitz MR, Hoque A, Trizna Z et al (1994) Mutagen sensitivity as a risk factor for second malignant tumors following malignancies of the upper aerodigestive tract. J Natl Cancer Inst 86(22):1681–1684
16. Wu X, Gu J, Patt Y et al (1998) Mutagen sensitivity as a susceptibility marker for human hepatocellular carcinoma. Cancer Epidemiol Biomarkers Prev 7(7):567–570
17. World Health Organization IAfRoC (2004) IARC monographs on the evaluation of carcinogenic risks to humans. IARC Press. Report No.: 83-Tobacco Smoke and Involuntary Smoking
18. CDC (2010) Smoking and tobacco use: GATS: Fact Sheet: China. http://www.cdc.gov/tobacco/global/gats/countries/wpr/fact_sheets/china/2010/index.htm. Available from: http://www.cdc.gov/tobacco/global/gats/countries/wpr/fact_sheets/china/2010/index.htm
19. Collishaw N, Boyd N, Cantor K (2009) Canadian expert panel on tobacco smoke and breast cancer risk. OTRU Special Report Series 2009
20. Hsu TC, Johnston DA, Cherry LM et al (1989) Sensitivity to genotoxic effects of bleomycin in humans: possible relationship to environmental carcinogenesis. Int J Cancer 43(3):403–409
21. Hoeijmakers JH (2001) Genome maintenance mechanisms for preventing cancer. Nature 411(6835):366–374
22. Berwick M, Vineis P (2000) Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. J Natl Cancer Inst 92(11):874–897
23. Spitz MR, Wei Q, Dong Q, Amos CI, Wu X (2003) Genetic susceptibility to lung cancer: the role of DNA damage and repair. Cancer Epidemiol Biomarkers Prev 12(8):689–698
24. Burger RM, Peisach J, Horwitz SB (1981) Mechanism of bleomycin action: in vitro studies. Life Sci 28(7):715–727
25. Dar ME, Winters TA, Jorgensen TJ (1997) Identification of defective illegitimate recombinational repair of oxidatively-

induced DNA double-strand breaks in ataxia-telangiectasia cells. Mutat Res 384(3):169–179
26. Xu YJ, Kim EY, Demple B (1998) Excision of C-4′-oxidized deoxyribose lesions from double-stranded DNA by human apurinic/apyrimidinic endonuclease (Ape1 protein) and DNA polymerase beta. J Biol Chem 273(44):28837–28844
27. Jyothish B, Ankathil R, Chandini R et al (1998) DNA repair proficiency: a potential marker for identification of high risk members in breast cancer families. Cancer Lett 124(1):9–13
28. Xiong P, Bondy ML, Li D et al (2001) Sensitivity to benzo(a)-pyrene diol-epoxide associated with risk of breast cancer in young women and modulation by glutathione S-transferase polymorphisms: a case–control study. Cancer Res 61(23):8465–8469
29. Wang LE, Han CH, Xiong P, Bondy ML, Yu TK, Brewster AM, Shete S, Arun BK, Buchholz TA, Wei Q (2012) Gamma-ray-induced mutagen sensitivity and risk of sporadic breast cancer in young women: a case–control study. Breast cancer Res Treat 132(3):1147–1155
30. Reynolds P, Goldberg D, Hurley S, Nelson DO, Largent J, Henderson KD, Bernstein L (2009) Passive smoking and risk of breast cancer in the California teachers study. Cancer Epidemiol Biomarkers Prev 18(12):3389–3398
31. Pirie K, Beral V, Peto R et al (2008) Passive smoking and breast cancer in never smokers: prospective study and meta-analysis. Int J Epidemiol 37(5):1069–1079